

UNIVERSIDAD POLITÉCNICA DE MADRID
Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de
Biosistemas



**The price of the plant induced defence:
insights from *Arabidopsis* and
Tetranychus urticae interaction**

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Alejandro García García

BSc. in Biotechnology
MSc. in Computational Biology

Madrid, 2024



UNIVERSIDAD POLITÉCNICA DE MADRID
Escuela Técnica Superior de Ingeniería Agronómica,
Alimentaria y de Biosistemas

**Doctoral Degree in Biotechnology and Genetic Resources of
Plants and Associated Microorganisms**

**The price of the plant induced defence:
insights from *Arabidopsis* and
Tetranychus urticae interaction**

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Alejandro García García

BSc. in Biotechnology
MSc. in Computational Biology

Under the supervision of:

Dra. M^a Estrella Santamaría Fernández

Madrid, 2024

Title: The price of the plant induced defence: insights from *Arabidopsis* and *Tetranychus urticae* interaction

Author: Alejandro García García

Doctoral Programme: Biotechnology and Genetic Resources of Plants and Associated Microorganisms

Thesis Supervision:

Dra. M^a Estrella Santamaría Fernández, Assistant Proffesor, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas – UPM (Supervisor)

External Reviewers:

Thesis Defense Committee:

Thesis Defense Date:

This thesis has been supported by Predoctoral Programa Propio 2019 UPM fellow which finances Alejandro García García and the Grants APOYO-JOVENES-SUR6Q9-22-YTFC3Z funded by the Convenio Plurianual between Comunidad de Madrid and Universidad Politécnica de Madrid (UPM) through the Apoyo Jóvenes Investigadores Program, PID2020-112756RA-I00 and CNS2022-135194 funded by MCIN/AEI/10.13039/501100011033 and by European Union NextGeneration EU/PRTR and 22-17435S from Czech Science Foundation.

A Florencio, a Faustina, a Felipe y a Eugenia,

Nunca sabes cuando algo es para siempre.

Acknowledgements

Desde que empecé la carrera, siempre tuve en mente llegar a ser doctor, no por el título en sí, sino por lo que representaba para mí. Ser doctor significa convertirse en alguien que no solo domina un área específica, sino que también tiene la capacidad de hacer preguntas profundas y, más importante aún, de buscar respuestas nuevas y originales. Es una persona que encarna la curiosidad intelectual y el rigor científico, con la capacidad de enfrentar problemas complejos y encontrar soluciones que, idealmente, beneficien al mundo. Además, ser doctor no es solo tener habilidades técnicas, sino ser un ejemplo para otros, guiando con paciencia y transmitiendo conocimiento. Es ser una figura inspiradora, con una combinación de persistencia, dedicación y pasión por el aprendizaje y el progreso, alguien a quien admirar por su capacidad para ver más allá de lo evidente, para innovar y para enfrentar el fracaso con resiliencia. Han pasado muchos años desde entonces, y por fin puedo decir que he alcanzado este objetivo, irónicamente trabajando con la 'araña roja'.

Me gustaría comenzar agradeciendo a Estrella, creo que este es tu lugar. Muchísimas gracias por todo. Tengo el orgullo de ser tu primera tesis como directora única. Gracias por guiarme y enseñarme a lo largo de todos estos años, por tu paciencia, tus lecciones, tus opiniones, y por preocuparte por mí tanto a nivel profesional como personal. Agradezco profundamente la confianza depositada en mí, así como por compartir no solo tu visión científica, sino también tu manera de entender la vida. Gracias por ser faro y guía, por tu constancia y esfuerzo. Siempre miras hacia el futuro, pero a veces, aunque sea difícil, frena un poco y mira atrás: has llegado muy lejos, y no tengo dudas de que lo llegarás aún más. No podría haber tenido una mejor mentora. Siempre recordaré que Vega casi se llamó Alejandra; faltó muy poco.

A Isabel, por acogerme en tu laboratorio durante casi seis años, comenzando desde mis prácticas de grado. Gracias por integrarme en el equipo, por tus sabios consejos y tu visión científica. La vida hace que las personas vayan y vengan, y su constante movimiento nos acerca a algunas más que a otras. Cuando llegue el momento, echaré de menos nuestros cafés, donde conseguíamos arreglar el mundo antes de volver al trabajo. Si alguien me pregunta por una científica exitosa, no dudaré en nombrarte.

Gracias también al resto de los integrantes del laboratorio. A Manu y a Pablo, por vuestra ayuda y consejo científico. A Dairon, Álvaro y Laura, gracias por hacer mucho más amenas las horas en el laboratorio. Dairon, sigo esperando ese café cubano tan suave que mencionas. Álvaro, mi

enciclopedia humana de deportes y de casi cualquier tema. Mucha suerte en tu nueva etapa; ya no tendrás que soportar mis chistes malos. Laura, acabas de empezar, y habrá días que serán duros, pero mantén siempre esa alegría que llevas al laboratorio. Si no "gomitas", todo irá bien. A las Martas, por vuestra experiencia y positividad. Marta Pérez, pocas personas tienen un humor tan básico como el mío, pero siempre hay gente como nosotros por ahí. Marta Boter (C. Cristo), gracias por tu ética, tu apoyo incondicional y tu forma de ver la ciencia. Es inspirador contar con personas como tú. Gracias por traer alegría al laboratorio y por las charlas en mis momentos finales de la tesis; viste mi lado más oscuro y aun así seguiste ahí al lado.

Agradecer en especial a Lucía. Sin ti el laboratorio no habría sido para nada igual. Gracias, básicamente, por ser como eres. Hay veces que simplemente encajas con alguien. Gracias por escucharme, por darme tu punto de vista y transmitir tranquilidad cuando hacía falta. Me quedo con nuestros ratos de experimentos escuchando podcasts, nuestras charlas al sol enfrente del invernadero y por ser mi cómplice en la grabación de vídeos de tesis (qué difícil habría sido hacerlos solos jajaja). Me alegra ver cómo hemos ido cambiando desde que empezamos en el laboratorio (lo hemos hecho a mejor). Siempre seremos parte del team trade-off.

Por supuesto también agradecer a mis compañeros de batallas que ya no están en el laboratorio. A Ana, por asentar las bases de lo que sería mi tiempo en el laboratorio. A Gara, por las risas y los buenos ratos juntos, esos TFG y TFM se consiguieron sacar con esfuerzo adelante. A la ya mamá Fany, por tu humor, bondad y compañía; estaría bien volver a coincidir con mi compañera de videojuegos. Y a Irene, por tu sinceridad y esas charlas de "salseo" por la tarde. Olivia tiene la suerte de tener una madre luchadora como tú.

Agradecer también al resto de amigos del doctorado, porque siempre una cerveza y un buen rato ayudan a pasar mejor los momentos malos. A Alique (parte 1), Daniela, Arturo, Martí, Clara, Ana, Claudia, Adrián... y espero no dejarme muchos más sin mencionar. Daniela, tus abrazos y charlas siempre fueron lo mejor. Arturo y Martí, porque, aunque tuvierais un mal día, siempre intentabais escuchar y mantener el buen humor. Ana, desde que empecé siempre te he visto por aquí, será extraño dejar de verte. Claudia, hemos ido prácticamente a la par, y siempre fue de gran ayuda tener apoyo en esos momentos finales de escritura. Adrián, por tu simpatía y porque con ayuda, finalmente derrotamos a Antonio.

I would also like to thank to the wonderful people at the LGR in Olomouc. Your kindness made a challenging time so much easier for me. I want to especially thank Ondrej for always finding time to talk with me when I needed it—often with a *huuuuge* cup of coffee in the early morning

hours. A special shout-out goes to the "Four Musketeers" of Olomouc: Ivan, Aleš, Radjim (Simi), and Ivo. You treated me like a brother, and to me, you are part of my family as well. I will never forget our morning coffees, beers at U Kuděje, the laughter, and all the moments we shared. Wishing all of you the very best, and I truly hope to see you again soon!

Sin embargo, no todo se reduce a mi experiencia en el laboratorio, esto también es fruto de toda mi formación académica. Desde que empecé la carrera en Madrid me han acompañado muchas personas que también se han hecho un hueco como parte de mi familia. Gracias a mis amigos del grado: Pablo, Jorge, Alique (parte 2), Robles, Clara, Chofi, Coral, Dani, y al resto de los Elemelons por los viajes y ratos juntos, y por hacer Madrid mucho más divertido. También gracias a mis compañeros de residencia talaveranos: Pablo, Juan y Ángel, por sobrevivir conmigo en la RUDA y hacer una pequeña familia durante toda la carrera. Y cómo no, también quiero agradecer a mis amigos de Segovia, el grupo de los Bereberes, que, aunque no sepáis a veces ni qué hago, siempre estáis ahí cuando hace falta.

Especialmente, quiero agradecer a Isa, crrrrreeeeooo que ahora es tu turno. Te ha tocado aguantarme durante un periodo que se ha hecho cuesta arriba a veces, y aún así quieres aguantarme un poco más. Gracias por ser mi apoyo diario, por mantenerme a flote cuando veo que las cosas se hunden, por valorarme y darme las energías que a veces me faltan, por ver en mí algo más que el resto de la gente. Gracias por hacerme saber que siempre estás ahí para lo que necesite, no fue fácil encontrarnos y haces que mi día a día sea mucho más especial. Te quiero mucho.

Finalmente, ante todo, quiero agradecer a mi familia. Sois la base por la que todo empieza. No habría llegado a realizar un doctorado sin toda la educación recibida, las tardes de estudio, los valores que me habéis transmitido y, en resumen, la persona en la que me he convertido gracias a vosotros. A Jesús y M^a Jesús, sois mi respaldo incondicional, siempre vais a estar ahí en cada paso que dé, y quiero agradecer los esfuerzos que habéis hecho para que yo lograra mis metas. Ya podéis decir que tenéis dos hijos doctores. A mi hermano Álvaro, mi otro gran apoyo. Ninguno hemos cogido caminos fáciles, pero estoy orgulloso del hermano que tengo y de los pasos que poco a poco vamos dando. A mi tía Azu, porque estás siempre muy orgullosa de tus sobrinos y de los pasos que damos, gracias por preocuparte cada día y estar siempre atenta por nosotros. Y también a mi tío Félix, que siempre me ha apoyado durante todos estos años y sé la ilusión que le hace verme cumplir esta etapa.

Finalmente, agradecer, sobre todo, a mis abuelos, sé lo orgullosos que estaríais de verme cumplir esta nueva etapa. No hay día que no me acuerde de vosotros. ¡Que sí! ¡Que vuestro nieto es Doctor! Os quiero muchísimo.

Abstract

Being sessile, plants constantly face a wide variety of environmental stresses present in the environment. These challenges disrupt their metabolism, ultimately leading to a decrease in their overall fitness and productivity. Consequently, plant survival relies on their ability to adapt to the continuous external events they confront. Among these stresses, biotic stresses, and specifically, phytophagous arthropods, cause significant crop yield losses, leading to substantial costs in agriculture. Particularly, *Tetranychus urticae*, commonly known as the two-spotted spider mite, is a polyphagous acari considered a serious threat to global agriculture, that in the current context of climate change, it is expected to increase their performance and distribution range. Therefore, the development of new alternative pest management strategies becomes a necessity for achieving an effective control of this pest.

Most previous studies evaluating plant responses to *T. urticae* have focused on the characterization of the plant defence response with the aim of increasing plant resistance. However, plant growth, development and reproduction throughout the infestation process have not been previously analysed. For this reason, this thesis provides a comprehensive evaluation of the defence-growth responses to *T. urticae* infestation, revealing the cost paid by the plants for implementing defence responses to this pest.

To achieve this goal, by evaluating previous research we identified relevant parameters to estimate plant fitness and determined the specific experimental conditions for evaluating the defence-growth trade-off. Growth, photosynthesis and reproductive parameters were found to be valuable in the final evaluation of plant fitness. Additionally, factors such as pest feeding guild, plant host and developmental stage, and the duration and intensity of infestation were found to significantly influence final responses.

Using this information, the experimental design for the evaluation of the defence-growth trade-off in spider mite infestations was established. Concretely, the evaluation was performed throughout the entire plant life cycle using the *Arabidopsis thaliana* – *T. urticae* model system. This analysis included physiological, transcriptomic, and hormonal data collected across plant life cycle, providing a comprehensive perspective on the dynamic of plant responses that enables plant adaptation to the stress. In addition, during the analysis of physiological responses, we developed a fast, accessible and user-friendly automated method for trichome quantification, making this process a more reachable option for the scientific community. The

integration of all the data enabled the generation of a transcriptional and hormonal model explaining the events of the plant-mite interaction during plant growth, development and reproduction. Interestingly, these molecular changes allowed plant adaptation with a penalty in plant fitness, showing the relevance of evaluating the long-term effects of mite infestation on plants.

From the data generated, the importance of the growth hormones in regulating defence-growth responses led us to analyse the performance of mutant and transgenic lines with altered gibberellin levels and DELLA regulation in response mite infestation. The results emphasized the importance of growth hormone regulation in modulating both defence and growth responses.

Overall, the present study provides new insights into the complexity of the defence-growth processes and their implications on plant fitness upon spider mite infestation. This research provides additional tools for the design of new pest management strategies and provides new potential directions for research in the evaluation of plant-pest interactions.

Resumen

Debido a su carácter sésil, las plantas están continuamente expuestas a una gran variedad de estreses presentes en el medio ambiente, lo que produce una alteración en su metabolismo que disminuye su *fitness* y su productividad. Por ello, para sobrevivir dependen de su habilidad para adaptarse a los cambios externos. Entre estos estreses, los estreses bióticos, y concretamente, los artrópodos fitófagos, producen pérdidas de rendimiento sustanciales en los cultivos que suponen importantes costes en agricultura. En particular, el ácaro *Tetranychus urticae*, conocido como araña roja, constituye una de las principales plagas a nivel mundial, que, en el contexto actual de calentamiento global, se estima que acelere su desarrollo y aumente su área de distribución. Por lo tanto, para lograr un control efectivo de esta plaga, el desarrollo de nuevas estrategias de manejo de plagas es necesario.

La mayoría de los estudios que evalúan las respuestas de las plantas frente a *T. urticae* se han centrado en la caracterización de su respuesta de defensa, con el objetivo de aumentar la resistencia de las plantas. Sin embargo, los efectos de la infestación sobre el crecimiento, desarrollo y reproducción de las plantas no han sido previamente estudiados. Esta tesis proporciona una evaluación exhaustiva de las respuestas de defensa y crecimiento frente a *T. urticae*, demostrando el precio que pagan las plantas al defenderse de este ácaro.

Para lograr este objetivo, mediante el análisis previo de otros estudios, identificamos parámetros relevantes que miden la *fitness* de las plantas y determinamos las condiciones experimentales adecuadas para evaluar el compromiso entre defensa y crecimiento. La evaluación de parámetros de crecimiento, fotosíntesis y reproducción proporcionaron datos valiosos para la estimación final de la *fitness* de las plantas. Además, factores como la forma de alimentación de la plaga, la planta huésped y su estadio de desarrollo, así como la duración e intensidad de la infestación, influyeron significativamente en las respuestas finales de la planta.

Con esta información, se estableció el diseño experimental para la evaluación del compromiso entre defensa y crecimiento. Utilizando el sistema *Arabidopsis thaliana* – *T. urticae*, el estudio incluyó análisis fisiológicos, transcriptómicos y hormonales recopilados a lo largo del ciclo de vida de la planta, proporcionando una perspectiva completa sobre la dinámica de las respuestas que les permiten adaptarse al estrés. Adicionalmente, durante el análisis de las respuestas fisiológicas, desarrollamos un método automatizado accesible y fácil de usar para la cuantificación de tricomas de potencial uso para la comunidad científica. La integración de

todos los datos permitió generar un modelo transcripcional y hormonal detallando los eventos de la interacción planta-ácaro durante el desarrollo de la planta. Curiosamente, a pesar de que estos cambios permitieron la adaptación de las plantas al estrés, su *fitness* se vio afectada negativamente, demostrándose la importancia de los efectos a largo plazo de la infestación por ácaro.

A partir de los datos generados, se observó la importancia de las hormonas de crecimiento en la regulación de las respuestas de defensa y crecimiento. Esto llevó a analizar el rendimiento de líneas mutantes y transgénicas con alterados niveles de giberelinas y de su regulación en respuesta a la infestación por ácaro. Los resultados destacaron la relevancia de la regulación de esta hormona en la modulación de las respuestas de defensa y crecimiento.

En conjunto, el presente estudio proporciona nuevas perspectivas sobre los procesos de defensa y crecimiento y sus implicaciones en la *fitness* de las plantas tras la infestación con ácaros. Esta investigación aporta herramientas adicionales para el diseño de estrategias de manejo de plagas y abre la puerta a nuevas investigaciones en el campo de las interacciones planta-plaga.

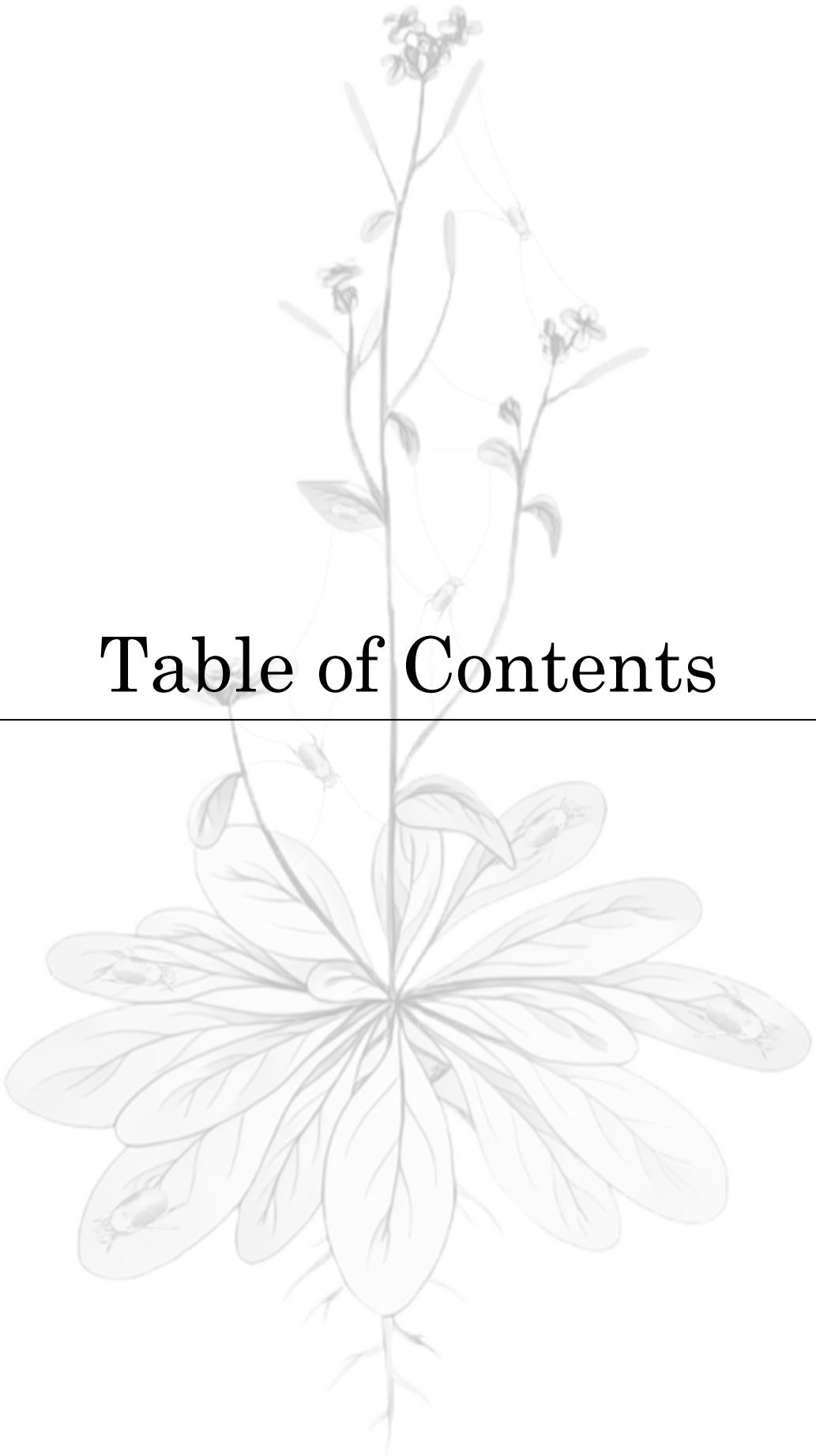
A detailed botanical illustration of a plant, likely a species of Verbena. The plant features a basal rosette of large, ovate leaves with prominent venation. A single, tall, slender stem rises from the center of the rosette, bearing several small, tubular flowers at the top. The illustration is rendered in a light, sketchy style with fine lines and subtle shading.

Table of Contents

Table of Contents

Acknowledgements.....	v
Abstract.....	ix
Resumen	xi
List of Figures.....	xix
List of Tables.....	xxxi
Abbreviations and Acronyms.....	xxxiii
1. INTRODUCTION	1
1.1. The challenge of pest in agriculture	1
1.2. Herbivore stress classification	2
1.2.1. Categorization of plant pest based on feeding mode and plant host	3
1.3. Plant response to herbivore pests	5
1.3.1. General features of the defence response of plants to pests	5
1.3.2. Plant response to spider mite: state of the art.....	8
1.3.2.1. <i>Tetranychus urticae</i> as a threat	8
1.3.2.2. Plant response to <i>T. urticae</i>	11
1.3.2.2.1. Constitutive defences.....	11
1.3.2.2.2. Inducible defences	12
1.3.2.2.2.1. Perception and signal transduction	12
1.3.2.2.3. Phytohormones	14
1.3.2.2.4. Direct and indirect defences.....	17
1.4. Plant defence-growth trade-off	18
1.4.1. The importance of the defence growth trade-off studies	18
1.4.2. Defence-growth theories	20
1.4.3. Resource diversion.....	21
1.4.3.1. Carbohydrate Metabolism	22
1.4.3.2. Nitrogen Metabolism.....	24
1.4.4. Phytohormonal regulation of plant defence-growth trade-off	25
1.4.4.1. Defence module induction modifies plant growth.....	26
1.4.4.2. Growth module interaction with defence module	26
1.4.4.2.1. Auxins.....	26
1.4.4.2.2. Cytokinins.....	29
1.4.4.2.3. Brassinosteroids.....	30
1.4.4.2.4. Gibberellins	32
1.4.4.3. The multitasking hormone ABA.....	35
1.4.4.4. Growth hormone crosstalk.....	38
2. OBJECTIVES.....	43

3. MATERIAL AND METHODS	47
3.1. Plant material and growth conditions.....	47
3.2. Spider mite maintenance.....	48
3.3. Spider mite infestation assays.....	48
3.3.1. Leaf damage quantification.....	48
3.3.2. Fecundity assays	49
3.4. Plant physiological and biochemical characterization	50
3.4.1. Growth parameters evaluation.....	50
3.4.2. Reproductive parameters evaluation	50
3.4.2.1. Reproductive parameters measurements.....	50
3.4.2.2. Germination assays.....	51
3.4.3. Trichome quantification	51
3.4.3.1. Image acquisition, processing and area estimation	52
3.4.3.2. Trichome counting methods	53
3.4.3.2.1. Manual counting	53
3.4.3.2.2. Automated counting.....	53
3.4.3.2.2.1. Automated approaches	53
3.4.3.3. Method reliability	55
3.4.4. Photosynthetic performance and anthocyanin determination	56
3.4.5. Cuticle evaluation	56
3.4.5.1. Cuticle permeability test.....	56
3.4.5.2. Confocal imaging	57
3.5. Hormone analysis.....	57
3.6. Gene expression analysis	58
3.6.1. Real-time qPCR	58
3.6.2. RNA-sequencing.....	59
3.7. <i>In silico</i> analysis.....	60
3.7.1. Meta-analysis	60
3.7.1.1. Compilation of the databases.....	60
3.7.1.2. Variable categorization.....	61
3.7.1.3. Data extraction.....	62
3.7.2. Transcriptomic analysis	65
3.7.2.1. Database collection.....	65
3.8. Statistical analysis.....	66
4. RESULTS.....	69
4.1. The price of the induced defences against pests: a meta-analysis.....	69
4.1.1. Meta-analysis data.....	69
4.1.2. Evaluation of growth, photosynthesis and reproduction parameters exerts negative effects on plant fitness upon arthropod infestation conditions.....	72
4.1.3. Effects on plant growth of the plant-pest interaction variables	72
4.1.4. Effects on plant photosynthesis of the plant-pest interaction variables	75

4.1.5.	Effects on plant reproduction of the plant-pest interaction variables	75
4.1.6.	Risk bias assessment	77
4.2.	An automatic method to quantify trichomes in <i>A. thaliana</i>	78
4.2.1.	Scanner trichome imaging and selection of experimental conditions.....	79
4.2.2.	Comparison of manual and automated approaches	81
4.2.3.	Reliability assessment of the selected automated method in Arabidopsis	83
4.2.4.	Method adaptability to other plant species	88
4.3.	Spider mite infestation triggers coordinated hormonal trade-offs enabling plant survival with a fitness cost	89
4.3.1.	Phenotypic traits reveal plant morphological and physiological changes upon mite infestation	90
4.3.2.	Transcriptomics analysis reveals a defence-growth contrasting interplay in the plant response to mite infestation.....	92
4.3.3.	Phytohormone profiling unveils dynamic interplay among defence and growth hormones in response to <i>T. urticae</i> infestation	94
4.3.4.	Reproductive parameter evaluation reveals a negative effect of mite infestation in plant fitness at short- and long-term level	99
4.4.	Gibberellin homeostasis regulates plant defence-growth trade-off in response to spider mite	102
4.4.1.	Commonalities and specificities in the GA response to <i>T. urticae</i>	102
4.4.2.	Altered plant GA levels and DELLA regulation modifies plant growth and resistance to <i>T. urticae</i>	105
4.4.3.	Transcriptomic evaluation of the GA and defence pathways across the different GA mutants and transgenic lines.....	107
4.4.4.	Plant GA homeostasis regulates plant defence response to spider by specific metabolic routes	114
5.	DISCUSSION.....	119
5.1.	Pest infestation produces an overall impact on plant fitness that variates depending on the different parameters surrounding the plant-pest interactions.....	119
5.2.	The automated trichome counting method is robust and reliable for the evaluation of trichomes with basic equipment.....	122
5.3.	The fine-tune regulation of transcriptomic and hormonal trade-offs allows plant survival with an induced plant developmental delay and a negative effect on seed production and viability	126
5.4.	GA homeostasis regulates plant defences upon spider mite infestation.....	131
5.5.	General discussion	136
6.	CONCLUSIONS.....	145
	References	147
	Annexes	175
	Annex A. Steps and parameters used for the automated approaches.....	175
	Annex B. Macros used for the trichome quantification analysis	179
	Annex C. Supplementary Figures.....	182
	Annex D. Supplementary Tables.....	190

List of Figures

Figure 1.1. Different feeding styles of different phytophagous arthropods.	4
Figure 1.2. Plant inducible defences against phytophagous arthropods require appropriated recognition systems to trigger signal transduction pathways that connect herbivore specific elicitors to the expression of suitable defence genes. CMs: calmodulins, CDPKs: Ca ²⁺ dependent protein kinases, MAPKs: mitogen activated protein kinases, TFs: transcriptional factors. Adapted from Santamaría <i>et al.</i> (2013).	8
Figure 1.3. <i>Tetranychus urticae</i> life cycle and feeding. (A) <i>T. urticae</i> female adult. (B) <i>T. urticae</i> life cycle. From Arnaiz <i>et al.</i> (2019a). (C) Cross section of mite feeding on Arabidopsis leaf. Scale bar: 100µm. From Bensoussan <i>et al.</i> (2016).	10
Figure 1.4. Summary of the studies developed on the evaluation of plant defences responses against spider mites. Adapted from Santamaría <i>et al.</i> (2018a).	11
Figure 1.5. Simplified scheme of the JA biosynthesis (A) and signalling transduction pathways (B). The intermediate OPDA is synthesized in the chloroplasts. JA is synthesized in the peroxisomes and exported to the cytosol, where it is converted to other bioactive derivates (i.e., JA-Ile). α-LA: α-linolenic acid; ACX1: Acyl-CoA-oxidase 1; AOC: Allene oxide cyclase; AOS: Allene oxide synthase; COI1: Coronatine insensitive 1; JA: jasmonic acid; JA-Ile: jasmonic acid-isoleucine conjugate; JAR: jasmonate resistant; JAZ: jasmonate ZIM domain; JMT: JA methyl transferase; LOX: 13-lipoxygenase; MeJA: methyl jasmonate; MED: mediator; NINJA: novel interactor of JAZ; OPDA: 12-oxophytodienoic; OPR3: OPDA reductase 3; TPL: topless. From Li <i>et al.</i> (2019a).	15
Figure 1.6. Simplified scheme of the SA biosynthesis (A) and signalling transduction pathways (B). BA2H: benzoic acid 2-hydroxylase; ICS: isochorismate synthase; IPL: isochorismate pyruvate lyase; NPR: non-expressor of pathogenesis-related genes; PAL: phenylalanine ammonia lyase; SA: salicylic acid; TGA: TGACG-binding factor. From Li <i>et al.</i> (2019a).	16
Figure 1.7. Conceptual diagram of plant fitness determination via the combined effects of resource allocation to competing plant functions (green arrows), resource-based trade-offs (gray lines), and ecological interactions (black arrow and lines). Adapted from Züst & Agrawal (2017).	20
Figure 1.8. Two hypotheses explaining the growth–defence trade-off. (A) Resource limitation (ceiling) drives the trade-off between growth (green) and defence (orange). (B) The trade-off is independent of resource limitation. Left axis indicates % of total available plant resource used for growth or defence. Adapted from He <i>et al.</i> (2022).	22
Figure 1.9. Plant classical hormonal modules participating in the defence-growth trade-off against biotic stress.	25
Figure 1.10. Simplified scheme of the (A) auxin metabolism and (B) signalling in the absence or presence of indole-3-acetic acid (IAA). ASA: anthranilate synthase alpha subunit; ANT: anthranilate; CAM: camalexin; Glu: glucose; IAAld: indole-3-acetaldehyde; IAEt: indole-3-ethanol; IAM: indole-3-acetamide; IAN: indole-3-acetonitrile; IAOx: indole-3-acetaldoxime; IBA: indole-3-butyric acid; IGP: indole-3-glycerol phosphate; IGs: indole glucosinolates; IPyA: indole-3-pyruvic	

acid; L-Trp: L-tryptophan; NITs: nitrilases; oxIAA: 2-oxindole-3-acetic acid; TAA: tryptophan aminotransferase; TAR: tryptophan aminotransferase-related protein; TDCs: tryptophan decarboxylases; TRA: tryptamine; TIR1: transport inhibitor response 1 F-box protein; Aux/IAA: auxin/indole-3-acetic acid proteins; TPL: topless co-repressor protein; ARF: auxin response factor; AuxRE: auxin responsive elements; Ub: ubiquitin. From Ortiz-García *et al.* (2023). 28

Figure 1.11. Simplified scheme of the CK metabolism and signalling. *tZ*: *trans*-zeatin; *tZR*: *tZ* riboside; *tZRMP*: *tZR* monophosphate; *cZ*: *cis*-zeatin; *cZR*: *cZ* riboside; *cZRMP*: *cZR* monophosphate; *iP*: isopentenyladenine; *IPR*: *iP* riboside; *iPRMP*: *iPR* monophosphate; *IPT*: isopentenyltransferase; CYP735A: cytochrome P450 monooxygenase; LOG: lonely guy; CKX: cytokinin oxidases/dehydrogenase; HK: histidine kinase; HPT: histidine phosphotransfer protein; RRB: type b response regulator; RRA: type a response regulator. From Cortleven *et al.* (2019). 30

Figure 1.12. Illustration of networked pathways for brassinosteroid biosynthesis. Yellow, purple and green colours represent the early C-22, the late C-6 and the early C-6 oxidation pathways. CPD: constitutive photomorphogenesis and dwarfism; DET2: de-etiolated2; DWF4: dwarf 4; ROT3: rotundifolia 3. Adapted from Chung & Choe (2013). 31

Figure 1.13. Schematic representation of BR signalling. BRI1: brassinosteroid insensitive 1 receptor; BAK1: brassinosteroid insensitive 1-associated receptor kinase 1; BSK: BR-signaling kinase; BSU1: BRI1-suppressor 1; CDG: constitutive differential growth kinase; BKI1: BRI1 kinase inhibitor 1; DWF: dwarf; PP2A: protein phosphatase 2A; BIN2: brassinosteroid insensitive 2; BZR: brassinazole resistant. From Wang *et al.* (2012). 32

Figure 1.14. Generalized scheme of (A) gibberellin (GA) biosynthesis, deactivation and (B) signalling in higher plants. GGDP: geranylgeranyl diphosphate; CPS: *ent*-copalyl diphosphate synthase; KS: *ent*-kaurene synthase; KO: *ent*-kaurene oxidase; KAO: *ent*-kaurenoic acid oxidase. From Katyayani *et al.* (2020) and Colebrook *et al.* (2010). 34

Figure 1.15. Simplified scheme of the (A) ABA metabolism and (B) signalling. VDE: violaxanthin de-epoxidase; ZEP: zeaxanthin epoxidase; NSY: neoxanthin synthase; NCED: enzyme 9-*cis*-epoxycarotenoid dioxygenase; AAO: abscisic aldehyde oxidase; GT: ABA glucosyltransferase; BG: β -glucosidase; ABA-GE: ABA-glucose Ester. From Wu *et al.* (2023). 36

Figure 1.16. Schematic summary of the hormonal crosstalk established between the classical “growth” hormone module and the “defence” module and ABA. Orange and green represent those hormones mainly classified as “defence” and “growth” hormones. Blue colour indicates the ABA, hormone involved in both defence and growth processes. JA: jasmonic acid; ET: ethylene; ABA: abscisic acid; CK: cytokinin; BR: brassinosteroid; GA: gibberellin; SA: salicylic acid. 38

Figure 1.17. Schematic representation of the (A) general hormonal crosstalk between growth hormones (auxins, BRs, GAs and CKs) and (B) of the specific BAP module (BZR1–ARF6–PIF4 complex) and DELLA regulation. ARF6: auxin response factor 6; PIF: phytochrome interacting factor; BZR1: brassinazole resistant 1; GA: gibberellin; BRs: brassinosteroids; CK: cytokinins. 39

Figure 3.1. Experimental design for the evaluation of the *A. thaliana* - *T. urticae* interphase during the plant life cycle. Three-week-old Col-0 plants were infested or non-infested with 20 mites of *T.*

urticae and collected at different time points of the mite infestation (30 min, 24 h, 4 d, 7 d, 12 d and 15 d). The timeline indicates the different times of infestation and the ontogenetic stage of the plants at the collection time of the samples. Green colour of the timeline indicates times of infestation where the plant is on its vegetative stage, while orange colour indicates reproductive stage. In the case of the samples collected for parameter evaluation, three biological replicates (n=5-12), were used. For RNA-seq analysis, four replicates of four plants for each time and treatment were collected. Finally, for hormonomics analysis, five biological replicates of four plants were used..... 49

Figure 3.2. Scanning process and area estimation. A total of 24 rosettes were scanned in three different rounds of n=8 (A). Three leaves from each rosette were individualized and their area was calculated using Photoshop (B). White scale bars indicate 1 cm. 52

Figure 3.3. Flow chart of the trichome quantification procedure using the Ilastik (pixel classification) + Fiji approach with noisy background. A total of 24 rosettes were scanned in three different rounds of n=8 and a randomized sample of individual leaves (n=44) were imported to Ilastik (A) and used to train the program (B) to differentiate trichomes (yellow) from leaf tissue (blue) and background (red). All the images were processed, renormalized from [0,3] to [0,255] and exported (C). Then, they were imported to Fiji where trichomes are extracted and exported as black and white images (D). The number of trichomes was estimated for each leaf after filtering by a specific noise threshold (each group of pixels with a size above a specific threshold was considered a trichome) (E). White scale bars indicate 1 cm. 54

Figure 4.1. PRISMA flow diagram detailing screening process of articles included in analysis. 70

Figure 4.2. A diagram depicting the concept of growth defence trade-offs, the parameters to evaluate the fitness and the main variables involved. Resource allocation is related to different processes by arrows. Solid arrows refer to natural processes occurring in plants, while arrows with dashed lines refer to events in which resource allocation is altered by herbivore infestation. 72

Figure 4.3. Effect sizes on general parameters related to fitness. Sample sizes are provided in brackets. Symbols specify mean values of Hedge's *g* with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's *t*-test. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number. 73

Figure 4.4. Growth effect sizes classified by subgroups. Fitness was analysed based in different parameters related to growth. Subgroups included feeding guild (A), type of plant host (B), plant ontogenetic stage (C), infestation length (D) and magnitude of infestation (E). Sample sizes are provided in brackets. Symbols specify mean values of Hedge's *g* with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's *t*-test. If more than two groups

were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenthal's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number. 74

Figure 4.5. Photosynthesis effect sizes classified by subgroups. Fitness was analysed based in different parameters related to photosynthesis. Subgroups included feeding guild (**A**), type of plant host (**B**), plant ontogenetic stage (**C**), infestation length (**D**) and magnitude of infestation (**E**). Sample sizes are provided in brackets. Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the non-parametric Mann-Whitney U test. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number. 76

Figure 4.6. Reproduction effect sizes classified by subgroups. Fitness was analysed based in different parameters related to reproduction. Subgroups included feeding guild (**A**), type of plant host (**B**), plant ontogenetic stage (**C**), infestation length (**D**) and magnitude of infestation (**E**). Sample sizes are provided in brackets. Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. No statistical differences are present between subgroups ($p > 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's t -test with Welch's correction for unequal variances. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number. 78

Figure 4.7. Manual and automated trichome quantification on Arabidopsis rosettes and leaves. Visualization of trichome quantification performed on a whole Arabidopsis rosette (**A**, **C**) or a detached leaf (**B**, **D**) with both manual and automated methods. Blue marks indicate the presence of a trichome by manually annotation (**A**, **B**). The automated quantification method (**C**, **D**) produces a grayscale image where the trichomes are identified by a darker grey region. Black and white scale bars indicate 1 cm. 80

Figure 4.8. Flow chart depicting the general steps and tools for both manual and automated approaches tested. Image acquisition initial step using Photoshop is common for all the methods. After image processing, all automated methods start with the use of the machine learning tool Ilastik. Each square represents a specific step performed with a specific software inside each approach. 82

Figure 4.9. Concordance analysis of trichome densities manually estimated against all three automated approaches. Bars plot (**A**) represents absolute density values for each method. No significant differences were found using One-Way ANOVA test. Lin's concordance correlations ($n=24$). (**B**) show the agreement between all different combinations of methods. The solid lines are the lines of concordance which indicate perfect agreement. The dashed lines indicate the best fit to

the values. The coefficient (CCC) and its respective 95% confidence interval (CI) are represented for each case. Pearson correlation coefficients (r_p) describing the relationship between the trichome density estimation by manual and automated methods are also depicted (n=24). The computing time (C) for each method is expressed as the time taken to process one image from a whole-rosette-image to the number of trichomes identified in a single leaf. 84

Figure 4.10. Reliability of trichome densities obtained by our automated quantification method. Absolute density values estimated manually and automatedly are represented as bar plots (A) Data are means $\pm$ SE of nine independent replicates. Statistical analysis for differences between values obtained by manual and automated methods were performed following a Student's *t*-test for each accession ($p > 0.05$). The Pearson correlation coefficient r_p (B) describes the relationship between trichome densities estimated by Hilscher *et al.* (2009) and our automated method (n= 6). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability. The Spearman correlation coefficient r_s (C) describes their relationship (n = 27). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability. The Bland-Altman plot (D) studies differences between both manual and automated methods. The central solid line indicates the mean difference, the two outer dashed lines indicate ± 1.96 standard deviations. Different colours indicate values for different *A. thaliana* ecotypes (n = 27). 86

Figure 4.11. Bland-Altman plots (A, B, C, G, H, I) and correlation coefficients (D, E, F, J, L, L) of estimated trichome densities describing the relationship between trichome densities estimated manually (Fiji) and by the automatic method developed. For the Bland-Altman plots the central solid line indicates the mean difference, the two outer dashed lines indicate ± 1.96 standard deviation. For the correlations, Pearson (r_p) or Spearman (r_s) coefficients were calculated according to the data distribution (n = 27). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability. 88

Figure 4.12. Trichome identification on leaves of different plant species. Original images are compared against manual quantification in Fiji and our automated method with Ilastik. Leaves from *Solanum tuberosum* and *Brassica napus* are good candidates for trichome quantification with our automated method. 89

Figure 4.13. Infestation of *A. thaliana* plants by *T. urticae* leads to a delay in plant aerial development with rosette phenotype changes related to plant growth and defence. Three-week old Col-0 plants were infested with 20 mites of *T. urticae*. (A) Rosette phenotype of the control and infested Arabidopsis Col-0 rosettes along plant development. (B) Aerial phenotypes of the control and infested Arabidopsis Col-0 plant after 7 d, 12 d and 15 d of infestation. Control and infested plants were evaluated for (C) trichome number and density (n=9), (D) rosette area (n=9), (E) petiole length (n=9), (F) number of rosette leaves (n=12), (G) plant height (n=12), (H) number of branches (n=12) at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of mite infestation. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p <$

0.01; and a triple asterisk indicates that $p < 0.001$. Significant differences in trichome number and trichome density are represented by grey and black asterisks, respectively..... 91

Figure 4.14. *T. urticae* infestation reduces plant growth, photosynthetic performance and increases the anthocyanin levels in Arabidopsis. Three-week old plants were collected after 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. (A) Fresh weight (n=5), (B) water content (n=5), (C) instantaneous fluorescence (n=12), (D) quantum yield (n=12) and (E) anthocyanin content (n=9), were evaluated at the different time points of *T. urticae* infestation in *A. thaliana* Col-0 plants. Coloured shadows represent the standard error of the samples. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$ 92

Figure 4.15. RNA-seq transcriptomic analysis evidences the presence of a defense-growth trade-off in which mites are responsible of a delay in plant development. (A) Principal Component Analysis (PCA) of control and mite-infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation in the two first PCA dimensions. Ellipses define confidence areas (95%) for each specific treatment and time. Each circle represents a biological replicate. Samples are identified using a specific colour code for different time and treatment. (B) Bar diagrams showing the number of total, up- or down-regulated genes at each infestation time point. (C) Heatmap showing hierarchical clustering analysis of samples and of the log₂FC values exhibited by the differentially expressed genes (DEGs) found in mite-infested plants compared to control plants through all time points. Clusters of genes are represented in the left dendrogram with a specific colour for each cluster. General biological function of the DEGs, belonging to defence or growth processes, is indicated on the right part of the heatmap. 94

Figure 4.16. Spider mite infestation rapidly triggers the accumulation of the main hormones involved in plant defence response. (A) Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of biotic stress hormones. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. (B) Hormone levels of the different defence hormone-related compounds. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from 5 biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. cisOPDA: cis-12-oxophytodienoic acid; dnOPDA: dinor-OPDA; JA: jasmonic acid; JA-Ile: jasmonic acid-isoleucine; Σ 11/12-OH-JA: 11/12-hydroxyjasmonic acid; SA: salicylic acid..... 96

Figure 4.17. Plant growth response to spider mite infestation is orchestrated by a complex coordination of the different growth hormones. (A) Principal Component Analysis (PCA) biplot of

control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of plant growth hormones. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. **(B)** Hormone levels of the different auxin-related compounds, **(C)** brassinosteroid-related compounds, **(D)** active gibberellins and **(E)** cytokinin-related compounds classified by its biological activity. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. IAN: indole-3-acetonitrile; IAA: indole-3-acetic acid; oxIAA: 2-oxindole-3-acetic acid; TY: typhasterol; 28homoCS: 28-homocastasterone; BL: brassinolide; GA: gibberellic acid; CK-B: active cytokinin free bases; CK-R: cytokinin ribosides; CK-OG: cytokinin O-glucosides. 98

Figure 4.18. The dual role of ABA in both defence and growth responses is revealed in response to spider mite infestation. **(A)** Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h and 4 d (left) during the vegetative stage of the plant, and at 7 d, 12 d and 15 d of infestation, during the reproductive stage of the plant. PCA was performed on the matrix of hormonal levels of ABA-related compounds. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. **(B)** Hormone levels of the different ABA-related compounds at the vegetative stage of the plant (30 min, 24 h and 4 d of infestation) and **(C)** at the reproductive stage of the plant (7 d, 12 d and 15 d of infestation). Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. ABA: abscisic acid; PA: phaseic acid; DPA: dihydrophaseic acid. 100

Figure 4.19. Mite infestation does not alter the final production of flowers and siliques, but it does affect the final plant fitness and germination of the offspring. Three-week-old Col-0 plants were infested with 20 adult mites of *T. urticae*. Parameters were evaluated for control and infested plants: **(A)** number of flowers, **(B)** number of siliques, **(C)** seed parameters (seed number, total seed weight, individual seed weight, number of seeds per silique, and seed size) and **(D)** the germination rate of the offspring. For **(A)** and **(B)**, parameters were measured until the end of its life cycle. In the case of **(C)**, the evaluation of seeds can only be performed at the end of the life cycle, when seeds are collected. Mean of the data is represented by a circle or a square with black border for "Control" or "Mites", respectively. Solid lines within a violin represents Q1, Q2 (median) and Q3, from bottom to top, respectively. For **(D)**, 'Control' and 'Mites' refers to seeds of three weeks after seed collection, while 'Control aged' and 'Mites aged' refers to seeds of eighteen months after seed collection. In all cases, 11 and 16 independent replicates were evaluated for mite-infested and control plants,

respectively. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$ 101

Figure 4.20. Transcriptomic profile across different plant species of all the DEGs involved in the GA biosynthetic and signalling pathways in response to spider mite infestation. (A) Schematic representation of the GA biosynthesis and signalling pathway with (B) heatmaps representing the $\log_2FC$ for DEGs at different time points of *A. thaliana*, *C. sativus*, *H. vulgare*, *S. lycopersicum*, *V. vinifera* and *Z. mays* (FDR adjusted p -value of 0.05). Upregulation or downregulation of target genes is given in red and blue, respectively (see legend). Grey colour indicates genes not differentially expressed for that time point and plant species. Genes that were not differentially expressed in none of the time points within a plant species were not plotted. GL: geranylinalool; GES: geranylinalool synthase; CYP82G1: cytochrome P450 monooxygenase; CPS: copalyl diphosphate synthetase 1; KS: ent-kaurene synthase; KO: ent-kaurene oxidase; KAO: ent-kaurenoic acid hydroxylase; GA20OX: gibberellin-20 oxidase; GA3OX: gibberellin-3 oxidase; GA2OX: gibberellin-2 oxidase; GID1: GA-insensitive dwarf 1; PIF3: phytochrome interacting factor 3. 104

Figure 4.21. Rosette area of control and mutant plants with altered GA levels and regulation in control and mite infested conditions. Three-week-old plants of the different GA mutant and transgenic Col-0 (*35S:GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and *35S:RGL3*) lines were infested with 20 mites of *T. urticae*. (A) Rosette phenotype of the control and infested three-week-old Arabidopsis rosettes. (B) Rosette area of control and infested plants after 4 dpi (n=9). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Foliar areas were compared by means of a GLM, using a gamma distribution and a logarithm link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 106

Figure 4.22. Mite performance on mutant and transgenic plants with different levels of GA and altered *DELLA* expression. (A) Foliar damage of rosettes after 4 dpi in Col-0 (*35S:GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and *35S:RGL3*) genotypes (n=9). (B) Effects on *T. urticae* fecundity measured 36 h after the infestation with synchronized mite females (n=12). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Boxplots represents the Q1, Q2 (median) and Q3, from bottom to top, respectively, and whiskers above and below the box plot indicate 1.5 times the interquartile range. Foliar damaged areas and egg counting data were compared by means of a GLM, using normal distribution and an identity link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 108

Figure 4.23. Gene expression evaluation of different genes involved in the GA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels (2^{-dCt}) of

(A) *GA20OX1*, (B) *GA2OX6*, (C) *RGL3* and (D) *EXPA8* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:*GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and 35S:*RGL3*) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a weibull distribution for *GA20OX1*, *GA2OX6* and *RGL3*, and a gamma distribution with a logarithm function for *EXPA8*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 109

Figure 4.24. Gene expression evaluation of different genes involved in the JA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels (2^{-dCt}) of (A) *AOS*, (B) *MYC2*, (C) *VSP2* and (D) *PDF1.2* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:*GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and 35S:*RGL3*) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a normal distribution with logarithm function for *AOS* and *MYC2*, and a weibull distribution for *VSP2* and *PDF1.2*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 111

Figure 4.25. Gene expression evaluation of different genes involved in the JA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels (2^{-dCt}) of (A) *ICS1*, (B) *NPR1* and (C) *PR1* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:*GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and 35S:*RGL3*) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a normal distribution with logarithm function for *ICS1*, a gamma distribution with logarithm function for *NPR1*, and weibull distribution for *PR1*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 113

Figure 4.26. Anthocyanin determination in control and mutant plant upon infestation conditions. Quantification of anthocyanins was performed after 4 dpi in Col-0 (35S:*GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and 35S:*RGL3*) genotypes (n=6). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Anthocyanin content was compared by means of a GLM, using a normal distribution and a logarithm link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$). 114

Figure 4.27. Leaf cuticle permeability and trichome density in *RGL3* mutant and overexpressing lines in control and after 4 d of mite infestation. (A) Toluidine blue test for cuticle permeability. A droplet was placed on the leaf surface for 2 h. After rinsing the leaf surface with water, the remaining blue stain attached to the cell wall is indicative of a permeable cuticle. (B) Percentage of leaf area stained after toluidine blue treatment. Leaf area covered by the toluidine blue droplet was measured

before quantification and compared to the remaining blue stained area. Data are means ($\pm$ SE) from 12 biological replicates. (C) Trichome densities of the different lines upon control and mite infested conditions. No trichomes were observed in the *35S:RGL3* lines. Data are means ($\pm$ SE) of measurements from eight biological replicates. Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Cuticle permeability was compared by means of a GLM, using an exponential distribution. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. For trichome density, two-way ANOVA followed by Tukey test was performed after logarithmic transformation of the original data. Different letters indicate significant differences ($p < 0.05$)...... 116

Figure 5.1. Hormone transcriptomic and quantification profiles explain plant phenotypes upon spider mite infestation. The induction of plant defenses (1) entails an initial growth arrest (2) that enable plant adaptation to the stress and subsequent growth resume (3). This growth slow down entails a delay of plant development maintained until the end of the plant life cycle with negative consequences on plant fitness. Transcriptional profiling represents the mean value of the gene expression of the differentially expressed genes involved on each hormonal pathway at each time points. Hormone effect of each hormone at each time point was quantified through Hedges' g coefficients using hormone levels between control and mite infestation conditions. 130

Figure 5.2. Evaluation of the defence-growth trade-off established upon spider mite infestation and the influence of growth hormones in the defence-growth responses. At the top, a summary of the variables and parameters affecting the final evaluation of the plant fitness found in the meta-analysis that enabled the experimental design of the defence-growth trade-off analysis. Physiological, transcriptomic and hormonal responses were measured upon mite infestation. Main results upon mite infestation are depicted. The trichome quantification protocol developed allowed the quantification of trichomes in the different plants. As a result of all these phenotypical, genetic and molecular adaptations, plant fitness is negatively affected. Data integration allowed the generation of a model of response to the mite involving defence-growth responses. In addition, from the data generated, the evaluation of growth hormones suggested the modulation of plant defence-growth responses. Functional validation of GA mutant and transgenic lines revealed that low GA levels or increased *RGL3* expression increase resistance and reduce growth by different regulatory mechanisms, while the role of the rest of hormones in response to spider mite remains unexplored. Publications derived from this thesis are depicted in the step of the analysis they are involved in..... 142

Supplementary Figure C.1. RNA-seq analysis and validation show patterns of defense and growth processes. (A) Principal Component Analysis (PCA) of control and mite-infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation in the first 3 PCA dimensions. Ellipses define confidence areas (95%) for each specific treatment and time. Each circle represents a biological replicate. Samples are identified using a specific color code for different time and treatment. (B) Bar plot representing the expression values in $\log_2$ FC from RNA-seq and quantitative real-time polymerase chain reaction assays for thirteen selected genes. (C) Dispersion graphic representing the correlation between the $\log_2$ FC values from RNA-seq and quantitative real-time polymerase chain reaction assays for the selected genes. Spearman rank correlation coefficient (r_s) is showed in combination

with the regression coefficient (R^2). **(D)** UpSet plots showing the number of specific (distinct) and shared (intersect) DEGs among infestation time points. Vegetative (30 min, 24 h and 4 d) and reproductive stages (7 d, 12 d and 15 d) were grouped together as independent groups of interest. **(E)** Enriched biological processes of the clusters with up- (red) or down-regulated (blue) detected DEGs upon mite infestation during the vegetative and reproductive plant stages..... 182

Supplementary Figure C.2. Mite infestation induces IAN and auxin accumulation. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Levels of different auxin-related compounds of the auxin biosynthesis were quantified and differentially expressed genes involved participating in each step of the pathway were plotted. Red colour indicates differential upregulation of genes at a specific time point. Blue colour indicates differential downregulation of genes for a specific time point. For metabolomic analyses, results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. TRP: tryptophan; IAOx: indole-3-acetaldoxime; IGs: indole glucosinolates; IAN: indole-3-acetonitrile; IAM: indole-3-acetamide; IPyA: indole-3-pyruvic acid; IAA: indole-3-acetic acid; oxIAA: 2-oxindole-3-acetic acid; IAA-Asp: indole-3-acetylaspatic acid; oxIAA-Asp: 2-oxindole-3-acetylaspatic acid..... 183

Supplementary Figure C.3. Mite infestation induces changes in the BRs levels. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Levels of different BR-related compounds of the BR biosynthesis were quantified. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. TE: teasterone; TY: typhasterol; 28-norCS: 28-norcastasterone; 28-homoCS: 28-homocastasterone; BL: brassinolide; homoDL: homodolicholide. 184

Supplementary Figure C.4. Levels of different GA compounds in control and mite infestation conditions. GA compounds were classified according to its involvement in the **(A)** 13-non-hydroxylated biosynthetic pathway for GA₄ biosynthesis or in the **(B)** 13-hydroxylated biosynthetic pathway for GA₁ and GA₃ biosynthesis. Red colour is used to highlight the active gibberellins. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. GA: gibberellin. 185

Supplementary Figure C.5. Mite infestation induces changes in the CK levels. Levels of the different CK-related compounds were classified into four groups based on its biological activity: (A) active CK bases (CK-B), (B) CK ribosides (CK-R), (C) CK O-glucosides (CK-OG) and (D) CK N-glucoside metabolites (CK-NG). Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. *tZ*: trans-zeatin; *cZ*: cis-zeatin; *iP*: isopentenyl adenine; *tZR*: trans-zeatin riboside; *cZR*: cis-zeatin riboside; *DHZR*: dihydrozeatin riboside; *iPR*: isopentenyl adenosine; *tZROG*: *trans*-zeatin riboside *O*-glucoside; *cZROG*: *cis*-zeatin riboside *O*-glucoside; *DHZROG*: dihydrozeatin riboside *O*-glucoside; *tZ7G*: *trans*-zeatin-7-glucoside; *cZ7G*: *cis*-zeatin-7-glucoside; *DHZ7G*: dihydrozeatin-7-glucoside; *iP7G*: isopentenyladenin-7-glucoside; *tZ9G*: *trans*-zeatin-9-glucoside; *iP9G*: isopentenyl adenine-9-glucoside..... 186

Supplementary Figure C.6. Evaluation of the ABA-related compounds from both control and mite-infested samples. Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of ABA-related compounds. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. 187

Supplementary Figure C.7. Transition of Col-0 plants to the flowering state is delayed during mite infestation conditions. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Percentage of plants with floral button (FB), flower (F) and siliques (S) was checked each day after mite infestation. Eleven and 18 independent replicates were evaluated for mite-infested and control plants, respectively. 188

Supplementary Figure C.8. Leaf cuticular lipids in in RGL3 mutant and overexpressing lines in control and after 4 d of mite infestation. Adaxial cuticular lipids of Arabidopsis were stained with Auramine O. Stacks of xz planes along the y-axis were collected using a confocal microscope at 40X magnification. The leaves are from mite infestation and control treatments for Col-0, *rgl3*, *Ler* and *35S:RGL3* plants. Images show a representative cell of the leaf epidermis for each genotype. 189

List of Tables

Supplementary Table D.1. Oligonucleotide sequences for RT-qPCR analysis.	190
Supplementary Table D.2. RNA-seq data quality of fastqc files.	191
Supplementary Table D.3. RNA-seq quality mapping.	193
Supplementary Table D.4. Reference list for studies included in the meta-analysis.	195
Supplementary Table D.5. Publication bias risk assessment. Fail-safe numbers obtained using the Rosenberg approach ($\alpha < 0.05$) and Egger's test p -values for each analysis. Fail-safe numbers greater than $5N+10$ indicate that the data are robust. N = number of experiments included in the meta-analysis. P -values higher than 0.05 suggest that no publication bias are present in the data. When trim and fill procedure was applied, fail-safe number and Egger's test p -values shown represent the values obtained after the procedure was used. An asterisk indicates robust data rejecting publication bias.	198
Supplementary Table D.6. Standards errors for both manual and automated approaches for each of the Arabidopsis accessions tested. Statistical analysis was performed on nine biological replicates for each accession.	199
Supplementary Table D.7. List of transcriptomic studies included in the transcriptomic meta-analysis for GA pathway evaluation.	201

Abbreviations and Acronyms

28-homoCS	28-homocastasterone
AAO	Absciscic Aldehyde Oxidase
ABA	Absciscic Acid
ABA-GE	ABA-Glucose Ester
ABI	Absciscic acid-Insensitive
AHK	Arabidopsis Histidine Kinases
AMI	Amidase
ANT	Anthranilate
AOC	Allene Oxide Cyclase
AOS	Allene Oxide Synthase
AREB	ABA-Responsive Element Binding proteins
ARF	Auxin Responsive Factors
ASA	Anthranilate Synthase Alpha subunit
ATP	Adenosine Triphosphate
Aux/IAA	Auxin/Indole-3-Acetic Acid
AuxRE	Auxin Responsive Elements
B&A	Bland-Altman
BAM	Binary Alignment Map
BAK	BRI1-Associated receptor Kinase
BES	BR1-EMS-Suppressor
BG	β -Glucosidase
BHHR	Becker, Hedges, and Hunter-Rosenthal
BIN	Brassinazole-Insensitive
BLAST	Basic Local Alignment Search Tool
BPH	Brown Planthopper
BR	Brassinosteroid
BRI	Brassinosteroid-Insensitive
BTH	Benzothiadiazole
BZR	Brassinazole-Resistant
CAM	Camalexin

Can-0	Arabidopsis Canary island accession
CBL	Calcineurin B-Like protein
CCC	Concordance Correlation Coefficient
cDNA	Complementary DNA
CDPK	Ca-Dependent Protein Kinases
CI	Confidence Interval
CK	Cytokinin
CK-B	Cytokinin active Bases
CK-NG	Cytokinin N-Glucoside metabolites
CK-OG	Cytokinin N-Glucoside metabolites
CK-R	Cytokinin Ribosides
CKX	Cytokinin oxidases/dehydrogenase
cm	Centimetre
CML	Calmodulin-Like protein
COI1	Coronatine-Insensitive 1
Col-0	Columbia-0 accession
CPD	Constitutive Photomorphogenesis and Dwarfism
CPS	Chloroplast-Copalyl Diphosphate Synthase
CRE	Cytokinin Responsive
CYP	Cytochrome P450 Monooxygenase
<i>cZ</i>	<i>cis</i> -Zeatin
<i>cZR</i>	<i>cZ</i> Riboside
<i>cZRMP</i>	<i>cZR</i> Monophosphate
d	Day
DAMPs	Damage-Associated Molecular Patterns
DEG	Differentially Expressed Gene
DET	De-Etiolated
DHZROG	Dihydrozeatin Riboside- <i>O</i> -Glucoside
DNA	Deoxyribonucleic Acid
dnOPDA	dinor-Oxo-Phytodienoic Acid
DPA	Dihydrophaseic Acid
dpi	Days Post-Infestation
DTT	Dichlorodiphenyltrichloroethane

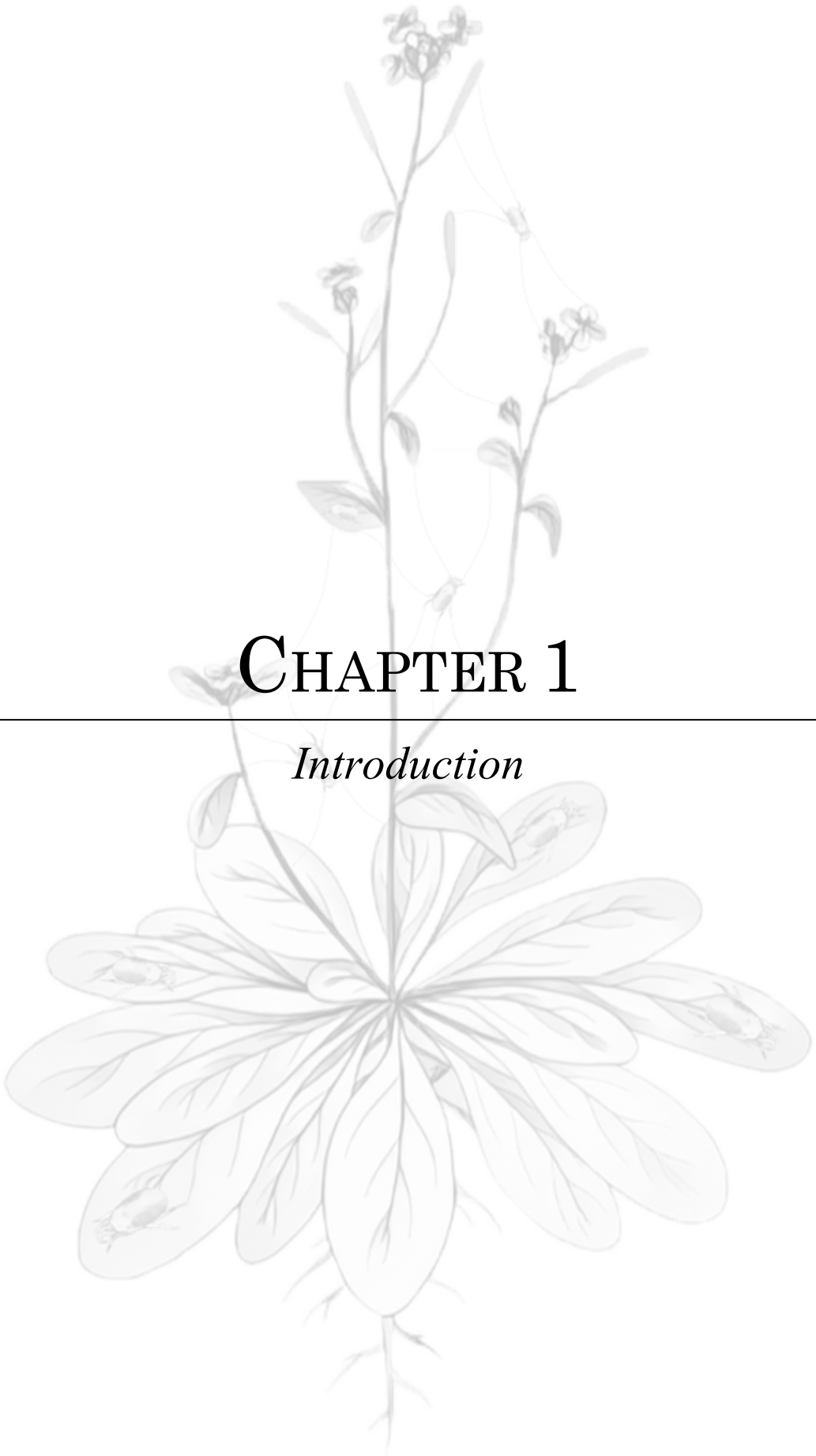
DW	Dry Weight
DWF	Dwarf
eATP	extracellular Adenosine Triphosphate
EGL	Enhancer of Glabra
EIN	Ethylene Insensitive
ET	Ethylene
ETI	Effector-Triggered Immunity
FACs	Fatty acid Amino Conjugates
FDR	False Discovery Rate
FPKM	Fragments Per Kilobase of exon per Million mapped reads
Ft	Continuous Fluorescence
FW	Fresh Weight
g	Gram
g	G-Force
GA	Gibberellin
GA13ox	GA-13-hydroxylases
GA20OX	Gibberellin 20-oxidases
GA2OX	Gibberellin 2-oxidases
GA3OX	Gibberellin 3-oxidases
GAI	Gibberellin Insensitive
GEO	Gene Expression Omnibus
GFP	Green Fluorescent Protein
GGDP	Geranylgeranyl Diphosphate
GL	Glabra
GLM	Generalised Linear Model
Glu	Glucose
GO	Gene Ontology
GOGAT	Glutamine Oxoglutarate Aminotransferase
GOX	Glucose Oxidase
GR-1	Arabidopsis Graz-1 accession
GT	Glucosyltransferase
h	Hour
H ₂ O ₂	Hydrogen peroxide

HAEs	Herbivore-Associated Elicitors
HAMPs	Herbivore-Associated Molecular Patterns
HISAT	Hierarchical Indexing for Spliced Alignment of Transcripts
HK	Histidine Kinases
HPT	Histidine Phosphotranfer protein
IAA	Indole-3-Acetic Acid
IAAld	Indole-3-Acetaldehyde
IAEt	Indole-3-Ethanol
IAM	Indole-3-Acetamide
IAN	Indole-3-Acetonitrile
IAOx	Indole-3-Acetaldoxime
IBA	Indole-3-Butyric Acid
ICS	Isochorismate Synthase
IGP	Indole-3-Glycerol Phosphate
IGs	Indole-Glucosinolates
iP	Isopentenyladenine
iP9G	Isopentenyladenine-9-Glucoside
IPM	Integrated Pest Management
iPR	iP Riboside
iPRMP	iPR Monophosphate
IPTs	Isopentenyl Transferases
IPyA	Indole-3-Pyruvic Acid
JA	Jasmonic Acid
JA-Ile	Jasmonic Isoleucine
JAZ	Jasmonate-Zim domain
KAO	Ent-Kaurenoic Acid Oxidase
KEGG	Kyoto Encyclopedia of Genes and Genomes
KO	Ent-Kaurene Oxidase
KS	Ent-Kaurene Synthase
KTI	Kunitz Trypsin Inhibitors
L	Liter
LC	Liquid Chromatography
Ler	Arabidopsis Landsberg <i>erecta</i> accession

LOG	Lonely Guy
LOX	Lipoxygenases
M	Molar
MAMPs	Microbe-Associated Molecular Patterns
MAPK	Mitogen-Activated Protein Kinase
MeJA	Methyl Jasmonate
MES	2-(N-Morpholino)Ethanesulfonic acid
MeSA	Methyl Salicylate
mg	Milligram
min	Minute
ml	Milliliter
mM	Milimolar
MS	Mass Spectrometry
NCED	9- <i>cis</i> -Epoxycarotenoid Dioxygenase
n _{fs}	Fail-safe number
ng	Nanogram
NITs	Nitrilases
NLR	Nucleotide binding Leucine rich repeat Receptor
Nm	Nanometer
NMF	Negative effect of the Mite infestation on plant Fitness
NO	Nitric Oxide
NPR1	Non-expressor of PR gene 1
NSY	Neoxanthin Synthase
°C	Celsius
OG	Oligogalacturonides
OPDA	12-oxo-Phytodienoic acid
OS	Oral Secretions
oxIAA	2-Oxindole-3-Acetic Acid
PA	Phaseic Acid
PAL	Phenylalanine Ammonia-Lyase
PAMPs	Pathogen-Associated Molecular Patterns
PAP	Production of Anthocyanin Pigment
PCA	Principal Component Analysis

PCD	Programmed Cell Death
PCR	Polymerase Chain Reaction
PIFs	Phytochrome Interacting Factors
PIL	PIF-Like
PIs	Proteinase Inhibitors
pmol	Picomolar
PP2C	Type 2C Protein Phosphatases
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
psc	Partially Suppressing Coil
PTI	Pattern-Triggered Immunity
PYL	Pyrabactin Resistance-Like
Qy	Quantum Yield
RGA	Repressor of GA1-3
RGL	RGA-Like
RGR	Relative Growth Rate
RNA	Ribonucleic Acid
RNA-seq	RNA-Sequencing
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
ROT	Rotundifolia
r_p	Pearson Correlation Coefficient
RRA	Type A Response Regulator
RRB	Type B Response Regulator
r_s	Spearman Correlation Coefficient
RT-qPCR	Real Time Quantitative PCR
Rub-1	Arabidopsis Rubenzhoe-1 accession
RuBisCO	Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
SCF	SKP1, CUL1, F-Box Protein
SG	Seed Germination
Sha	Arabidopsis Shakh dara accession
SN	Seed Number

TAA	Tryptophan Aminotransferase
TAIR	The Arabidopsis Information Resource
TAM	Tryptamine
TAR	Tryptophan Aminotransferase-related Protein
TE	Teasterone
Tet	Tetranin
TF	Transcription Factor
TIR	Toll/Interleukin-1 Receptor Resistance
TIRK	Thioredoxin Interacting Receptor Kinase
TMTT	4,8,12-Trimethyltrideca-1,3,7,11-Tetraene
TPL	Topless
tRNA	Transfer Ribonucleic Acid
Trp	Tryptophan
TT	Transparent Testa
TY	Typhasterol
<i>tZ</i>	<i>trans</i> -Zeatin
<i>tZR</i>	<i>trans</i> -Zeatin Riboside
<i>tZRMP</i>	<i>tZR</i> Monophosphate
Ub	Ubiquitin
UPM	Universidad Politécnica de Madrid
v/v	Volume/Volume
VDE	Violaxanthin De-Epoxidase
VOCs	Volatile Organic Compounds
YUC	Flavin Monooxygenase YUCCA
ZEP	Zeaxanthin Epoxidase
μg	Microgram
μl	Microliter
Σ11-OH-JA/12-OH-JA	11-Hydroxy-/12-Hydroxyjasmonic Acids

A detailed botanical illustration of a plant, likely a species of Verbena. The plant features a basal rosette of large, ovate leaves with prominent venation. A single, tall, slender stem rises from the center, bearing several small, tubular flowers at the top. The illustration is rendered in a light, sketchy style with fine lines and subtle shading.

CHAPTER 1

Introduction

1. INTRODUCTION

1.1. The challenge of pest in agriculture

As sessile organisms, plants are subjected to the continuous interaction with an extensive range of stresses present in the environment. Exposure of plants to these stresses induce a disruption in the plant metabolism that ends up with a reduction in their final plant fitness and productivity (Rejeb *et al.*, 2014). Consequently, plant survival relies on the capacity of plant adaptation to the continuous external events they confront.

Among all stresses, biotic stress arises from the damage caused through pathogen or herbivore attack. Biotic stress poses a significant challenge that induces a strong pressure on plants (Strauss *et al.*, 2002; Brown *et al.*, 2002), leading to a coevolutionary process marked by the pest/pathogen-plant interactions. Each of the 391,000 plant species in the world is exposed to the interaction with other organisms and estimations indicate that several plant diseases and invasive insect supposes a cost of ~\$220 billion to the world economy, with a damage caused by plant pests of ~20-40% of loss in the total crop production (Charaya *et al.*, 2021; Sawicka & Egbuna, 2019).

Since the Green Revolution, the introduction of high-yielding crop varieties, chemical fertilizers, and pesticides, boosted the agricultural productivity (Matson *et al.*, 1997). However, this also led to an induced crop biodiversity loss that enabled the proliferation and prevalence of disease and pests (He *et al.*, 2019). Nevertheless, in the recent decades, the goal for a boosted agricultural productivity has been shaped. Agriculture economics has included issues related to sustainability, environmental impact, and globalization, including concerns about climate change and resource depletion (Adisa *et al.*, 2024). In the case of pests, the globalization of agriculture and the current context of climate change have promoted their expansion and impact on plants, posing a substantial threat for agricultural productivity and sustainability (Bale *et al.*, 2002). Increasing temperatures also lead to a drift in the current pest-host geographical distribution (Coakley *et al.*, 1999) and help both the spread of temperature insect species and their speed of development, thus, increasing the number of insect generations within a year (Bale *et al.*, 2002). This pest dispersal is expected to provoke an alteration of the ecology of the ecosystems through a developmental impact on community dynamics, gene flow and speciation, resulting not only in an important adverse effect on crop yields, but additionally in

the forthcoming establishment of new pests (Mazzi *et al.*, 2012). Therefore, understanding the spatial and temporal abundance of pest is essential for meeting the food demands in a continuously growing population, allowing the establishment of proper management programs that secures a sustainable production.

One of the main methods used to control pests in agriculture is the application of pesticides. The use of pesticides increased since 1945 with the discovery of the dichlorodiphenyltrichloroethane (DDT), and from then on, technologies have continued the generation of a wide range of new chemicals (Tudi *et al.*, 2021). However, their continued use provokes harmful effects to the environment, including biodiversity loss and destruction (Tudi *et al.*, 2021), and imposes high costs on farmers, prompting the search for new alternative pest management methods. In this context, integrated pest management (IPM) arises as a potential alternative that could help to minimize the number and volume of pesticides used. Furthermore, the advances in the field of biotechnology and nanotechnology allow the development of new pesticides with less adverse effects and plant resistant genotypes that reduce the pesticide use and contributes to increased crop yields (Khan *et al.*, 2023).

For this reason, to continue developing optimal pest management strategies, it is required a thorough knowledge of the plant-pest interactions. During coevolution with pests, plants have evolved specific mechanisms of resistance which allow them to detect the stress and respond in a precise manner, eliciting a complex cellular and molecular response system to anticipate damage and survive (Herms & Mattson, 1992). These responses are complex to understand because different types of organisms, such as chewing, piercing/sucking herbivores and bacteria or other pathogens, trigger different types of defensive responses (Bruce & Pickett, 2007). Therefore, understanding plant defence responses to specific stresses is crucial for reducing crop failures and ensuring survival while maintaining its growth and reproductive capabilities.

1.2. Herbivore stress classification

Phytophagous arthropods are a major group of plant attackers belonging to the Arthropoda phylum that are known for their vast diversity, varied feeding modes, and their high adaptation capability (Erb & Reymond, 2019). The main characteristic of the arthropods relies on the presence of an exoskeleton made of chitin and a segmented body with bilateral symmetry bearing jointed appendages and legs. The phylum is additionally categorized into four different subphyla, including Crustacea (crustaceans), Myriapoda (millipedes and centipedes),

Chelicerata (arachnids) and Hexapoda (insects and springtails). Interestingly, insects are the most predominant group of arthropods, being nearly half of them phytophagous insects. For this reason, phytophagous arthropods, with more than 500,000 species, include a wide variety of insect orders, being only a few families in the Acari subclass of the Chelicerata subphyla able to feed on plants in addition to insects (Resh & Cardé, 2009). Particularly, the main phytophagous groups in this Acari subclass belong to the superfamilies Tetranychidea and Eriophyoidea (Vacante, 2015).

1.2.1. Categorization of plant pest based on feeding mode and plant host

Phytophagous arthropods exhibit a wide diversity of feeding modes that have evolved to exploit various plant tissues and resources. The primary feeding modes include chewing, siphoning, piercing-sucking, mining and gall-inducing, or a combination of them (**Figure 1.1**). Chewing arthropods, such as caterpillars, grasshoppers and beetles, consume important amounts of plant leaves, stems, or roots by biting and tearing the tissues that are mixed with their saliva before swallowing them (Weihmann & Wipfler, 2019). In contrast to their chewing larvae, adult lepidopterans, such as butterflies or moths, are siphoning insects carrying a curled long tube that use to feed without causing insignificant damage (Kant *et al.*, 2015). Piercing-sucking arthropods, including aphids, thrips and spider mites, use specialized mouthparts (stylets) to puncture plant cells and suck cell content fluids, often transmitting plant pathogens in the process (Nault, 1997; Bensoussan *et al.*, 2016). Within this group, depending on their specific target tissue, it can be found vascular-feeding insects such as aphids, whiteflies or mealybugs, and mesophyll- or epidermal-feeders such as thrips and spider mites (Kindt *et al.*, 2003; Bensoussan *et al.*, 2016). Leaf miners, such as certain fly and moth larvae, create tunnels within the leaves as they consume the mesophyll tissue, leaving characteristic trails (Kirichenko *et al.*, 2019). Finally, gall-inducing arthropods such as some wasps and mites, manipulate plant growth to form galls, providing themselves both nutrition and protection for their development (Raman *et al.*, 2005).

Nevertheless, in addition to their feeding mode, phytophagous arthropods can also be classified depending on their plant host range in monophagous, oligophagous and polyphagous species. Monophagous herbivores specialize in feeding on a single plant species or genus, which often results in highly specialized adaptations to the specific host plant they attack, including chemical defences and nutritional profile. This is the case of the yellow stem borer *Scirpophaga incertulas* (Walker) that feeds on rice (*Oryza sativa*) plants (Kattupalli *et al.*, 2021).

Oligophagous species, instead, feed on several related plant families and have a broader diet. Due to this behaviour, these herbivores must adapt to a broader variety of plant defences and nutritional qualities, but still maintain some level of specialization. For example, the Colorado potato beetle *Leptinotarsa decemlineata* or the mite *Tetranychus evansi* restricts its feeding to several species of the Solanaceae family (Hare, 1990; Ximénez-Embún *et al.*, 2017). Finally, polyphagous insects have a wide-ranging diet, feeding on many different plant species across multiple families. These generalist feeders, such as the gypsy moth caterpillar *Lymantria dispar* or the two-spotted spider mite *Tetranychus urticae*, can exploit diverse plant resources, making them highly adaptable but less specialized on overcoming specific plant defences (Migeon & Dorkeld, 2006-2014; Boukouvala *et al.*, 2022).

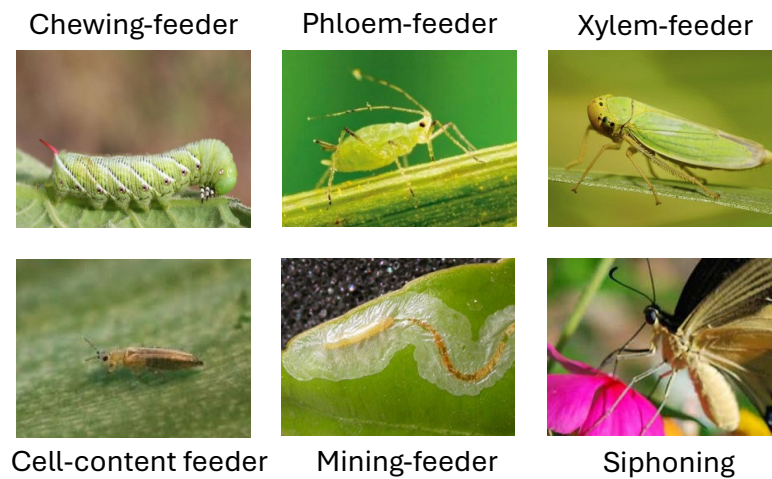


Figure 1.1. Different feeding styles of different phytophagous arthropods.

Based on this previous classification, an important factor that facilitates nutrient uptake and modify plant defences are the oral secretions (OS) and regurgitants secreted by the arthropods. These fluids, generated in the foregut, midgut and salivary glands (Peiffer & Fenton, 2009; Eichenseer *et al.*, 1999), are complex mixtures containing a variety of compounds such as enzymes, proteins, amino acids, fatty acids, and plant derived-secondary metabolites (Stahl *et al.*, 2018). Hydrolytic and proteolytic enzymes differ among herbivore species according to their specific gut pH (Holtorf *et al.*, 2019). Proteins, carbohydrates and lipids are hydrolysed by the action of endo-proteases (serine- and cysteine-proteases), exo-proteases (amino- and carboxypeptidases), peptinases, cellulases and α -amylases, lipases and phospholipases (Tunaz & Stanley, 2004; Terra & Ferreira, 2012; Sharma *et al.*, 2014; Mohamed *et al.*, 2019). These enzymes in combination with other components such as the glucose oxidase (GOX), that catalyzes the production of D-gluconic acid and H_2O_2 (Eichenseer *et al.*, 1999) or fatty acid

amino conjugates (FACs) (Yoshinaga *et al.*, 2010) can act as elicitors or suppressors of the plant defence (Musser *et al.*, 2002; Alborn & Schmetz, 2008).

In conclusion, the array of strategies employed by the plant to overcome the stress and the mechanisms displayed by arthropods varies according to the specific nature of the interaction between arthropods and hosts. For this reason, understanding the different feeding modes employed by each type of arthropod is crucial for the proper management pest populations and the reduction of agricultural losses.

1.3. Plant response to herbivore pests

1.3.1. General features of the defence response of plants to pests

Plant defence responses to arthropod herbivores involves a species-specific interaction between the arthropod and the host plant attacked. In this sense, the establishment of proper plant defences allows the plant to survive by the induction of two different defence strategies: tolerance or resistance. While resistance refers to the capacity of the host to reduce or neutralize the herbivore performance and fitness, tolerance refers to the response of the plants induced after herbivore consumption to buffer the negative fitness effect of the damage. Particularly, plant tolerance response is usually attributed to the growth, vigour and capacity of the plant to produce an acceptable yield (Nunez-Farfán *et al.*, 2007; Pagán & García Arenal, 2018). Furthermore, these strategies, having evolved during millions of years, are widely dynamic and involve an array of different defensive traits generally classified in constitutive and induced defences.

While constitutive defences are always present independently of the herbivore presence, inducible defences are activated only in the presence of the attackers. Constitutive defences offer an immediate response to threat and danger signals, but they are limited in the ability to amplify the response. In this sense, inducible defences have evolved to reduce the cost of the constitutive defences, allowing saving energy and avoiding self-intoxication (Heil & Baldwin, 2002; Zangerl, 2003; Cipollini & Heil, 2010).

Constitutive defences encompass physical and chemical barriers that can act in a synergistic manner. Variations in the amount and presence of specific constitutive defences are related to the genetic plant host differences and environmental factors, indirectly determining a higher resistance or susceptibility to a specific herbivore (Lombardero *et al.*, 2000; Moreira *et al.*,

2014). Physical barriers include surface waxes, cuticular and cell wall modifications, spines, trichomes and thorns, or sclerophylly, a process involving the toughening of leaves, among others. These morphological features obstruct herbivore accessibility, hindering the establishment of arthropod pests on plants and reducing its feeding and oviposition. Chemical defences also act as barriers decreasing phytophagous detectability through the alteration of their growth, development and digestion, extremely leading to arthropod mortality (Santamaría *et al.*, 2013). These defences involve the production of primary and secondary metabolites including peptides, proteins, protease inhibitors (PIs), and various other compounds possessing properties that deter, inhibit nutrition, or exhibit toxicity against potential threats. In the case of secondary metabolites, these compounds mainly arise from the phenylpropanoid, the isoprenoid and the alkaloid metabolic pathways and are not essential for plant growth and development. Phytoanticipins and phytoalexins, terpenes and terpenoids, flavonoids, alkaloids or other phenolic compounds such as coumarins, lignins or tannins, are secondary compounds that affect to arthropod feeding behaviour or performance (Rehman *et al.*, 2012; Boncan *et al.*, 2020; Gupta & Roy, 2021). Additionally, the aromatic and aliphatic glucosinolates, or the acyl sugars, are secondary compounds commonly found in the Brassicaceae or Solanaceae families, respectively, being the latter synthesized and released from glandular trichomes, providing the plants tissues a sticky texture to impede arthropod establishment (Moghe *et al.*, 2023).

On the other hand, inducible defences are determined by the plant host's ability of recognition and detection of the specific stress (Wu & Baldwin, 2009). Upon landing on the plant surface, herbivore activity such as feeding, walking, oviposition, faeces, synthesis of silk or cell disruption can leave arthropod compounds that can be recognized as herbivore-associated elicitors (HAEs) triggering the pattern-triggered immunity (PTI) response (Reymond, 2013; Santamaría *et al.*, 2015; 2021; Ojeda-Martínez *et al.*, 2021).

PTI is initiated by the activation of specific pattern recognition receptors (PRRs) through the detection of HAEs (Erb & Reymond, 2019) such as herbivore-associated molecular patterns (HAMPs) present in the saliva, regurgitants, oral or oviposition secretions, cuticle, faeces or pheromones; pathogen-associated molecular patterns (PAMPs), such as the chitin found in exoskeletons (Zhu-Salzman *et al.*, 2008); microbe-associated molecular patterns (MAMPs) derived from endosymbiont of herbivores, such as *Wolbachia* spp. (Bi & Wang, 2020); or damage-associated molecular patterns (DAMPs) from herbivore-induced injury including peptides (systemins or plant elicitor peptides), nucleotides (eATP), or cell wall fragments such as oligogalacturonides (OGs) (Acevedo *et al.*, 2015; De Lorenzo & Cervone, 2022). Once

recognized, PRRs suffer a conformational change that triggers a signal transduction pathway that activates defence responses. This signalling cascade involves a sequence of molecular events including the depolarization of the plasma transmembrane potential (V_m) associated with Ca^{2+} influx from the apoplast and some organelles (Park & Shin, 2022), the production of reactive oxygen and nitrogen species (ROS and RNS), the increase of the mitogen-activated protein kinase (MAPK) activity, the synthesis of different phytohormones, and finally, the transcriptional activation of specific defence genes that lead to the production of compounds functioning as toxins, repellents and/or antifeedants such as glucosinolates, phenylpropanoids or sesquiterpenes (**Figure 1.2**).

Concretely, the phytohormone alteration is an essential module that determines the final defence response of the plant. Among all the phytohormones involved, jasmonates (JA) and salicylic acid (SA) are the main regulators of the induced defence response. These hormones in combination with ethylene (ET) comprises the hormonal core of the plant defence response (Glazebrook *et al.*, 2005). Activation of SA signalling can inhibit JA-responsive genes and *vice versa*, allowing plants to prioritize their defence mechanisms depending on the type of attacker (Li *et al.*, 2019a). In general, while chewing insects are known to behave as necrotrophic insects, triggering mainly plant JA- and ET-mediated responses, phloem-sucking insects mainly induce the SA pathway, showing similarities to the biotrophic induced responses (de Vos *et al.*, 2005). In addition to these hormonal core, abscisic acid (ABA) has been also reported as signalling molecules in plant defence against herbivores (Erb *et al.*, 2012; Erb & Reymond, 2019). These results suggest that plant resistance or susceptibility to herbivores is modulated by the action of different hormones that depends on the nature of interaction, in which hormonal crosstalks determine the establishment of different defence responses.

Nevertheless, during plant-herbivore coexistence, certain herbivores have developed the capacity to modulate or suppress PTI through the secretion of molecules in their faeces, OS or regurgitants, termed as effectors (Hogenhout & Bos, 2011; Ray *et al.*, 2020). For this reason, in addition to the PTI response, plant inducible defences are also established through the induction of the effector-triggered immunity (ETI). In contrast to PTI, ETI initiates after the recognition of effectors by nucleotide binding leucine rich repeat receptors (NLRs) (Alhoraibi *et al.*, 2019). However, the signalling cascade seems to be interconnected with the PTI responses by inducing similar downstream cellular responses (Tao *et al.*, 2003; Ngou *et al.*, 2022). In fact, recent research suggests that PRRs are also required for the proper operation of ETI (Ngou *et al.*, 2021) and that PTI inhibit ETI signalling sectors to reduce unnecessary fitness

costs (Hatsugai *et al.*, 2017). Further research will determine the mechanisms of interaction between both immune responses, but PTI and ETI seem to work together to establish proper defence responses to external attacks.

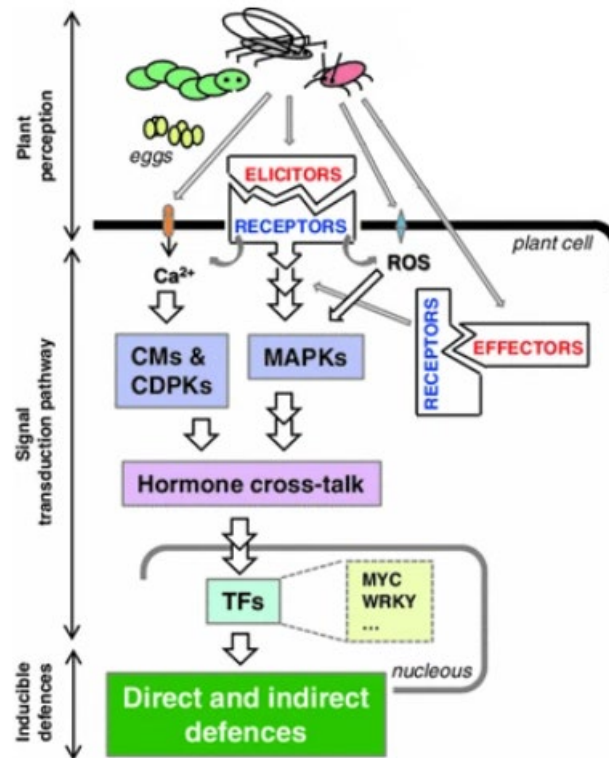


Figure 1.2. Plant inducible defences against phytophagous arthropods require appropriated recognition systems to trigger signal transduction pathways that connect herbivore specific elicitors to the expression of suitable defence genes. CMs: calmodulins, CDPKs: Ca^{2+} dependent protein kinases, MAPKs: mitogen activated protein kinases, TFs: transcriptional factors. Adapted from Santamaría *et al.* (2013).

1.3.2. Plant response to spider mite: state of the art

1.3.2.1. Tetranychus urticae as a threat

T. urticae Koch is a small arthropod with 0.5 mm long belonging to the Arachnids class and Tetranychidae subfamily. This species is commonly known as the “two-spotted spider mite” due to the presence of two dark patches on its body. These patches can vary in colour depending on diet or physiological stage and are visible through its semitransparent cuticle, revealing the internal gut content (Bensoussan *et al.*, 2018) (**Figura 1.3A**). The life cycle of this arthropod is composed of five different stages including the egg, the larvae, two nymphal stages known as protonymph and deutonymph, and the adult stage (**Figura 1.3B**) (Cagle, 1949; Dosse, 1952). During the larvae stage mites present three pair of legs, developing a fourth pair at the

protonymph stage (Naher *et al.*, 2008). The influence of the temperature and the different plant host on the development and fecundity of the mite are key factors altering the optimal mite fitness. Under standard temperate climate conditions, the duration of the life cycle usually takes around 10 days, when female mites can produce, at 25 °C, over 100 eggs of varying sizes depending on the sex of the embryo (Shih *et al.*, 1976; Riahi *et al.*, 2013). However, increasing temperatures and optimal host plants accelerates its development and modify mite fecundity (Praslička *et al.*, 2004). Remarkably, *T. urticae* reproduces through a specific way of parthenogenesis called arrhenotoky, characterized by the peculiarity that unfertilized eggs develop into male mites, while fertilized eggs generate diploid females (Macke *et al.*, 2010). Additionally, these mites can produce silk that use to construct complicated three-dimensional webs on plant leaves that provides them protection against environmental conditions and other predators. These silks enhance female oviposition and reproductive success, also assisting the communication and the spatial distribution of the mites to neighbour plants (Saito, 1997; Bell *et al.*, 2005; Oku *et al.*, 2009). In conditions the of overcrowding and food depletion, mites create silk balls containing aggregations of hundreds of mites, that serves as aerial dispersal elements carried away by the wind or a passing animal (Clotuche *et al.*, 2011).

The two-spotted spider mites are pierce-sucking arthropods possessing an elongated and retractable cheliceral stylet that use to feed from plants. They use the stylet to penetrate between epidermal pavement cells or through stomatal openings without harming the epidermis and to inject saliva to predigest the mesophyll cells and extract its content (Bensoussan *et al.*, 2016) (**Figure 1.3C**). The OS introduced in the plants contain a repertoire of molecules that can function as elicitors or suppressors of the plant defence response (Jonckheere *et al.*, 2016; Villarroel *et al.*, 2016). Finally, mites suck the content of one cell each time and they perform a proteolytic digestion using cysteine- and aspartyl-proteases (Santamaría *et al.*, 2015). As a result of the feeding process, mites cause cell death and lead to a loss of chlorophyll in the plants, resulting in the formation of a characteristic chlorotic spot lesion on the leaves.

T. urticae, is one of the most successful polyphagous species, with an extremely wide range host range including more than 1,500 plants species of greenhouse crops, ornamental plants and annual and perennial field cultivars, including about 150 plants of relevant agronomic importance (Migeon & Dorkeld, 2024). With a cosmopolitan distribution, this species is considered a serious threat for agriculture due to the important yield losses that originates in greenhouse and field crops (Vacante, 2015). The main control systems used against this pest are based on the use chemical synthetic acaricides and biological agents composed by bacteria,

fungi, insects or predatory mites (Chandler *et al.*, 2005; Sato *et al.*, 2005; Pakyari *et al.*, 2011; Van Leeuwen *et al.*, 2015; Emam, 2021; Mohammadi *et al.*, 2021; Van de Velde *et al.*, 2021). However, due to its short generation time and high fecundity rate, mites show a great capacity of adaptation to the different pesticides, showing a great record of pesticide resistance that complicates their control (Osakabe *et al.*, 2009; Van Leeuwen *et al.*, 2010; 2016; Jakubowska *et al.*, 2022).

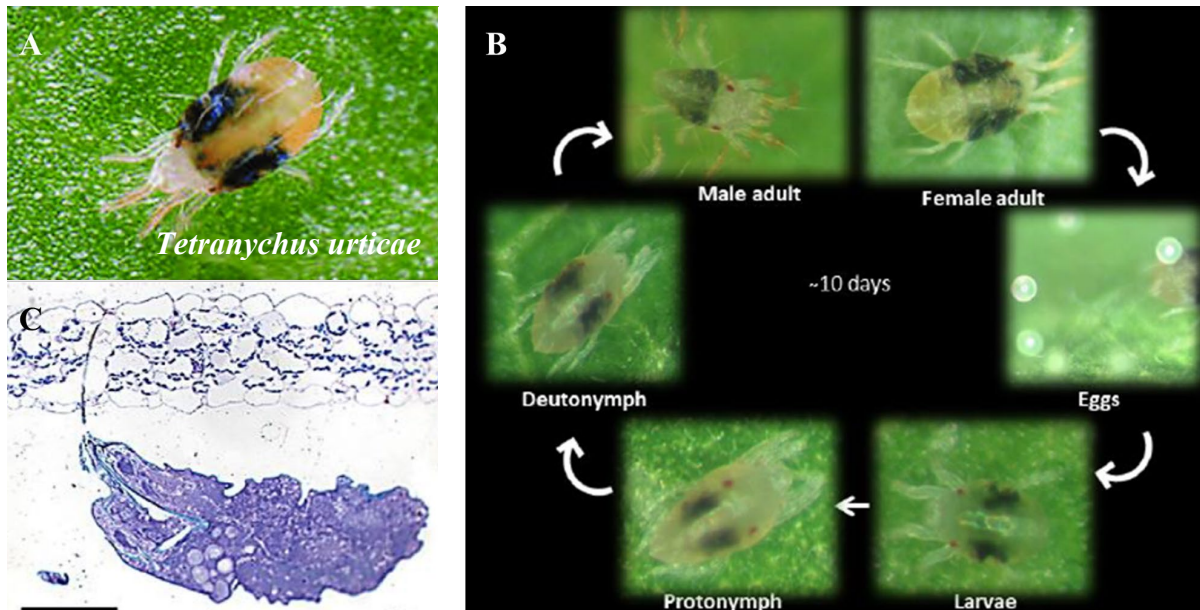


Figure 1.3. *Tetranychus urticae* life cycle and feeding. (A) *T. urticae* female adult. (B) *T. urticae* life cycle. From Arnaiz *et al.* (2019a). (C) Cross section of mite feeding on Arabidopsis leaf. Scale bar: 100µm. From Bensoussan *et al.* (2016).

Nowadays, in the current context of climate change, increasing temperatures are expected to shorten its life cycle, leading to a higher number of generations per year and to an expansion of its distribution range, aggravating the negative effect of this pests on crops (Delucia *et al.*, 2012; Ximenez-Embum *et al.*, 2017).

Finally, due to its short life cycle, the presence of a short genome completely sequenced (Grbić *et al.*, 2011), its ability to feed on Arabidopsis in combination with an easy laboratory maintenance, and the availability of a wide range of genomic and handling tools (Cazaux *et al.*, 2014; Santamaría *et al.*, 2017; Suzuki *et al.*, 2017; Ojeda-Martínez *et al.*, 2020), this species is considered as a valuable model species for studying molecular mechanisms underlying plant-pest interactions.

1.3.2.2. Plant response to *T. urticae*

Due to the importance of the impact of this pest in agriculture, the study of the plant defence response to *T. urticae* has become an interesting research focus with the aim of generating knowledge to be used in the design of pest management strategies (**Figure 1.4**). During the last decades, the analysis of the plant responses to this mite has revealed the establishment of a broad array of strategies, including both constitutive and inducible defences, that confer resistance or susceptibility to this arthropod. Interestingly, due to the mite ability to feed on *Arabidopsis thaliana* and the wide available toolkits for this plant species, many studies have used the system conformed by these two species as a model system for functional studies of plant-mite interaction.

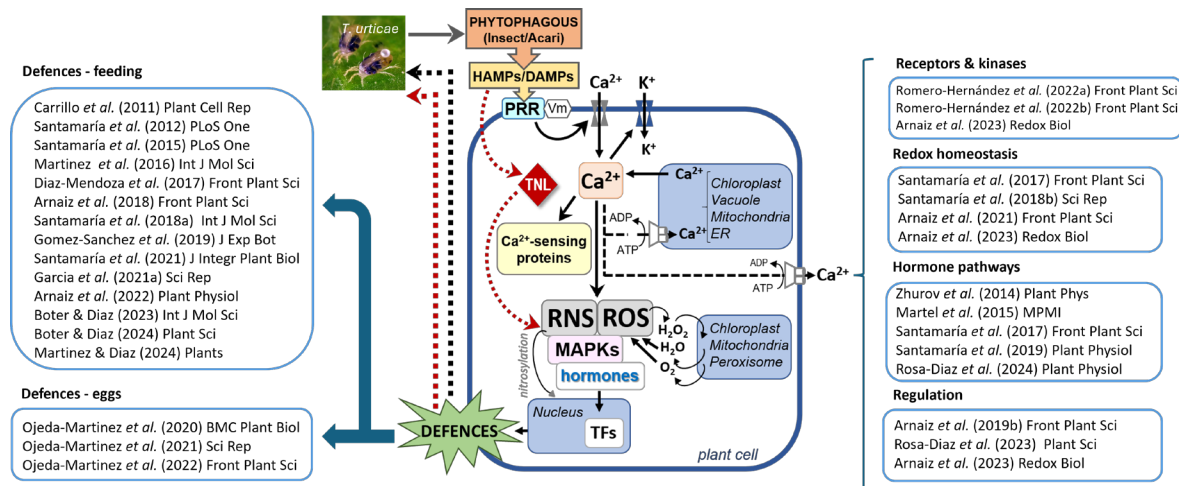


Figure 1.4. Summary of the studies developed on the evaluation of plant defences responses against spider mites. Adapted from Santamaría *et al.* (2018a).

1.3.2.2.1. Constitutive defences

The plant defensive response to the mite *T. urticae* involves a blend of constitutive defences already evolved to avoid pest damage. Particularly, trichomes, as hair-like appendages developed on the aerial epidermis of most of the plant species, has been proved to be an important barrier for this mite, reducing mite establishment through the restriction of their movement and fecundity (Rakha *et al.*, 2017; Resende *et al.*, 2020). Trichomes can be classified into glandular or non-glandular trichomes depending on the presence of glands that synthesize and secrete repellents and secondary metabolites for plant defence (Huchelmann *et al.*, 2017; Lange and Srividya, 2019). Remarkably, independently of the trichome type, higher trichome densities have shown a reduced amount of damage and eggs oviposited in strawberry, raspberry or tomato (Keskin & Kumral, 2015; Karley *et al.*, 2016; Resende *et al.*, 2020). However,

additional constitutive barriers are also effective against these mites. Interestingly, higher leaf thickness of several melon cultivars decreased mite population development (Xu *et al.*, 2019), while thicker leaf cuticles in the barley *HvPap-1* knockdown lines protected from *T. urticae* feeding. The slippery film or crystals formed by the epicuticular waxes hampers the introduction of the stylet on their leaves, interfering with the mite feeding and establishment (Gomez-Sanchez *et al.*, 2019).

1.3.2.2.2. Inducible defences

1.3.2.2.2.1 Perception and signal transduction

Besides the constitutive barriers, inducible defences are activated upon the detection of the mite elicitors or effectors. Iidia *et al.* (2019) characterized two proteins, tetranin 1 (Tet1) and tetranin 2 (Tet2) present in the salivary glands of *T. urticae* that elicit PTI responses. Moreover, Cui *et al.* (2023) recently identified a widely conserved disulfide isomerase from *T. evansi* (TePDI) as a PTI elicitor in Solanaceae plants. Apart from these elicitors, compounds excreted from faeces, eggs, silk and acari molts could act as alternative source of HAMPs alerting the plant about the spider mite attack (Santamaría *et al.*, 2019; 2021; Ojeda-Martínez *et al.*, 2021). On the other hand, effector proteins have also been found. Particularly, Villarroel *et al.* (2016) identified a collection of salivary-secreted peptides in which transient expression of two of these peptides Tu28 and Tu84 in *Nicotiana benthamiana* plants suppressed mite-induced SA defences promoting the reproductive performance of the mite.

Nevertheless, a low number of PRR have been identified. Some examples of specific receptors are the LYK4 receptor, a LysM receptor like-kinase involved in response to chitin, and the protein PP2-A5, composed by a Toll/Interleukin-1 receptor (TIR) and a phloem protein 2 with lectin activity (PP2). While the first one was induced by mite feeding in tomato, grapevine and *A. thaliana*, the second one was involved in defence against *T. urticae*, increasing the resistance of the plant (Santamaría *et al.*, 2019). Similarly, a recent study demonstrated the induction of two divergent genes regulated by a bidirectional promoter in response to *T. urticae* feeding (Arnaiz *et al.*, 2019b), where one of the genes encodes a receptor-like kinase, likely involved in perceiving phytophagous mites and initiating the defence transduction cascade (Santamaría *et al.*, 2020; Arnaiz *et al.*, 2023). In the case of NLRs, little is known about the R genes involved on the defence responses. However, it has been recently suggested the role of two putative *NLR* genes known as *MRT1* and *MRT2* to be potentially involved in ETI to *T. urticae* (Rosa-Díaz, 2024).

Once receptors are activated, the defence signalling cascade is initiated. Occurring within seconds or minutes, V_m alterations and Ca^{2+} influxes are the earliest elicited responses, producing electric signals along the entire plant. These signals involve the action of Ca^{2+} sensors regulating downstream signals, in which there are found calmodulin, calmodulin-like and calcineurin B-like proteins (CMLs and CBLs), Ca^{2+} -dependent protein kinases (CDPKs) and Ca^{2+} -ATPases (Zebelo & Maffei, 2015; Vincent *et al.*, 2017). Upon *T. urticae* infestations, alterations of V_m and cytosolic Ca^{2+} levels are followed by the production of reactive species (Iida *et al.*, 2019). It has been demonstrated that ROS, such as H_2O_2 , are primarily produced by NADPH oxidases [respiratory burst oxidase homologs (RBOHs)] located at the plasma membrane. High ROS levels cause oxidative stress and induce programmed cell death (PCD); however, low-moderate accumulations activate signalling responses to induce plant defence cascades (Mittler, 2017). In fact, the reduction of ROS levels in plants by silencing three genes responsible of ROS degradation, result in a higher susceptibility to *T. urticae* infestations (Santamaría *et al.*, 2018b). Furthermore, the overexpression of the mite attack triggered immunity (*MATT*) gene, involved in the Arabidopsis antioxidant status, produces a moderate accumulation of H_2O_2 that conferred a higher resistance to this mite, showing lower oviposition rates and damage on plant leaves (Santamaría *et al.*, 2017). Additionally, Arnaiz *et al.* (2023) recently demonstrated that altered expression of the thioredoxin *TRXh5*, a redox regulator involved in the denitrosylation of the TIRK transmembrane receptor, lead to unbalanced amounts of H_2O_2 and NO that correlate with changes in the susceptibility to mite attack and behaviour. In combination with ROS, RNS has also been involved in response to this mite. Concretely, Arnaiz *et al.* (2021) showed the rapid transcriptional alteration of nineteen nitric oxide (NO)-associated genes encoding nitrate transporters, nitrate reductases, and nitrilases after mite infestation, and suggested that RNS signalling is first activated at the cell membrane and then transmitted through the cytosol to the rest of the organelles to finally induce the expression of defensive genes.

After these initial steps, MAPKs also play a relevant role in the early signal transduction pathway upon plant-mite interaction. In Arabidopsis, highly activated MPK4 and MPK6 in mutant plants lead to an enhanced resistance to *T. urticae* (Schweighofer *et al.*, 2007). Additionally, Romero-Hernández & Martínez (2022a) demonstrated that the induction of *MAPKKK17* and *MAPKKK21* is required to balance the response of the plant to spider mites, acting as positive and negative regulators of the plant defence, respectively.

1.3.2.2.3. Phytohormones

As a result of the signalling cascade, there is an activation of defence responses by the induction and regulation of specific defence hormone pathways. Interestingly, in response to spider mite infestations, the establishment of plant responses requires the induction of both JA and SA pathways, suggesting that the defence core composed by SA and JA pathways may be acting in a synergistic rather than in an antagonistic manner in the plant-mite context (Agut *et al.*, 2014; Martel *et al.*, 2015; Santamaría *et al.*, 2017; 2019; Zhang *et al.*, 2020).

Jasmonates are fatty acid derivatives mainly synthesized through the octadecanoid acid pathway. The precursor α -linolenic acid (18:3) is transformed into 12-oxo-phytodienoic acid (OPDA) by several reactions involving 13-lipoxygenases (13-LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC). OPDA is then transported into the peroxisome to form JA, and later, into the cytosol, to synthesize jasmonoyl-L-isoleucine (JA-Ile) through the action of the jasmonoyl amino acid conjugate synthase JAR1 (**Figure 1.5A**). Concretely, JA-Ile has been identified as the most bioactive form of jasmonates, and its binding to the F-box protein coronatine-insensitive 1 (COI1), leads to the ubiquitination and degradation of JAZ repressors, enabling the release of JA-dependent transcription factors such as MYC factors, activating JA-defence responses such as anthocyanin accumulation and trichome formation (Howe *et al.*, 2018) (**Figure 1.5B**). Constitutively activated JA signalling pathway and JA-mediated responses exhibit a higher resistance degree to *T. urticae* infestations, while plants with deficiencies in the ability to synthesize JA or transmit JA signals are more susceptible to these infestations (Ament *et al.*, 2004; Martel *et al.*, 2015; Rioja *et al.*, 2017). For example, *myc2,3,4* mutant plants, defective in JA-responses, showed a reduction in mite mortality rates in comparison to control plants (Zhurov *et al.*, 2014). Other JA-derivates such as the hydroxylated jasmonate 12-OH-JA, thought to be inactive, also seem to play a role during mite infestations. Concretely, *Arabidopsis jao* mutant plants, defective in the production of 12-OH-JA, show enhanced resistance to *T. urticae*, reducing plant damage and mite oviposition rates (Rosa-Díaz *et al.*, 2023). In addition, methyl jasmonate (MeJA), a volatile compound derived from JA, also induces defence responses and reduces mite reproduction and longevity (Zhang *et al.*, 2023).

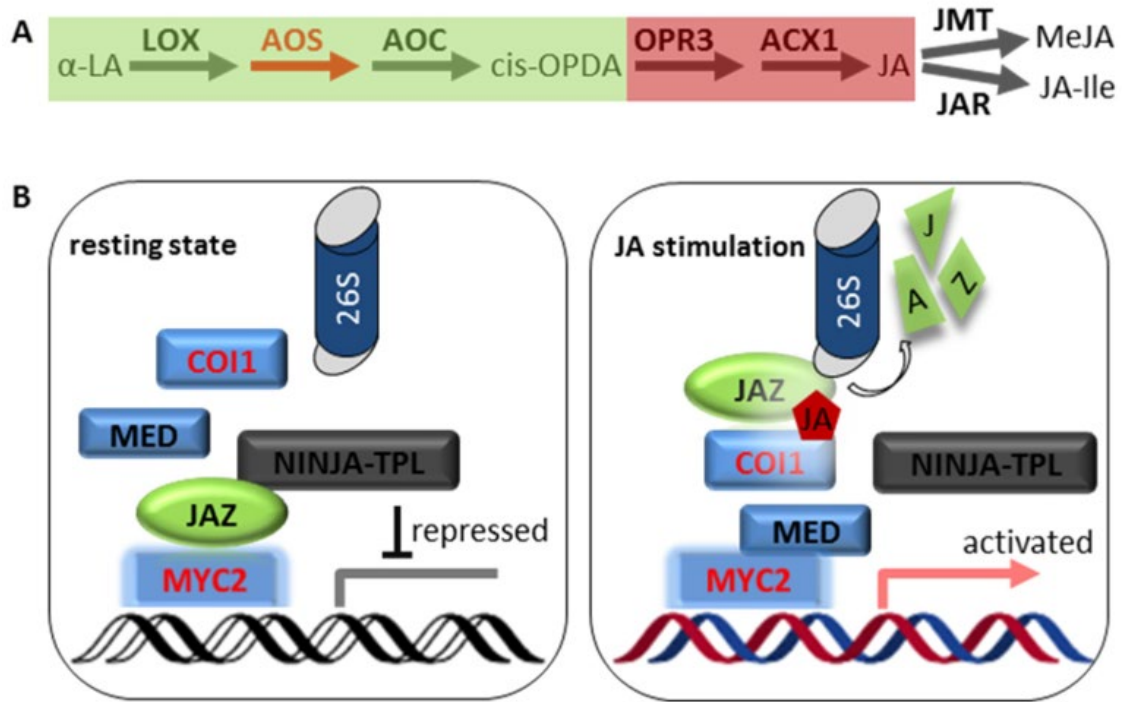


Figure 1.5. Simplified scheme of the JA biosynthesis (**A**) and signalling transduction pathways (**B**). The intermediate OPDA is synthesized in the chloroplasts. JA is synthesized in the peroxisomes and exported to the cytosol, where it is converted to other bioactive derivatives (i.e., JA-Ile). α -LA: α -linolenic acid; ACX1: Acyl-CoA-oxidase 1; AOC: Allene oxide cyclase; AOS: Allene oxide synthase; COI1: Coronatine insensitive 1; JA: jasmonic acid; JA-Ile: jasmonic acid-isoleucine conjugate; JAR: jasmonate resistant; JAZ: jasmonate ZIM domain; JMT: JA methyl transferase; LOX: 13-lipoxygenase; MeJA: methyl jasmonate; MED: mediator; NINJA: novel interactor of JAZ; OPDA: 12-oxophytodienoic; OPR3: OPDA reductase 3; TPL: topless. From Li *et al.* (2019a).

Likewise, SA is another important hormone regulating plant defence mechanisms against herbivores (Erb & Reymond, 2019). SA biosynthesis is mainly performed by the action of the isochorismate synthase (ICS) or the phenylalanine ammonia-lyase (PAL) (Zhang & Li, 2019) (**Figure 1.6A**). Once synthesized, SA binds to the non-expressor of PR gene 1 (NPR1) receptor (Wu *et al.*, 2012). SA accumulation leads to NPR1 activation through redox pathways, which is translated to the nucleus to activate SA-responses (Pieterse & Van Loon, 2004) (**Figure 1.6B**). SA responses are generally accepted to be more related to defence responses towards sucking feeders, inducing both local and systemic responses involving systemic acquired resistance (SAR) (De Vos *et al.*, 2005; Kawazu *et al.*, 2012). Additionally, SA also induces ROS production and plant volatile release, such as methyl salicylate (MeSA), to attract natural enemies such as the predatory mite *Neoseiulus womersleyi* (Ishiwari *et al.*, 2007).

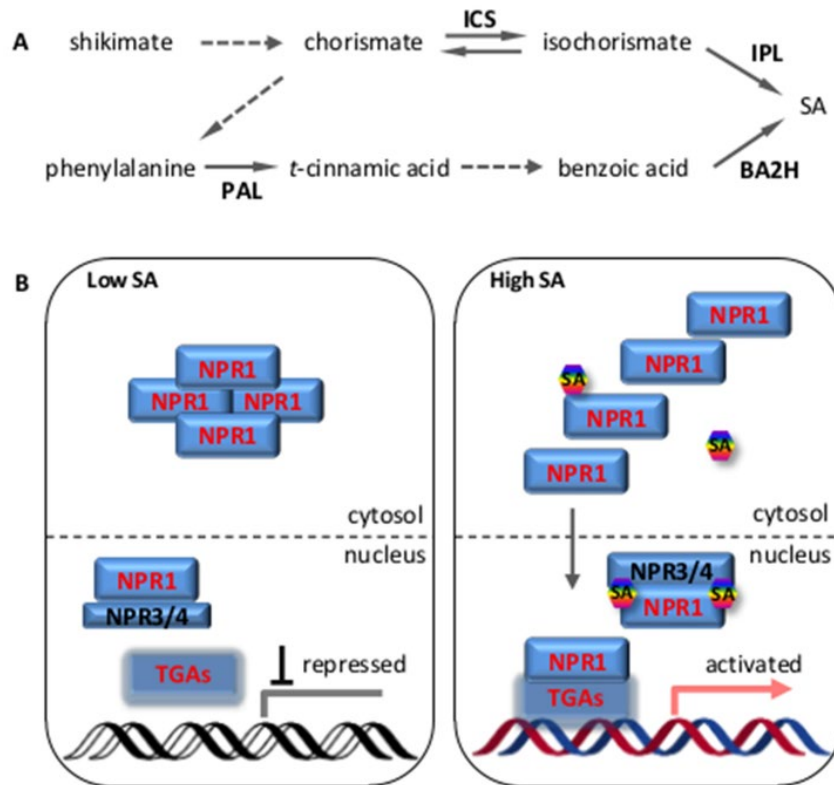


Figure 1.6. Simplified scheme of the SA biosynthesis (**A**) and signalling transduction pathways (**B**). BA2H: benzoic acid 2-hydroxylase; ICS: isochorismate synthase; IPL: isochorismate pyruvate lyase; NPR: non-expressor of pathogenesis-related genes; PAL: phenylalanine ammonia lyase; SA: salicylic acid; TGA: TGACG-binding factor. From Li *et al.* (2019a).

Besides, to JA and SA, other phytohormones previously linked exclusively to plant growth, development and/or reproduction processes such as ethylene (ET), abscisic acid (ABA) or auxins have been also reported as signalling molecules in plant defence against mites (Arimura *et al.*, 2002; Rosa-Diaz *et al.*, 2024). While the ABA-insensitive 4 (ABI4) transcription factor has shown to regulate mite-associated plant defences directed by ABA (Barczak-Brzyżek *et al.*, 2017), very recently, Rosa-Diaz *et al.* (2024) demonstrated that ABA-induced stomatal closure after mite infestations, hinders mite feeding and minimizes leaf cell damage. In addition, while variations in auxin levels have also been found in spider mite infestations (Santamaría *et al.*, 2019), mutations in YUCCA genes, involved in auxin biosynthesis also showed enhanced resistance to this mite (Santamaría *et al.*, 2019; Pérez-Alonso *et al.*, 2021). These results suggest that plant resistance or susceptibility to *T. urticae* is modulated by the action of different hormones involving hormonal crosstalk. However, further research including their dynamic levels and activation among plant species and developmental stage will determine the role of each of these hormones against spider mites.

1.3.2.2.4. Direct and indirect defences

As a result of the previous responses, plants deploy a sophisticated array of direct and indirect defences in response to mites. Direct defences include structures and compounds that are toxic, repellent or avoid herbivore feeding. One defence strategy involves the production of primary metabolites such as proteinase inhibitors (PIs), generally encoded by JA-dependent genes (Santamaría *et al.*, 2018b). Based on their substrate, PI can be classified as serine-, cysteine-, carboxy-, aspartic- or metallo-peptidase inhibitors, among others. Some PI involved in response to *T. urticae* are the cysteine-PI from barley, HvCPI-6, or the Kunitz trypsin inhibitors (KTI) from Arabidopsis, *AtKTI4* and *AtKTI5*, whose overexpression and silencing on plants confers higher resistance and susceptibility to this mite, respectively (Arnaiz *et al.*, 2018; Santamaría *et al.*, 2012, 2018b). In the case of these inhibitors, it has been demonstrated that they targeted the specific proteases in spider mite faeces and bodies respectively (Santamaría *et al.*, 2015). In tomato, two serine-PI genes, *PIN-I* and *PIN-II*, were shown to be induced and increase their enzyme activity upon mite infestation on leaves (Kant *et al.*, 2004; Martel *et al.*, 2015). In addition to PI, secondary compounds such as glucosinolates also induce resistance to spider mites. Interestingly, accumulation of indole-glucosinolates (IG), compounds derived from the tryptophan pathway, are involved in JA-dependent responses (Liu *et al.*, 2010) and have shown a positive correlation with plant resistance to *T. urticae*. In this sense, while *cyp79b2 cyp79b3* mutant plants, defective in IG production, showed higher damage and less mite mortality than control plants, plants overexpressing the ATR1 transcription factor, a positive regulator of IGs, made plants more resistant to *T. urticae* infestations (Zhurov *et al.*, 2014). Moreover, other compounds also have shown importance against mites. In strawberry leaves, there exist a significant correlation between the natural leaves of catechol-based phenolics and the plant's resistance to *T. urticae* (Luczynski *et al.*, 1990). Furthermore, the accumulation of flavonoids such as naringenin, hesperetin, and of the precursor of flavonoids and lignin, p-Coumaric acid in citrus, phenolic compounds in chrysanthemum or terpenoids in cucumber has also been associated with resistance to this pest by reducing plant palatability (Balkema-Boomstra *et al.*, 2003; Agut *et al.*, 2014).

Indirect defences, on the other hand, involve the recruitment of natural enemies of the herbivores or the repellence of specific herbivores through the release of volatile organic compounds (VOCs). Spider mite infestation has also shown to induce volatiles emissions of hormones such as ET or MeJA and other compounds in tomato and lima bean (Arimura *et al.*, 2002; Martel *et al.*, 2015). In addition, VOCs also work as interplant airborne signals in plant-

plant communication and its exposure to plants usually induces JA accumulation and induction of JA-related genes (Engelberth *et al.*, 2007). In fact, lima bean plants attacked by the spider mite *T. urticae* and predatory mites *Phytoseiulus persimilis* showed that not only the attacked plant, but also the neighbouring ones, become more attractive to predatory mites and less susceptible to mites (Bruin *et al.*, 1992; Arimuza *et al.*, 2000). Additionally, in mite-infested citrus plants, it was detected the production of the terpene volatiles α -ocimene, α -farnesene, pinene and D-limonene, and of the green leaf volatile 4-hydroxy-4-methyl-2-pentanone, showing a marked repellent effect on spider mites and a promoted mite-induced resistance in neighbouring citrus plants (Agut *et al.*, 2015).

However, despite all the knowledge generated around plant defence responses to spider mite infestations, the cost paid by plants to implement these defensive traits and its implications on the final plant fitness, has not been previously studied.

1.4. Plant defence-growth trade-off

1.4.1. The importance of the defence growth trade-off studies

The defence-growth theory establishes that the plant defences suppose a cost to the overall plant performance and fitness. Under stress conditions, plants fine-tune their phenotype to maximize their fitness, severely impacting plant growth and favouring the selection of individuals with a better defence response to the specific stress. As proper growth of crops in the presence of different stresses is a desirable trait that could help to achieve a sustainable agriculture, plant growth, development and reproduction evaluation is essential to understand the molecular underpinning and physiological basis of plant allocation to trade-offs.

However, the evaluation of the defence-growth trade-offs on fitness is extremely difficult to assess. To be precise, the term “*fitness*” is related with “*reproductive success*” and, generally, effects on plant fitness has been associated to seed production (Strauss, 1997). Indeed, fitness analysis through evaluation of the plant offspring is the most direct measure of fitness. Nevertheless, the difficulties and high costs of the maintenance of infestations on plants until the reproductive phase make a constraint that is usually overcome by evaluating surrogate fitness parameters of growth, photosynthesis or reproduction such as relative growth rate (RGR), leaf number, leaf length, leaf area, plant biomass, photosynthetic rate, CO₂ fixation, number of flowers or siliques (Nykänen & Koricheva, 2004; Erb, 2018; Buffon *et al.*, 2021).

Particularly, evaluation of biomass and growth rate are the most frequently used parameters, often positively associated with fecundity and overall fitness (Younginger *et al.*, 2017). Plant growth rate is understood as a measure of the increase in biomass of a plant over a specific time-period, where the conversion of primary metabolites into cells and molecular structures depends on the balance of resource sources and sinks. In this process, the presence of an external pressure such as herbivory, usually provokes the redirection of resources from primary metabolism to defence, commonly reducing growth and reproduction processes that modifies the final plant fitness. This resource diversion depends also on the specific costs of the defences deployed. While constitutive defences entail costs for the plants that outweigh their benefits when these defences are not needed (Karban, 2011), induced defences are suggested to have evolved to reduce the costs of constitutive defences (Heil & Baldwin, 2002; Zangerl, 2003; Cipollini & Heil, 2010). Nevertheless, induced defences still incur costs, and plants may suffer considerable damage during the time required to mount this induced defence response upon infestation (Frost *et al.*, 2008). For example, production of trichomes, being part of the constitutive physical barriers but also of the induced defences in newly grown tissues in presence of herbivores (Cipollini *et al.*, 2018), can impose a cost in growth and reproduction processes. Higher trichome densities show lower growth rates but an increased resistance to the herbivore *Trichoplusia ni* with a reduction on seed production in tomato (Züst *et al.*, 2011; Mymko & Avila-Sakar, 2019). This reduced seed production was also observed previously by Hare *et al.* (2003), where glandular trichome production was associated with reduced seed set, suggesting allocation trade-offs between growth and reproduction. Nevertheless, contrasting information has also been found, in which trichome production in tomato or *Arabidopsis lyrata* plants did not find significant cost of trichome expression affecting yield or tolerance to herbivory (Kaplan *et al.*, 2009; Puentes & Ågren, 2014).

On the other hand, it is well known that the effects of induced defence on plant fitness depend on the specific pest and host plant, but the effect of other critical variables surrounding the interaction remain largely unexplored. Different host plants can elicit different responses with a different cost on the final plant fitness. For example, Bekaert *et al.* (2012) estimated that glucosinolate biosynthesis in *A. thaliana* increases the plant's photosynthetic requirement by at least 15%, but production of glucosinolates is not a common strategy in higher plants. In addition, the impact of phytophagous specialization or different feeding guilds has been poorly studied, and its evaluation has been limited to sap-feeders on woody plants (Nykänen & Koricheva, 2004; Zvereva *et al.* 2010). Moreover, duration and severity of the infestation are

factors that could induce an excessive shift between primary and secondary metabolism, consequently leading to a diversion of resources to defence responses.

Furthermore, this diversion of resources between different physiological processes are greatly determined by the ecological context. In specific ecological contexts, differences in growth rates could additionally results in a reduction in plant fitness. Poor growth or defence are not commonly favoured by natural selection. In a context of simultaneous germination of different plants, those with lower growth rates have proved to be less competitive than those with higher rates, determining their dominance over other genotypes or plants with a less accessibility to nutrient and resources. In contrast, faster growth rates have been directly associated with an increased susceptibility to pests, apparently prioritizing growth over defence response (Züst & Agrawal, 2017). Therefore, resource allocation effects to different plant functions, in combination with other ecological interactions that impose selective pressures on plants such as herbivory and plant competition, conforms a resource-derivative network that determines the final plant fitness (**Figure 1.7**).

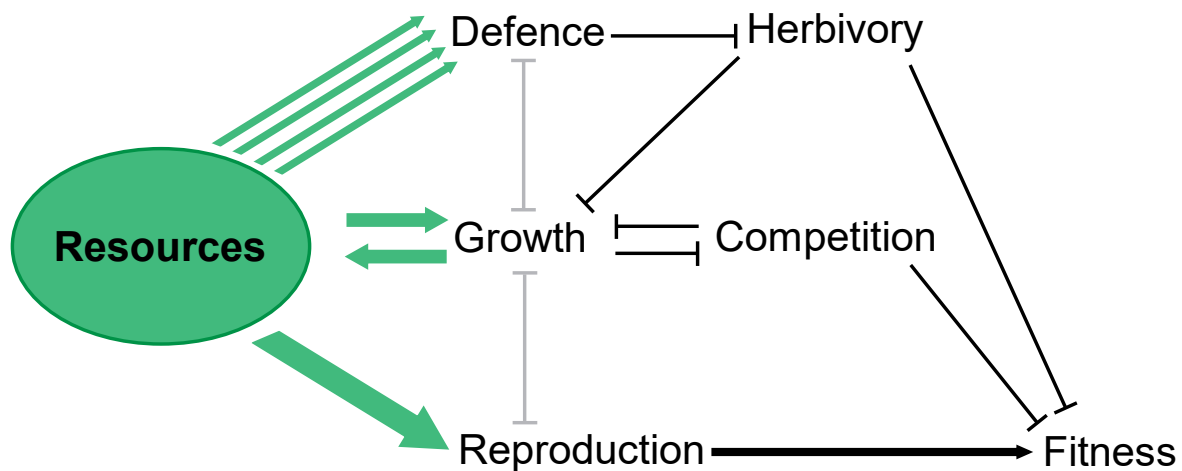


Figure 1.7. Conceptual diagram of plant fitness determination via the combined effects of resource allocation to competing plant functions (green arrows), resource-based trade-offs (gray lines), and ecological interactions (black arrow and lines). Adapted from Züst & Agrawal (2017).

1.4.2. Defence-growth theories

To explain the molecular basis underlying growth-defence trade-offs in plants, diverse theories have been suggested. The main prevailing hypothesis usually comes with the idea that, under a specific fixed budget of resources or ceiling, the induction of a variety of defence compounds involves a substantial demand for resources that leads to a huge redistribution of energy toward the defence response (**Figure 1.8A**). After simulated herbivore attack on *N. attenuata* plants,

labelled nitrogen derived from ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is reallocated into nicotine and phenolamide compounds, entailing an alteration of the normal metabolic flux to the production of defence compounds (Ullmann-Zeunert, *et al.*, 2013). In this scenario of limited resources, an increment in the number of resources should potentially lead to plants with promoted growth and enhanced defences. Supporting this idea, *Arabidopsis jaz* polymutants with enhanced JA-defence response and stunted growth, increase plant biomass and root length with exogenous sucrose supply, suggesting that high-nutrient conditions reduce or eliminate the inverse relationship between defence and growth processes (Guo *et al.*, 2018).

An alternative hypothesis establishes that these trade-offs can be also a result of temporal adaptations of the plant to stresses, in which the resource diversion between growth and defence is an active and hardwired strategy to cope the stress instead of a resource ceiling (He *et al.*, 2022) (**Figure 1.8B**). Upon sensing the stress, plants would incur in a transcriptional reprogramming and would temporarily shift its metabolism to the production of defence compounds and strategies and to prepare themselves from future attacks, while avoiding providing nutrients and food to pests. These two hypotheses are not mutually exclusive and may operate in different external environments. In fact, it is hypothesized that trade-offs between growth and defence could be originated by directly limited by competition for resources (allocation costs), by genetically linked pleiotropy or linkage disequilibrium (genetic costs) or penalized by depending on the plant's environment (ecological costs) (Züst & Agrawal, 2017).

1.4.3. Resource diversion

Independently of the defence-growth theory, the establishment of these trade-offs involves a diversion of resources. This diversion occurs at several levels, from precursors for primary metabolism to machinery involved in transcription, translation and protein secretion of defence responses (Jung *et al.*, 2007; Bilgin *et al.*, 2010; Chen *et al.*, 2011). Plant resources usually depend on the environmental conditions and come from the nutrient availability and from photosynthesis and carbohydrates. Thus, a reduction of the level of resources on stressful conditions could impose a constraint that increases the penalties of expressing high-demanding traits. Concordantly, different milkweed genotypes grown at two nutrient levels exhibited a higher negative correlation between growth rate and defence (concentration of cardiac glycosides) in nutrient-limited environments (Züst *et al.*, 2015). In the case of insect herbivory infestations, different studies have suggested the presence of nutrient limitations in primary

metabolism, including photosynthesis, carbon transport and nitrogen allocation (Zhou *et al.*, 2015).

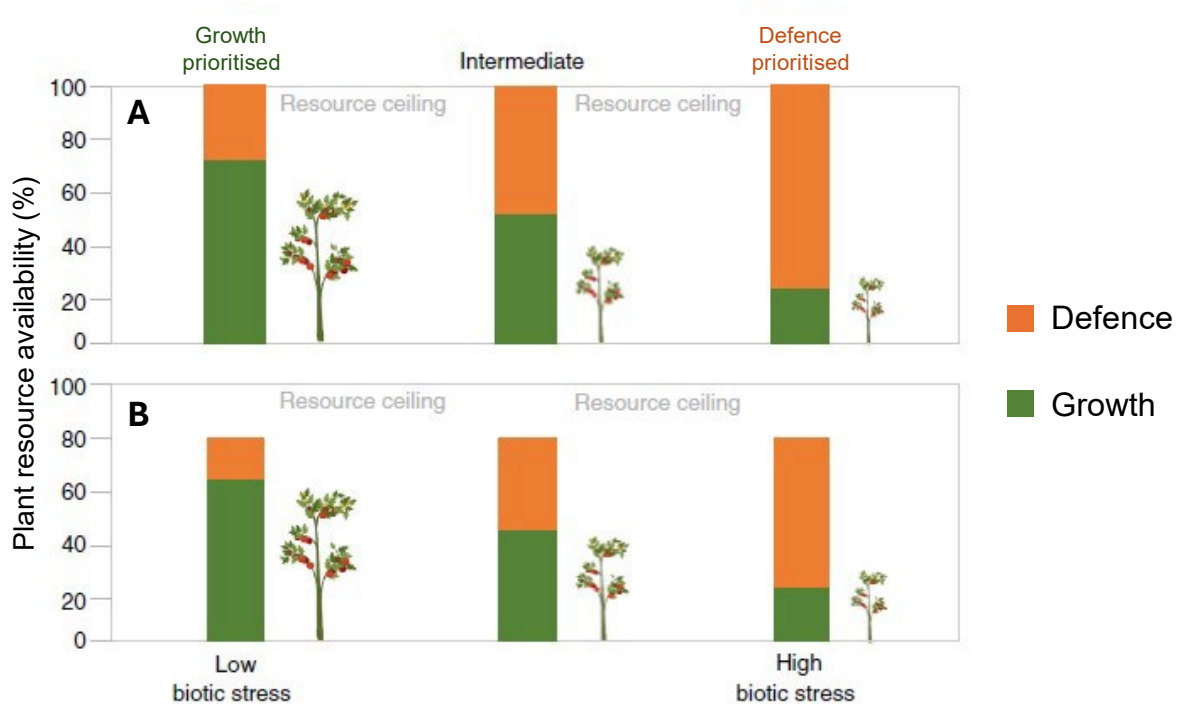


Figure 1.8. Two hypotheses explaining the growth–defence trade-off. **(A)** Resource limitation (ceiling) drives the trade-off between growth (green) and defence (orange). **(B)** The trade-off is independent of resource limitation. Left axis indicates % of total available plant resource used for growth or defence. Adapted from He *et al.* (2022).

1.4.3.1. Carbohydrate Metabolism

The carbohydrates are the major source of stored energy supporting plant growth and primary metabolism (Zhou *et al.*, 2015; Machado *et al.*, 2017). Photosynthesis, being the source of almost all carbohydrates in green plants, conforms a central process involving carbon allocation in response to herbivory. Upon herbivory attack, a reduction in the photosynthetic rate, transcriptional responses or production of photosynthetic-related genes have been observed (Giri *et al.*, 2006; Wei *et al.*, 2009; Bilgin *et al.*, 2010). The generation of the photosynthetic structures is an energy consuming process that could be compromised by the production of defensive metabolites. In addition, senescence and foliar abscission of damaged tissues could reduce photosynthesis, leading to a less carbon fixation that reduces the carbohydrates available for insect herbivores. Interestingly, JA defence responses has shown to decrease gene expression and the amount of components required for light harvesting and carbon fixation, and besides feeding, insect oviposition and plant volatiles also reduce the plant photosynthetic

capacity as a result of the defence process (Little *et al.*, 2007; Arimura *et al.*, 2010; Attaran *et al.*, 2014).

However, increased photosynthetic activity in response to herbivory has also been described as a plant tolerance strategy. Photosynthetic response can be promoted as a response for the additional energy requirement imposed by the production of secondary metabolites and may compensate the loss of area and activity after herbivory feeding. Nonetheless, possibly manipulation through insect effectors to increase the carbon fixation and obtain more resources could also occur. For example, in *N. attenuata* plants, there is an increase in the CO₂ fixation in response to the myriad bugs saliva of *Tupiocorus notatus*, without observing negative effects on plant fitness (Halitschke *et al.*, 2011). Similar responses have also been observed in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) in response the wheat aphid *Diuraphis noxia*, where an increase of photosynthetic-related genes was observed after the attack (Botha *et al.*, 2006; Gutsche *et al.*, 2009).

Interestingly, increasing demands of energy may require other sources of carbon. These alternative resources can be a result of the catabolism of energy compounds such as sucrose or starch. In *Arabidopsis*, feeding damage by four different chewing and sucking insects, showed increased expression of invertases and enzymes involved in the carbohydrate catabolism (Appel *et al.*, 2014). Nevertheless, although some studies have reported lower concentration of sucrose and starch in local tissues after herbivore infestations, other studies show no changes in sugar content, or reduction in distal tissues, suggesting a carbon reallocation from other sources (Steinbrenner *et al.*, 2011; Castrillon-Arbelaiz *et al.*, 2012). Treatments with the volatile MeJA or insect feeding can increase carbohydrate movement to attacked sites, supporting local defence (Allison & Schultz, 2005; Schultz *et al.*, 2013). Indeed, Ferrieri *et al.* (2012), using radioactively labelled carbohydrates, demonstrated that in MeJA-treated or wounded *Arabidopsis* plants there was a reallocation of carbon to the production of defence-related compounds such as cinnamic acid or phenolic glycosides.

However, reallocation may also be a tolerance strategy, preserving resources for regrowth post-herbivory (Millard & Grelet, 2010). For example, maize reallocates carbohydrates from infested roots by the western corn rootworm *Diabrotica virgifera* to stems, promoting regrowth (Robert *et al.*, 2014), while simulated herbivory in *N. attenuata* moves carbon to roots to delay senescence (Schwachtje *et al.*, 2006).

1.4.3.2. Nitrogen Metabolism

Plant amino acids are also crucial in plant metabolism serving as growth-limiting nutrients and as precursors for plant defence compounds. Upon herbivory, plants may increase amino acid production to synthesize defensive metabolites while also limiting the access to free amino acids to herbivores. Caterpillar feeding on *Arabidopsis* induces genes involved in amino acid biosynthesis and sulphur assimilation, promoting the production of glucosinolates, which are derived from the induced methionine and tryptophan biosynthesis (Appel *et al.*, 2014). Similarly, induction of the aromatic amino acids derived from the shikimate pathway on tobacco plants treated with MeJA or by caterpillar herbivory showed rapid increases in amino acids such as Trp, which can serve as a precursor of defensive metabolites (Hanik *et al.*, 2010; Steinbrenner *et al.*, 2011). In the case of mite infestation, Ximénez-Embún *et al.* (2018) showed that, although the production of defensive PIs in tomato plants was mainly induced after infestations with *T. evansi*, previous plant exposition to drought stress stimulated *T. urticae* feeding and fecundity by improving the plant nutritional values through the accumulation of essential amino acids. Likewise, changes in the amino acid homeostasis due to *T. urticae* infestation have also been described in legumes (Boter & Diaz, 2024).

In fact, herbivory also alters amino acid metabolism and movement between tissues. Nitrogen phloem transport, predominantly mediated by glutamine, glutamate, asparagine, and aspartate, was also affected by herbivory through transcriptional changes involved in the production of these amino acids, which are required for nitrogen assimilation (Coruzzi & Last, 2000). Some examples include the gene upregulation of nitrate and nitrate reductases, glutamine oxoglutarate aminotransferase (GOGAT) or glutamine synthetase. However, these changes can vary depending on the specific plant-herbivore interaction and tissue analysed (Zhou *et al.*, 2015).

Interestingly, as described for carbon metabolism, the observed changes could also be a consequence of insect herbivory manipulation to enhance free amino acid levels in their diets. Infestation by the aphids *Schizaphis graminum* and *Rhopalosiphum insertum* have shown to increase essential amino acids in the phloem sap of wheat and Japanese rowan leaves, respectively. This manipulation strategy likely involves increasing amino acid biosynthesis, inducing senescence and proteolysis, or altering sink-source relationships to boost nitrogen transport to infested leaves (Zhou *et al.*, 2015).

1.4.4. Phytohormonal regulation of plant defence-growth trade-off

One of the key elements directing this resource diversion and the plant defence-growth trade-off upon the presence of herbivores is the plant hormonal response. Plant hormones have been traditionally classified as “defence” and “growth” hormones. According to He *et al.* (2022), two different hormone modules can be established based on their involvement in defence and growth processes. Concretely, while JA, SA and ET have been described to conform a hormonal core directing main defence biotic responses; auxins, brassinosteroids (BRs), cytokinins (CKs) gibberellins (GAs) have been traditionally classified as “growth” hormones, mainly participating in the regulation of plant growth and development (**Figure 1.9**). However, although ABA does not appear in none of the modules, it has been shown to participate in response to abiotic stress and in the regulation of seed dormancy, embryo development or plant senescence, exhibiting a dual role by regulating plant response to stress and growth and developmental processes (Lata & Prasad, 2011; Chen *et al.*, 2014). Interestingly, in addition to ABA, during the last years, the role of the traditional growth hormones in modulating defence responses has been highlighted (Erb *et al.*, 2012), in which alterations of their levels determine both plant growth and defence responses. For this reason, additionally to the antagonistically described JA-SA crosstalk modifying defence responses, the integration of signals among different “defence” and “growth” hormonal pathways determines the final defence-growth responses established in plants upon stress.

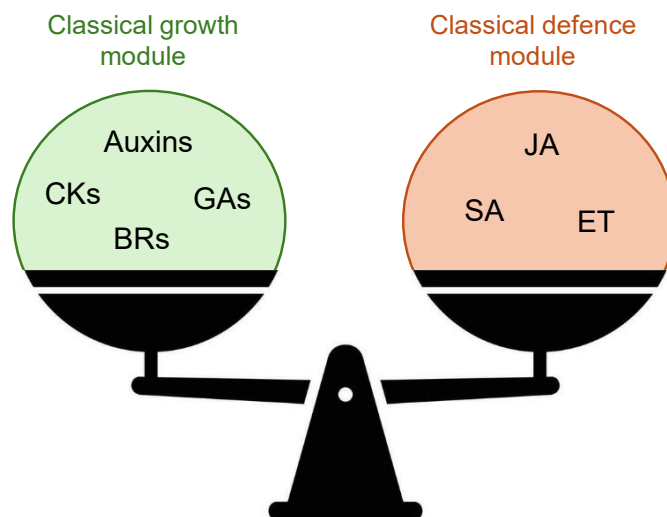


Figure 1.9. Plant classical hormonal modules participating in the defence-growth trade-off against biotic stress.

1.4.4.1. Defence module induction modifies plant growth

JA and SA are the masters' regulators of the biotic defence responses of the plant, in combination with ET. As a result of the defence establishment, accumulation of these hormones is commonly followed by a reduction in plant growth (Zhang *et al.*, 2003; Paponov *et al.*, 2021). The role of JA in inhibiting plant growth has been extensively described, being JA able to suppress mitosis and arrest the cell cycle in G1 prior to the S transition (Noir *et al.*, 2013; Paponov *et al.*, 2021). In addition, activation of both MYC and MYB transcription factors by JA accumulation is known to regulate stamen development and seed production in Arabidopsis (Qi *et al.*, 2015; Huang *et al.*, 2017). However, among all the members of the JA signalling pathway, JAZ repressors are the main regulators in defence-growth hormonal crosstalk, participating in the regulation of auxin, GA and ABA responses (Liu & Timko, 2021).

Regarding SA, the mechanisms by which SA induces growth suppression usually involves crosstalk with other hormones, specially highlighting the mutual antagonistic interaction between SA and auxin signalling. Some examples of the SA negative impact on growth are reflected in plants treated with benzothiadiazole (BTH), a chemical analogue of SA, which induces a reduction in plant biomass in a dose-dependent manner (Canet *et al.*, 2010), or in the induced SA accumulation and signalling in mutant plants with a constitutively induced defence and a dwarf phenotype (Clarke *et al.*, 2000; Zhang *et al.*, 2003).

Finally, ET additionally participates in various aspects of plant growth and development, regulating processes such as fruit ripening, leaf abscission or flower wilting (Schaller *et al.*, 2002; Hyodo, 2018). ET has also shown to negatively regulate plant growth through inhibition of cell elongation and reduction of stem and root growth (Street *et al.*, 2015). This reduction of root growth involves hormonal crosstalk antagonism with ABA and auxins, affecting to their biosynthesis and signalling (Mao *et al.*, 2016; Müller, 2021).

1.4.4.2. Growth module interaction with defence module

As previously described, the growth module is comprised by auxins, CKs, BRs and GAs, in which hormonal crosstalk with defence hormones occurs at several levels of their biosynthetic and signalling pathways.

1.4.4.2.1. Auxins

Auxins are central regulators of plant growth and development, functioning in cell fate determination and cell division, and controlling apical dominance, stem and petiole elongation,

root gravitropism and hair formation, responses to light or temperature, and flower formation (Kazan, 2013; Schaller *et al.*, 2015). Among auxins, the indole-3-acetic acid (IAA) is the most predominant form in plants, mainly produced tryptophan-dependent pathways. The indole-3-pyruvic acid (IPyA) pathway is the main biosynthetic pathway, mediated by tryptophan aminotransferase (TAA) and flavin monooxygenase YUCCA (YUC) (Dai *et al.*, 2013). However, additional biosynthetic pathways have been described: the tryptamine (TAM)-, the indole-3-acetamide (IAM)-, and the indole-3-acetaldoxime (IAOx) pathway (Kasahara, 2016). In the case of the IAOx pathway, it involves the action of the cytochrome P450 monooxygenases (CYP79B2, CYP79B3) (Mikkelsen *et al.*, 2000), and its synthesis is related to the production of IG, camalexins and the indole-3-carboxylic acid, all crucial for defence in *Arabidopsis* (Müller *et al.*, 2019). The TAM pathway is not described to be relevant for auxin de novo biosynthesis, and the IAM pathway involves the action of the IAM hydrolase gene *amidase1 (AM1)* for auxin biosynthesis (Ortiz-García *et al.*, 2023) (**Figure 1.10A**).

However, once IAA is synthesized, it can be deactivated through conjugation with amino acids or sugars by the action of GH3 enzymes, among others, or through oxidation processes by IAA oxidases (Casanova-Sáez & Voß, 2019). In addition to its synthesis, auxin signalling is controlled by a E3 ubiquitin ligase complex SCF^{TIR1/AFB}, the co-receptor/repressor proteins auxin/indole-3-acetic acid (Aux/IAA) and the DNA-binding auxin responsive factors (ARFs). In the presence of auxin, the repressor Aux/IAA is degraded and ARF activates the expression of auxin-responsive genes (**Figure 1.10B**).

The SA regulation of IAA homeostasis has important implications on defence-growth trade-offs (Huot *et al.*, 2014). In *Arabidopsis*, SA decreases the expression of auxin biosynthesis and signalling. Exogenous treatments of SA stabilize Aux/IAA repressors, which leads to an attenuation of the auxin signalling responses (Wang *et al.*, 2007). Additionally, SA represses auxin accumulation and transport by the induction GH3 enzymes, such as the *GH3.5* gene, to form the inactive IAA-Asp (Wang *et al.*, 2007), and through the control of the distribution of the PIN proteins, transporters of auxins (Zhong *et al.*, 2021). On the other hand, while dwarf *GH3.5* overexpressing lines promote *PR1* expression, auxin treatments suppress *PR1*, suggesting also a negative SA regulation by auxins (Zhang *et al.*, 2008; Robert-Seilantantz *et al.*, 2011).

Interestingly, contrary to the negative effect observed in SA-mediated responses, JA and auxin signalling has been mainly described to interact positively (Kazan *et al.*, 2009). While both MeJA and IAA synergistically increase *PDF1.2* expression (Qi *et al.*, 2012), JA production has

shown to increase auxin biosynthesis by the induction of the anthranilate synthases (*ASA1* and *ASB1*) and *YUCCA* (*YUC2*, *YUC8* and *YUC9*) (Jiaquiang *et al.*, 2009; Hentrich *et al.*, 2013). On the other hand, early auxin responses have also shown to induce *JAZ1*, negatively regulating JA signalling and supporting the idea of an intimate molecular interplay between auxin and JA signalling (Grunewald *et al.*, 2009).

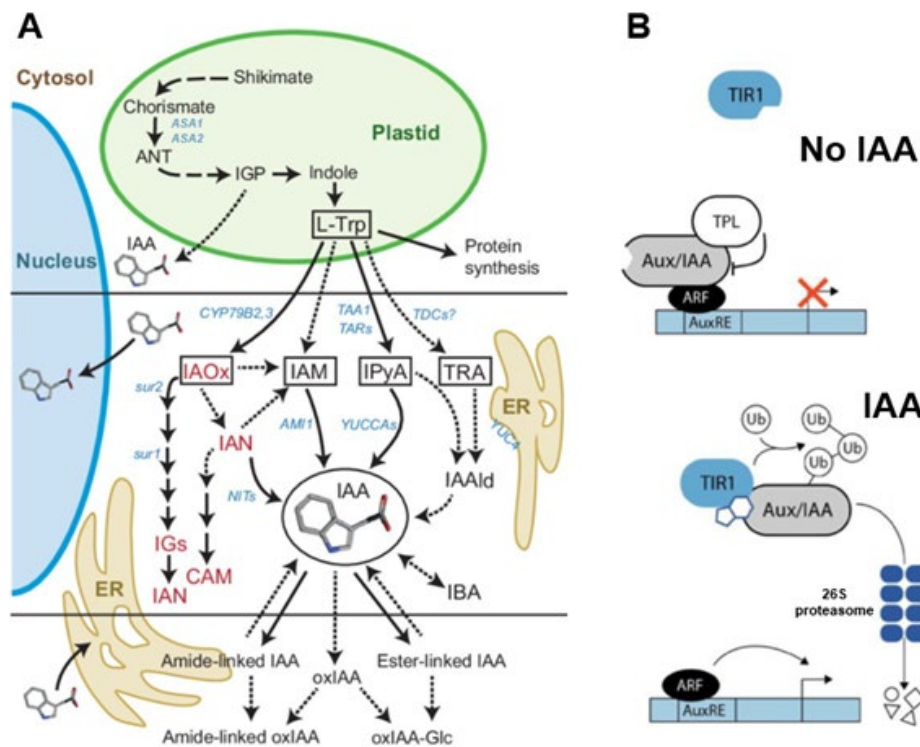


Figure 1.10. Simplified scheme of the (A) auxin metabolism and (B) signalling in the absence or presence of indole-3-acetic acid (IAA). ASA: anthranilate synthase alpha subunit; ANT: anthranilate; CAM: camalexin; Glu: glucose; IAAld: indole-3-acetaldehyde; IAEt: indole-3-ethanol; IAM: indole-3-acetamide; IAN: indole-3-acetonitrile; IAOx: indole-3-acetaldoxime; IBA: indole-3-butyric acid; IGP: indole-3-glycerol phosphate; IGs: indole glucosinolates; IPyA: indole-3-pyruvic acid; L-Trp: L-tryptophan; NITs: nitrilases; oxIAA: 2-oxindole-3-acetic acid; TAA: tryptophan aminotransferase; TAR: tryptophan aminotransferase-related protein; TDCs: tryptophan decarboxylases; TRA: tryptamine; TIR1: transport inhibitor response 1 F-box protein; Aux/IAA: auxin/indole-3-acetic acid proteins; TPL: topless co-repressor protein; ARF: auxin response factor; AuxRE: auxin responsive elements; Ub: ubiquitin. From Ortiz-García *et al.* (2023).

Finally, auxin has also shown both antagonism and synergism with ET, being context dependent. In light-grown seedlings of *Arabidopsis*, ET signals mediated by ethylene-insensitive 3 (EIN3) attenuate auxin response, reducing auxin-induced cell expansion and hypocotyl growth (Kim *et al.*, 2021). Moreover, during root growth regulation, both auxin and ET have shown a synergism by inducing root growth inhibition (Muday *et al.*, 2012; Miao *et al.*, 2018).

1.4.4.2.2. Cytokinins

CKs are N⁶-substituted adenine derivatives that regulates the maintenance of stem cell identity and cell proliferation, influencing processes such as root and shoot growth, leaf expansion or formation of buds (Kieber & Schaller, 2018). In addition to these roles, CKs are also crucial in delaying leaf senescence, enhancing chloroplast development, and promoting nutrient mobilization (Mok, 2019). These phytohormones are produced in the roots and transported to other parts of the plant where they exert their effects. The most common class of CKs have isoprenoid side chains, including the most abundant cytokinin in Arabidopsis, *trans*-zeatin (*tZ*). Zeatin exists in two isomeric forms: *trans*-zeatin (*tZ*) and *cis*-zeatin (*cZ*). *tZ* is the most active form of CK and is found in all plant species. On the other hand, *cZ* is less active than *tZ* and it is suggested to function as stress-response markers mediating plant development, pathogen resistance and anti-herbivore responses (Schäfer *et al.*, 2015). These forms are usually synthesized through the action of isopentenyl transferases (IPTs), and cytochrome P450 CYP735 (CYP735A1 and CYP735A2), becoming active by the activity of lonely guy (LOG) enzymes (**Figure 1.11**). In the case of *cZ*, this isomeric form is synthesized via a different route involving tRNA-IPTs catalysing the isopentenylation of adenine on tRNA, that after degradation liberates *cZ* nucleotides. Breakdown of CKs is produced by CK oxidase/dehydrogenase (CKX) enzymes, but they also can be inactivated by conjugation to sugars, in which glucosyl-conjugated CKs do not interact with CK receptors (Spíchal *et al.*, 2004). In Arabidopsis, the CK signalling initiates by the perception of CKs through histidine kinases (HKs) known as Arabidopsis histidine kinase2 (AHK2), AHK3, and CK responsive1 (CRE1)/AHK4. The activation of these receptors triggers the phosphorylation of histidine phosphotransfer proteins (HPTs or AHPs) that move to the nucleus to activate B-type Arabidopsis response regulator (ARR) TF, also activating CK-responses (Yan *et al.*, 2017). ARR are of two main types: type B ARRs that positively regulates CK signalling, and type A ARRs, negatively regulating it.

CK signalling is suppressed by negative crosstalk with both JA and SA. In the case of JA, it has been suggested that the inhibition of CK by JA occurs at the MYC2 level, in which MYC2 promotes the expression of AHP6, a specific negative regulator of cytokinin signalling (Jang *et al.*, 2020). Moreover, other studies show that JA inhibits the effect of CK on chlorophyll development on rice (Liu *et al.*, 2016) and suppresses the procambium-specific CK response probably by reducing the expression of *PIN7*, involved in xylem development (Jang *et al.*, 2017). In contrast, CKs have been described to increase JA and phenolamide levels, with a

repressing feedback loop by JA, being able to induce resistance to the herbivory *M. sexta* and necrotrophic fungi in IPT-overexpressing plants and suggesting a probable interaction of CK with the JA pathway of resistance (Choi *et al.*, 2010; Erb *et al.*, 2012).

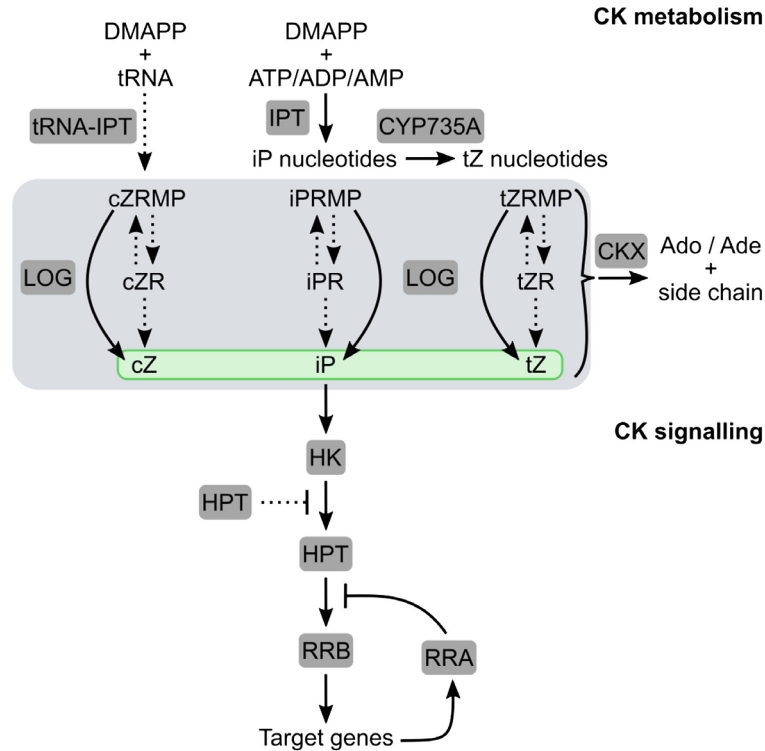


Figure 1.11. Simplified scheme of the CK metabolism and signalling. *tZ*: *trans*-zeatin; *tZR*: *tZ* riboside; *tZRMP*: *tZR* monophosphate; *cZ*: *cis*-zeatin; *cZR*: *cZ* riboside; *cZRMP*: *cZR* monophosphate; *iP*: isopentenyladenine; *iPR*: *iP* riboside; *iPRMP*: *iPR* monophosphate; *IPT*: isopentenyltransferase; *CYP735A*: cytochrome P450 monooxygenase; *LOG*: lonely guy; *CKX*: cytokinin oxidases/dehydrogenase; *HK*: histidine kinase; *HPT*: histidine phosphotransfer protein; *RRB*: type b response regulator; *RRA*: type a response regulator. From Cortleven *et al.* (2019).

Focusing on the CK-SA crosstalk, Johnston *et al.* (2024) demonstrated that although CK signalling was suppressed by negative crosstalk with both JA and SA, SA negative regulation was more evident. On the other hand, either an exogenous CK application or overexpression of *IPT* genes has been shown to induce *PR1* gene expression in Arabidopsis (Naseem & Dandekar, 2012), establishing a possible positive interaction of CK with SA.

1.4.4.2.3. Brassinosteroids

BRs are a class of steroidal growth-promoting plant hormones that play crucial roles in various physiological processes, including cell elongation, division and differentiation, as well as vascular development and reproductive growth (Gudesblat & Russinova, 2011). Starting from campesterol as a common precursor, three pathways including the early C-22 oxidation early C-6 oxidation, and late C-6 oxidation pathways, function as cascade for BR biosynthesis (Figure 1.12) (Chung & Choe, 2013). During this process, enzymes such as CYP90A1 (CPD),

CYP90B1 (DWF4), CYP90C1 rotundifolia3 (ROT3) and de-etiolated2 (DET2) are involved in the initial steps of their synthesis, while CYP85A1 (BR6OX1) and CYP85A2 (BR6OX2) are involved in the final steps of their synthesis.

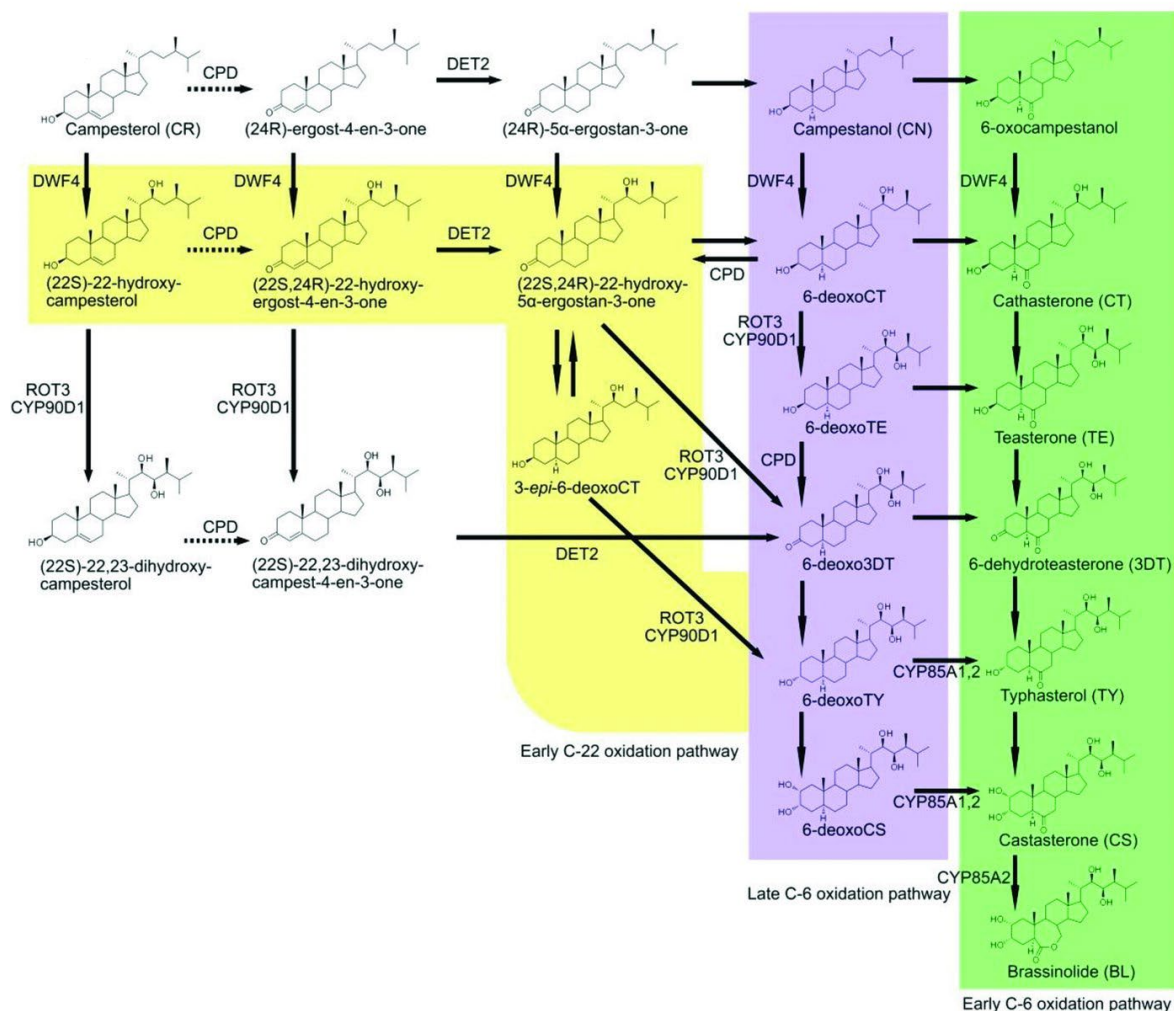


Figure 1.12. Illustration of networked pathways for brassinosteroid biosynthesis. Yellow, purple and green colours represent the early C-22, the late C-6 and the early C-6 oxidation pathways. CPD: constitutive photomorphogenesis and dwarfism; DET2: de-etiolated2; DWF4: dwarf 4; ROT3: rotundifolia 3. Adapted from Chung & Choe (2013).

BR signalling is mediated by the receptor kinase brassinosteroid-insensitive1 (BRI1). Once perceive by BRI1, there is an activation of the coreceptor kinase BRI1-associated receptor kinase1 (BAK1), that subsequently inactivates downstream the repressor brassinazole-insensitive2 (BIN2) through phosphorylation (Liao *et al.*, 2020). In response to BR, the transcription factors BRI1-ems-suppressor1 (BES1) and brassinazole-resistant1 (BZR1) are not phosphorylated and translocate to the nucleus to bind to BR-responsive gene promoters, by inducing or repressing the target gene expression (Wang *et al.*, 2012) (**Figure 1.13**).

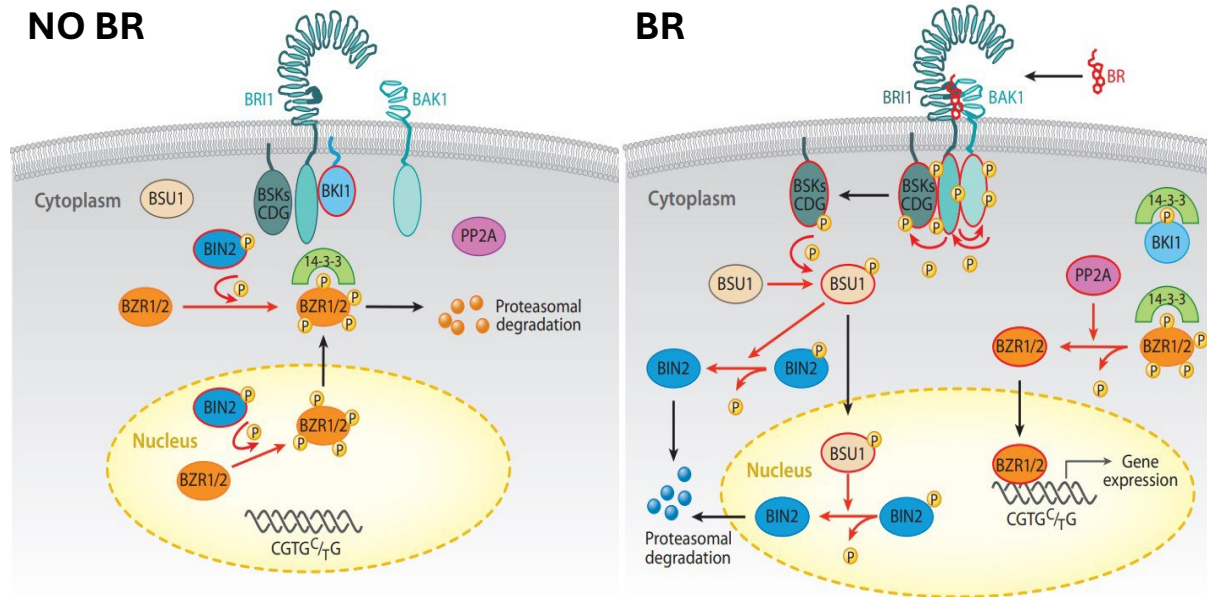


Figure 1.13. Schematic representation of BR signalling. BRI1: brassinosteroid insensitive 1 receptor; BAK1: brassinosteroid insensitive 1-associated receptor kinase 1; BSK: BR-signaling kinase; BSU1: BRI1-suppressor 1; CDG: constitutive differential growth kinase; BKI1: BRI1 kinase inhibitor 1; DWF: dwarf; PP2A: protein phosphatase 2A; BIN2: brassinosteroid insensitive 2; BZR: brassinazole resistant. From Wang *et al.* (2012).

BR signalling antagonizes JA-induced growth suppression (Wasternack, 2013). In *Arabidopsis*, the *partially suppressing coil* (*psc1*), which carries a mutation in the DWF4 protein, involved in BR biosynthesis, displays increased root growth suppression by JA (Huang *et al.*, 2010). Besides, in tomato, the negative impact of BR on JA signalling impacted JA-related antiherbivory traits such as trichome density and allochemical content (Campos *et al.*, 2009). Recently, Liao *et al.* (2020) found that BES1 repressed *PDF1.2a/PDF1.2b* expression and the JA-production of IG. In rice, it has also been shown that MeJA application suppresses the expression of the BR receptor BRI1 and co-receptor BAK1, and the biosynthetic DWF4 and CPD genes in a CO1 dependent manner (He *et al.*, 2017). However, Nolan *et al.* (2017) also indicates a probable positive regulation of JA by BZR1, as gain-of-function *bzr1-D* mutants also have shown induced expression of JA-inducible VSP genes against thrip feeding. Furthermore, it is shown that BRs can antagonize SA responses in rice, being proposed the use of BR by biotic stresses as a decoy to suppress SA signalling (De Vleeschauwer *et al.*, 2012).

1.4.4.2.4. Gibberellins

GAs are a broad class of tetracyclic diterpenoid compounds known to regulate plant growth and development, also participating in seed germination, root development, leaf expansion and reproductive flower and stamen development (Um *et al.*, 2018). However, due to their

involvement in the regulation of plant growth and productivity, variation in the levels of GAs are relevant for optimizing plant fitness. Therefore, GA control through interaction with other hormones such as JA and ABA are essential to regulate defence-growth trade-offs (Hou *et al.*, 2010; Yang *et al.*, 2012; Liu & Hou, 2018).

Among the 136 forms of GAs, there are only a few of them considered as biologically active GAs, including GA₁, GA₃, GA₄ and GA₇. Concretely, GA₁ and GA₄ are the most relevant biologically active compounds, showing higher abundance on plant tissues. The biosynthetic pathway starts from the geranylgeranyl diphosphate (GGDP), being transformed to the first C₂₀-GA compound of the route, GA₁₂, through the action of several enzymes: ent-copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurene oxidase (KO), ent-kaurenoic acid oxidase (KAO). Later, GA₁₂ can generate GA₅₃ through the GA 13-hydroxylase (GA13ox) by 13-hydroxylation, initiating two parallel routes of GA biosynthesis, the non-13-hydroxylation pathway and the 13-hydroxylation pathway. Action of GA 20-oxidases and GA 3-oxidases lead to the formation of the main active GA₄ and GA₁, while GA 2-oxidases lead to the formation of inactive GAs such as GA₅₁, GA₃₄, GA₂₉ and GA₈ (Hedden, 2020) (**Figure 1.14A**).

On the other hand, GA signalling is another key step controlling GA-responses. Binding of GA to its receptor GID1, promotes a conformational change that enables the interaction with DELLA proteins, which then, through association of an E3 ubiquitin ligase, are finally degraded in the proteasome (**Figure 1.14B**). The action of DELLA proteins as repressors of GA responses is essential in controlling GA homeostasis and are involved in plant growth suppression (Hedden, 2020). In Arabidopsis, there are five DELLAs: repressor of ga1-3 (RGA), gibberellin insensitive (GAI), RGA-like 1 (RGL1), RGL2 and RGL3. The proteolysis of DELLAs in the presence of GA leads to the release of GA-responsive factors such as phytochrome interacting factors (PIFs) in Arabidopsis and PIF-like (PIL) proteins in rice (*O. sativa*), triggering GA responses (Jang *et al.*, 2020).

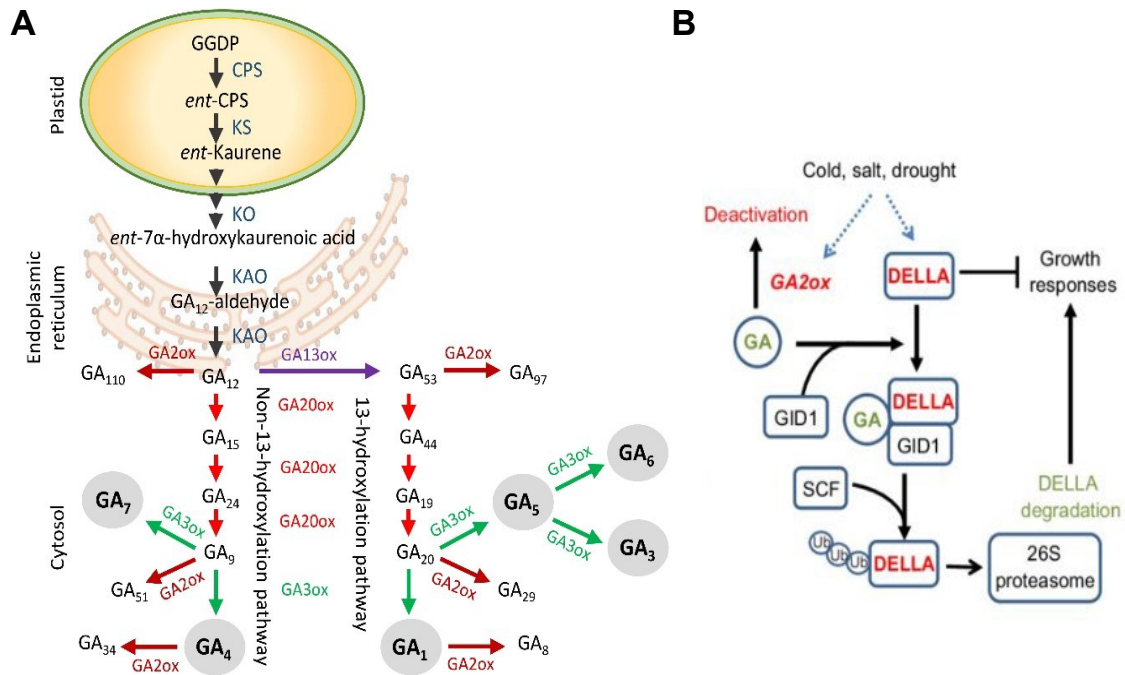


Figure 1.14. Generalized scheme of (A) gibberellin (GA) biosynthesis, deactivation and (B) signalling in higher plants. GGDP: geranylgeranyl diphosphate; CPS: *ent*-copalyl diphosphate synthase; KS: *ent*-kaurene synthase; KO: *ent*-kaurene oxidase; KAO: *ent*-kaurenoic acid oxidase. From Katyayini *et al.* (2020) and Colebrook *et al.* (2010).

Several studies have demonstrated the antagonism between JA and GA signalling pathways. Elevated levels of JA lead to a negative effect on the GA biosynthesis and signalling, resulting in growth inhibition and reduction of sugar accumulation (Heinrich *et al.*, 2013; Machado *et al.*, 2017). Wild *et al.* (2012) demonstrated that MYC2 directly binds to the promoter of *RGL3* gene, positive regulating the expression of this gene. Consequently, overexpression or mutation of the *RGL3* DELLA protein correlates with up- or down-regulation of MYC2, respectively. Additionally, DELLA repressors are also positive regulators of JA-mediated responses and direct interaction between DELLA and JAZ repressors has shown to be crucial for the defence-growth regulation (Yang *et al.*, 2012). DELLA can compete with MYC2 for binding to JAZ1, relieving MYC2 for JA signal activation. On the other hand, JAZ proteins also interact with DELLA proteins, allowing the release of PIF factors by DELLA and promoting GA-responses. Supporting this idea, the *Arabidopsis coi1* mutants and other JAZ overexpression lines showed enhanced growth, while *della* mutants are less sensitive to JA for growth inhibition (Yang *et al.*, 2012). In addition, Campos *et al.* (2016) demonstrated that this negative genetic relationship can be bypassed through dual inactivation in *A. thaliana* of five JAZ and phyB, a light photoreceptor that negatively regulates PIF factors, leading to phenotypes with enhanced growth and anti-insect defences. Interestingly, synergy between JA and GA has also been

described in anthocyanin biosynthesis and trichome formation in response to insect resistance (Gonzalez *et al.*, 2008; Zhao *et al.*, 2008). In these cases, transcription factors involved in these processes such as bHLH Glabra3 (GL3), enhancer of glabra3 (EGL3), transparent testa8 (TT8) and other MYB components, have been shown to be repressed by both JAZ and DELLA (Tian *et al.*, 2016).

In the case of the crosstalk between SA and GA, both synergism and antagonism responses have been observed. Huot *et al.* (2014) suggested that SA may increase DELLA stability, repressing GA responses. In fact, Gallego-Giraldo *et al.* (2011) showed that exogenous application of GA allowed growth recovery after loss of SA accumulation in plants with stunted growth and high SA levels. However, Emamverdian *et al.* (2020) reported synergetic roles between GA and SA in response to abiotic stress. In this line, exogenous applications of GA₃ and induction of GA-responsive genes lead to an upregulation of the biosynthesis and endogenous levels of SA, inducing plant tolerance under abiotic stress conditions.

1.4.4.3. The multitasking hormone ABA

ABA is a vital plant hormone classically involved in various aspects of plant growth and development, including seed dormancy, germination or senescence (Nguyen *et al.*, 2023). However, by regulating the expression of stress-responsive genes, the stomatal closure, and increasing the ROS production, ABA also modulates plant physiological and biochemical processes to enhance tolerance to biotic and mainly abiotic stresses such as water-limited conditions, salinity, cold, heat stress and wounding (Lata & Prasad, 2011; Bharath *et al.*, 2021; Mehra *et al.*, 2023).

The main ABA biosynthetic pathway in higher plants is the C₄₀ carotenoid indirect pathway. In this pathway, the precursor C₄₀ zeaxanthin is transformed into the C₁₅ xanthoxin in the plastids following a series of isomerization and cleavage reactions through the action of the zeaxanthin epoxidase (ZEP), an unknown isomerase and the enzyme 9-cis-epoxycarotenoid dioxygenase (NCED), considered to be the rate-limiting step in ABA biosynthesis. Once xanthoxin is generated, it can be converted into abscisic aldehyde and then into active ABA in the cytosol, through catalysis by the alcohol dehydrogenase (ABA2) and the abscisic aldehyde oxidase (AAO/ABA3), respectively. This active ABA, then, can be inactivated by reversible glycosylation to form ABA-glucose ester (ABA-GE) or by conversion to phaseic (PA) and dihydrophaseic acid (DPA), through the action of the cytochrome P450 monooxygenase (CYP707A) (**Figure 1.15A**). However, regarding its signalling, in the presence of ABA, ABA

receptors PYR/PYL/PCAR suffers a conformational change that allows their interaction with the type 2C protein phosphatases (PP2C). This interaction releases the subfamily 2 of the SNF1-related kinase (SnRK2), that is then phosphorylated by itself or other kinases and activates ABA responsive element binding proteins (AREB/ABFs) inducing ABA responses (**Figure 1.15B**).

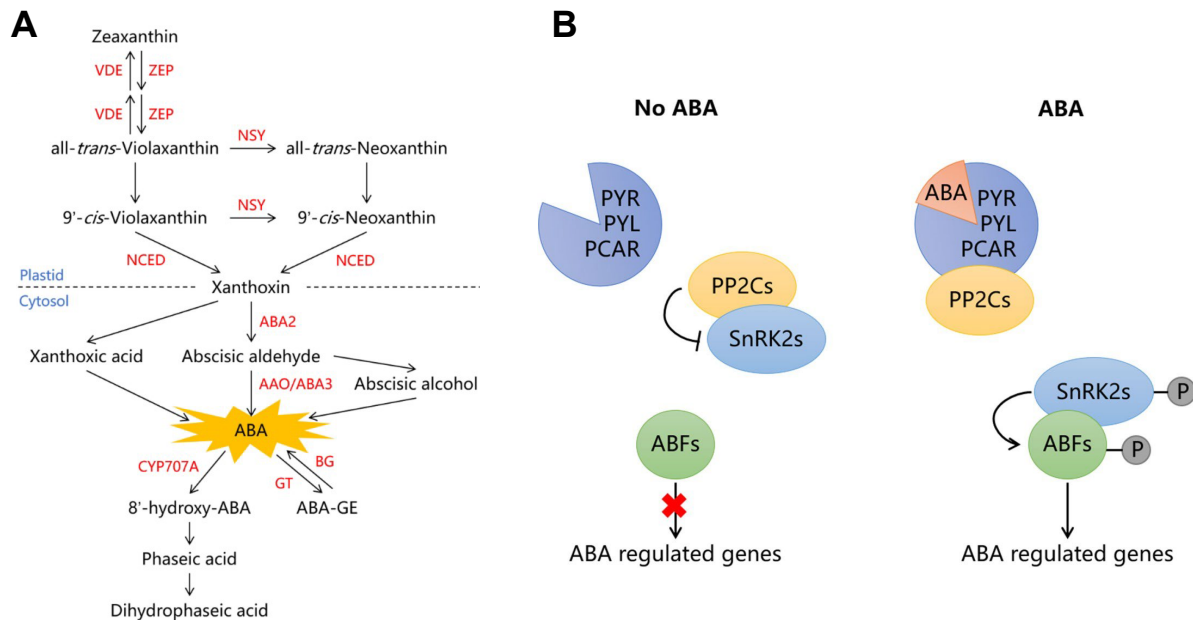


Figure 1.15. Simplified scheme of the (A) ABA metabolism and (B) signalling. VDE: violaxanthin de-epoxidase; ZEP: zeaxanthin epoxidase; NSY: neoxanthin synthase; NCED: enzyme 9-*cis*-epoxycarotenoid dioxygenase; AAO: absciscic aldehyde oxidase; GT: ABA glucosyltransferase; BG: β -glucosidase; ABA-GE: ABA-glucose Ester. From Wu *et al.* (2023).

Due to their involvement in defence, growth and developmental processes, ABA interaction with other plant hormones allows the fine-tuning of plant responses according to the different environmental cues.

Regarding the defence response, ABA show a variable interaction with JA and ET. ABA and JA act synergistically upon establishment of herbivore defence responses and inhibition of seed germination, and antagonistically in developmental processes (Yang *et al.*, 2019a). Jasmonate signalling involves the expression of PYL4, an ABA receptor (Lackman *et al.*, 2011). Conversely, the ABA receptor PYL6 directly interacts with MYC2 to negatively regulate its activity in an ABA dependent manner (Aleman *et al.*, 2016), suggesting a JA signalling dependent of ABA. Moreover, JAZ repressors also bind to the ABA positive transcription factors ABI3 and ABI5, in which JA induction produces JAZ degradation and subsequent activation of ABA signalling (Pan *et al.*, 2020). In the case of ABA interaction with ET, particularly, although ABA and ET generally act antagonistically regulating processes such as

seed germination (Ali *et al.*, 2022), they also act synergistically in inhibiting root growth under abiotic stress (Qin *et al.* 2019).

Ortiz-García *et al.* (2023) recently described the ABA-auxin antagonism in the regulation of cell division and expansion during abiotic stress responses. In the presence of a stress, the precursor of auxins, IAM, is accumulated with a reduction in the biosynthesis of the active IAA. This accumulation triggers the expression of the ABA biosynthetic gene *NCED3* and the negative growth regulator MYB74, also induced by ABA, leading to an enhanced tolerance to the abiotic stress with a repression on growth.

An additional negative regulation has been observed with CKs upon abiotic stress conditions (Zwack & Rashotte, 2015). ABA has shown to negatively regulate both the CK biosynthetic genes *IPT3* and *IPT8*, and the type-A *ARR4*, *ARR5* and *ARR6* transcription factors, leading to a reduction of the CK levels and altered CK signalling (Skubacz *et al.*, 2016). Similarly, the action of the CK signalling components, AHK4, AHP2, AHP3, AHP5 and ARR12, promote ABI5 degradation by proteasome, negatively regulating ABA and stimulating the greening of cotyledons (Guan *et al.*, 2014).

In the case of BRs, BRs and ABA regulate common sets of genes where ABA modulates BR responses (Nemhauser *et al.*, 2006). In rice, the ABA-inducible OsREM4.1, a membrane-anchored remorin protein, inhibits the BRI1–BAK1 receptor complex formation for BR perception (Nolan *et al.* 2017). In addition, the BR negative regulator BIN2 has been shown to stabilize ABI5 in an ABA dependent manner. On the other hand, BR also inhibits ABA signalling through repression of *ABI3* and *ABI5* expression by BES1 and BZR1 transcription factors (Nolan *et al.* 2017).

Finally, the control of the GA-ABA crosstalk is essential for inducing and maintaining seed dormancy, ensuring that seeds only germinate under favourable conditions. During germination, ABA levels decrease, allowing the GA to increase and promote of germination (Liu & Hou, 2018). This antagonism has also been shown during seed development, morphogenesis and maturation, where high ABA and low GA levels are observed during seed maturation. Recently, it has been described an association between DELLA repressors and ABI5, where a negative regulator of ABA signalling known as ICE1, has been shown to be also negative regulated by DELLA repressors (Hu *et al.*, 2019). Interestingly, all these repressors are targeted by JAZ proteins, being ABI5 a point of convergence between GA, ABA and JA.

(Gallego-Bartolomé *et al.*, 2012; Ben-Targem *et al.*, 2021). Previously, Hedden & Thomas, (2012) also described that gibberellins can enhance auxin transport, and Weiss & Ori (2007) enlightened the induction of GA by auxin and its biosynthetic repression by CKs, being CKs also negatively regulated by GA. Therefore, the interplay between all these hormones involves complex signalling networks where each hormone can modulate the sensitivity and signalling pathways of the others (**Figure 1.17A**). In contrast, Gilroy & Breen (2022) described the presence of essential hubs regulating hormonal crosstalks. The presence of a regulatory BAP module (BZR1–ARF6–PIF4 complex) coordinating cell proliferation, cell expansion, and differentiation underpins plant growth has been observed, where these individual transcription factors share significant overlap in the genes they regulate (Bouré *et al.*, 2019) (**Figure 1.17B**). Interestingly, under low GA, these transcription factors are inhibited by DELLAs, resulting in JA signalling (Gilroy & Breen, 2022).

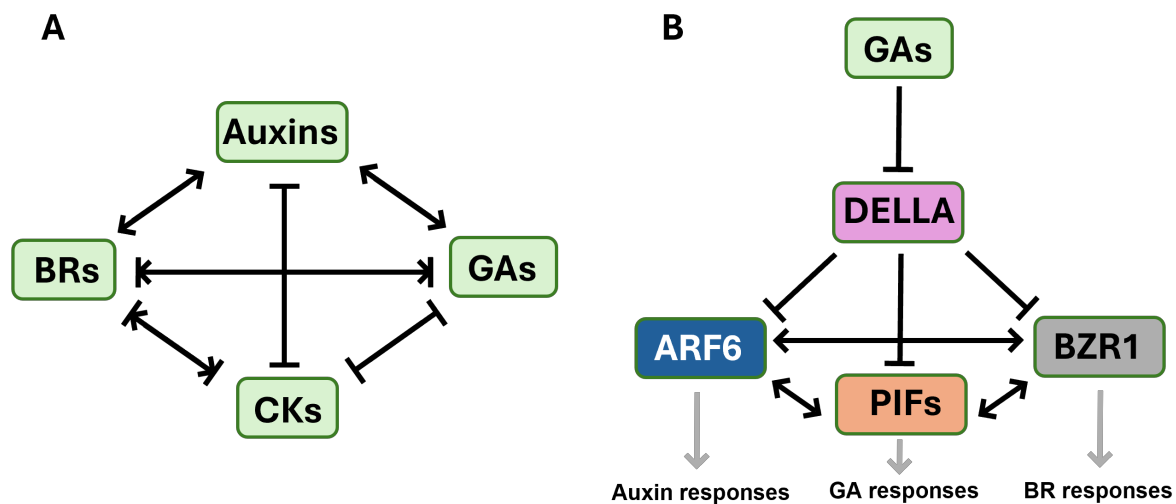
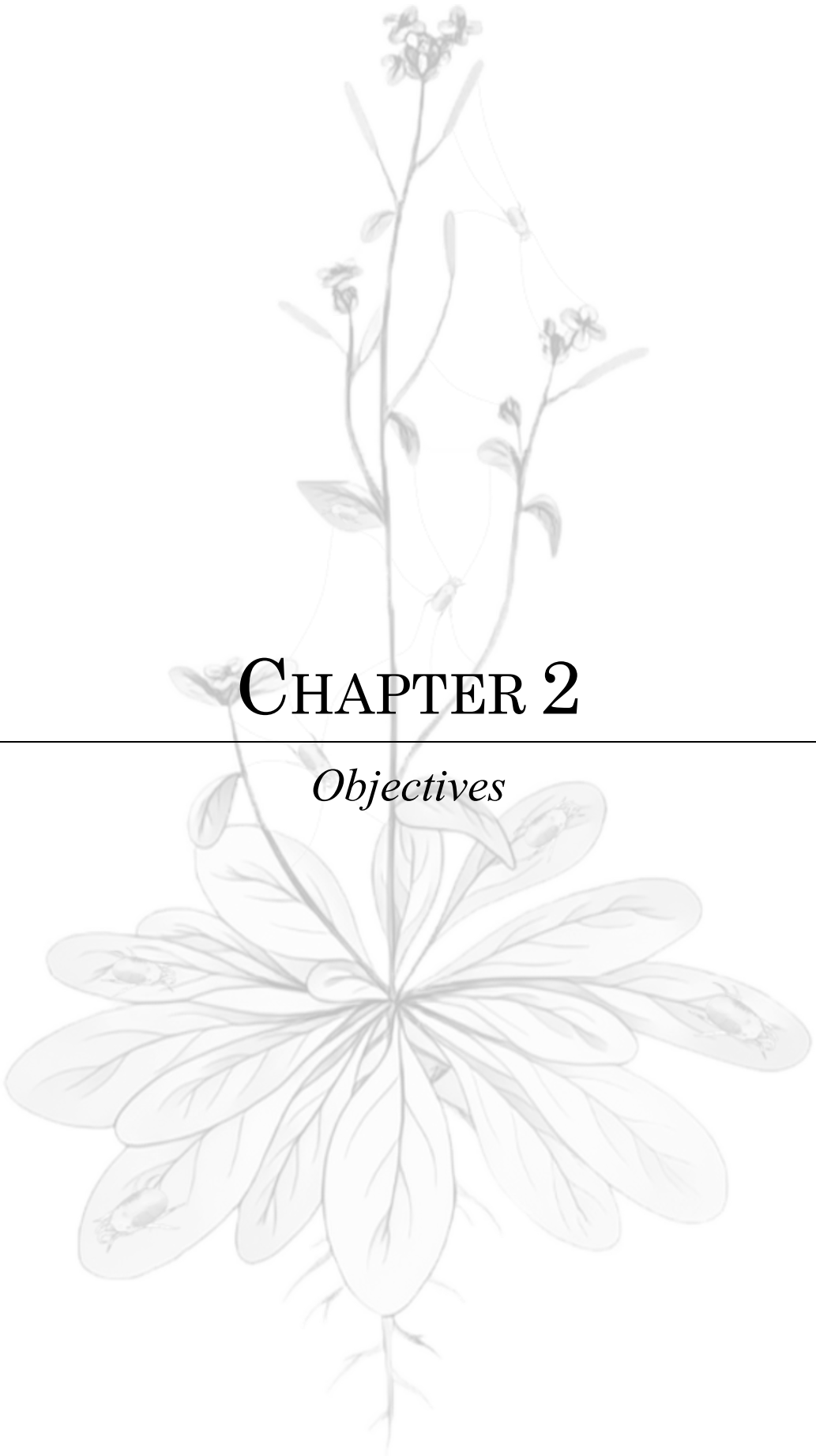


Figure 1.17. Schematic representation of the (A) general hormonal crosstalk between growth hormones (auxins, BRs, GAs and CKs) and (B) of the specific BAP module (BZR1–ARF6–PIF4 complex) and DELLA regulation. ARF6: auxin response factor 6; PIF: phytochrome interacting factor; BZR1: brassinazole resistant 1; GA: gibberellin; BRs: brassinosteroids; CK: cytokinins.

The balance between these hormones is crucial for adapting to environmental stresses, where changes in one hormone's levels can influence the overall growth and developmental outcomes (Emamverdian *et al.*, 2020). Understanding the crosstalk between BRs, CKs, GAs, and auxins is essential for advancing our knowledge of plant biology and improving agricultural practices through hormone manipulation.



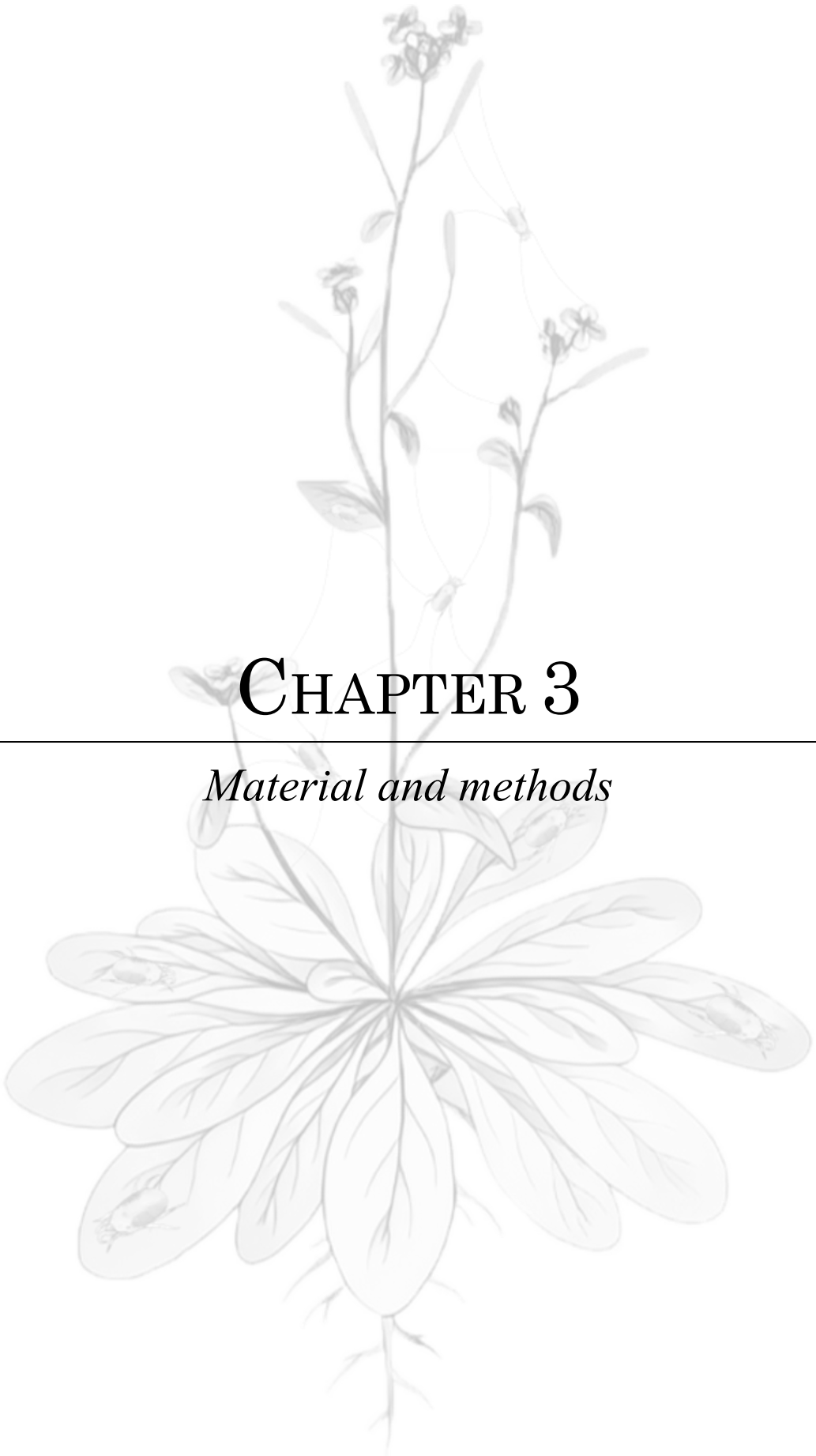
CHAPTER 2

Objectives

2. OBJECTIVES

During the last years, the knowledge on plant defence responses to *T. urticae* has been strengthened with the aim of generating resistant plants to this pest. However, enhanced resistance to the mite stress must be followed with an increased plant fitness in order to improve plant production upon stress. Therefore, based on the reduced number of studies covering this topic, this thesis has focused on the evaluation of the cost of the plant induced defences in the final plant fitness using the *A. thaliana* – *T. urticae* system, with the objective of generating new knowledge that could be implemented in the generation of new pest management strategies, as well as providing new insights in the defence-growth trade-offs established upon mite infestation on plants. Therefore, the main objectives of this thesis are:

1. To evaluate the impact of the defence induction on plant fitness and the main parameters influencing the final outcome in the defence-growth trade-off.
2. To analyse possible alterations in plant growth, development, and reproduction throughout plant life cycle due to spider mite infestation and its implications on plant fitness.
3. To identify key genes and/or pathways involved in establishing the defence-growth trade-off in *Arabidopsis* after red spider mite infestation.
4. To validate the transcriptional results through the quantification of metabolites and/or hormones identified as main regulators of the defence-growth trade-off.
5. To integrate all the data to understand the processes directing the *Arabidopsis* defence-growth upon mite infestation throughout plant life cycle and their impact on plant fitness.
6. To functionally characterise the impact on the plant defence-growth trade-off of mutant plants with altered levels or signalling of growth hormones.



CHAPTER 3

Material and methods

3. MATERIAL AND METHODS

3.1. Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh. cv. Columbia-0 accession (Col-0) was acquired from Nottingham Arabidopsis Seed Collection. *A. thaliana* plants from the accessions Graz (Gr-1; N1198), Shakh dara (Sha; N929), Canary Islands (Can-0; N1064) and Rubezhnoe-1 (Rub; N28718) were acquired from the ABRC (Arabidopsis Biological Resource Center). *A. thaliana* Heynh. Landsberg *erecta* (Ler) seeds were kindly provided by Dr. Lucia Jordá from the Centro de Biotecnología y Genómica de Plantas (UPM-INIA). *A. thaliana* Col-0 overexpressing *GA20OX1* line (*p35S:GA20OX1*), quintuple *ga2ox*, *ga20ox1/2* and *ga3ox1/2* mutant lines were kindly provided by Dr. Peter Hedden (Olomouc, Czech Republic). These lines were generated in Col-0 background and previously characterized by Coles *et al.* (1999), Mitchum *et al.* (2006), Rieu *et al.* (2008a; 2008b) and Plackett *et al.* (2012), respectively. *A. thaliana* Ler-0 quadruple mutant line *della4x* (*gai-t6 rga-t2 rgl1-1 rgl2-1*) containing homozygous transposon insertion mutations for *GAI*, *RGF*, *RGL1* and *RGL2* DELLA genes was kindly provided by Dr. Miguel A. Blázquez (IBMCP, Valencia). Overexpressing *RGL3* Ler-0 line (*p35S:RGL3-GFP*) and mutant *rgl3-5* Col-0 line (SALK_082546) were kindly provided by Patrick Achard (Strasbourg, France). Characterization of these lines was performed by Wild *et al.* (2012). For soil growth, a mixture of peat moss and vermiculite (3:2 v/v) was used. Sterilized seeds were stratified in the dark at 4°C for 5 d. Plants were then grown in growth chambers (Aralab D1200PLH) under control conditions (23°C ± 1°C, >60% relative humidity and a 16 h/8 h day/night photoperiod).

Solanum tuberosum L. cv. Désirée explants were obtained from *in vitro* propagated shoots kindly provided by Dr. Joaquín Medina from the Centro de Biotecnología y Genómica de Plantas (UPM-INIA). Shoots were proliferated on MS (Murashige & Skoog, 1962) media supplemented with 4.4 g/L vitamins, 0.5 g/L MES, 20 g/L sucrose, with a media pH of 5.8. Explants were grown in culture chambers under control conditions (21 °C ± 1 °C, > 60% relative humidity and a 16 h/8 h day/night photoperiod). After four weeks, plants were transplanted to soil and were grown in climate chambers (Panasonic MLR-352-PE) at 23 °C ± 1 °C, > 70% humidity and 16/8h day/night photoperiod.

Brassica napus L. RV31 seeds were kindly provided by Dr. Luis Oñate from the Centro de Biotecnología y Genómica de Plantas (UPM-INIA). Seeds were planted and incubated for 3 d

at 4 °C and plants were then grown in climate chambers (Aralab 18,000EH) under control conditions (23 °C $\pm$ 1 °C, > 60% relative humidity and a 16 h/8 h day/night photoperiod).

3.2. Spider mite maintenance

A colony of *T. urticae* Koch, London strain (Acari: Tetranychidae), provided by Dr. Miodrag Grbic (UWO, Canada) was reared on beans (*Phaseolus vulgaris*) and maintained on growth chambers (Sanyo MLR-350-H, Sanyo) at 25 °C $\pm$ 1 °C, > 70% relative humidity and a 16 h/8 h day/night photoperiod.

3.3. Spider mite infestation assays

Three-week-old Arabidopsis plants were infested with 20 *T. urticae* female adults per plant. Control plants without mites were carried out in parallel. Each plant was covered by a cylinder that enabled the correct growth and development of the plant while preventing the mites from escaping from infested plants to control plants. To evaluate the effects of the infestation on plants throughout the entire plant life cycle, plant material was analysed at specific ontological stages of the plant development, coinciding with 30 min, 24 h, 4 d, 7 d, 12 d and 15 d post-mite infestation (dpi) (**Figure 3.1**).

3.3.1. Leaf damage quantification

Quantification of plant damage after mite feeding was performed on Arabidopsis whole rosettes from selected transgenic lines (*p35S:GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2*, *p35S:RGL3-GFP*, *rgl3-5* and *della4x*) and non-transformed Col-0 and Ler-0 controls. Three-week-old plants were infested with 20 *T. urticae* adult females per plant. In general, after 4 d of mite feeding, leaf damage was assessed by scanning the entire rosette using a scanner (HP Scanjet 5590 Digital Flatbed Scanner series). In the case of the evaluation of the damage throughout plant life cycle, damage quantification was performed at the specific time points of evaluation previously described (**Figure 3.1**). Leaf damage was calculated in mm² using Ilastik and Fiji to calculate damaged area and Adobe Photoshop CS to calculate total rosette area (Ojeda *et al.*, 2020). Six to 12 replicates from independent plants were used for each genotype and time point.

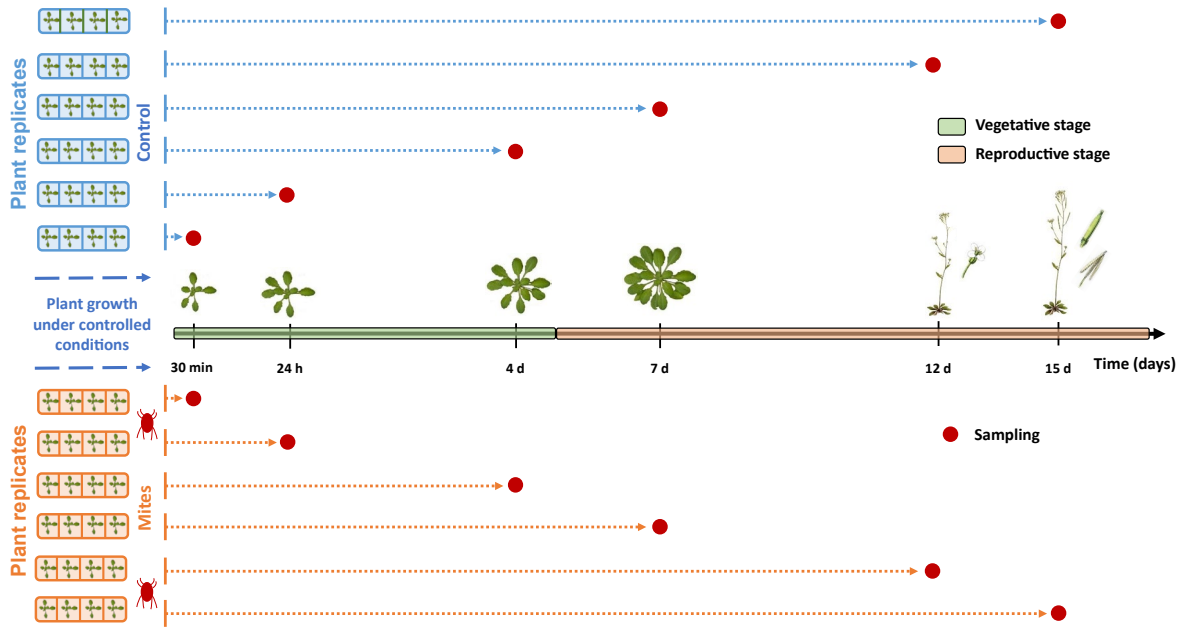


Figure 3.1. Experimental design for the evaluation of the *A. thaliana* - *T. urticae* interphase during the plant life cycle. Three-week-old Col-0 plants were infested or non-infested with 20 mites of *T. urticae* and collected at different time points of the mite infestation (30 min, 24 h, 4 d, 7 d, 12 d and 15 d). The timeline indicates the different times of infestation and the ontogenetic stage of the plants at the collection time of the samples. Green colour of the timeline indicates times of infestation where the plant is on its vegetative stage, while orange colour indicates reproductive stage. In the case of the samples collected for parameter evaluation, three biological replicates (n=5-12), were used. For RNA-seq analysis, four replicates of four plants for each time and treatment were collected. Finally, for hormonomics analysis, five biological replicates of four plants were used.

3.3.2. Fecundity assays

Mite performance was evaluated through evaluation of the mite fecundity. Mites were synchronized by inoculating 225 adult females (random age) on three leaves of bean confined in a closed system under water-soaked cotton. After 24 h, adult females were removed leaving only the eggs oviposited, and 10 d after, population on the leaf was synchronized. Spider mite fecundity was recorded on mutant and non-transformed Col-0 and *Ler-0* controls. *T. urticae* fecundity assay was performed on detached leaves from three-week-old plants. The newest emerged leaf (about 1 cm long) from each plant was placed in special dishes and infested with 12 synchronized adult females. After 36 h of infestation, the number of eggs was counted (Santamaría *et al.*, 2017). Twelve replicates were used for each plant genotype.

3.4. Plant physiological and biochemical characterization

3.4.1. Growth parameters evaluation

To evaluate the effect of the mite infestation in the plant growth, number of rosette leaves, rosette area, petiole length, plant height, number of branches of the primary inflorescence, plant biomass and water content were measured for Col-0 control and infested plants at 30 min, 24 h, 4 d, 7 d, 12 d and 15 dpi (**Figure 3.1**).

Non-destructive methods were followed for the quantification of number of rosette leaves, plant height and number of branches, using the same plants through the whole experiment without any tissue removal or damage. Twelve replicates coming from three independent experiments were used. In the case of rosette area and petiole length, these parameters were quantified from plant images. Entire rosettes were collected and scanned using a size reference on a HP Scanjet (HP Scanjet 5590 Digital Flatbed Scanner series). The scanning conditions and the area estimation protocol were established following Ojeda-Martinez *et al.* (2020). For petiole length evaluation, images were analysed using ImageJ (Abràmoff *et al.*, 2004). Petiole measurements were performed on the three longest leaves of each rosette and mean values were used as reference values for each rosette. Nine biological replicates coming from three independent experiments were used.

For biomass and water content quantification, aerial parts of the plants were collected, and fresh weight (FW) was recorded. Three replicates of four plants were used. The samples were then oven-dried at 70 °C for at least 48 h before dry weight (DW) was recorded. FW and DW per plant were calculated respectively. Water contents were estimated by using the formula: $(FW - DW)/DW$ (Wang *et al.*, 2016).

3.4.2. Reproductive parameters evaluation

3.4.2.1. Reproductive parameters measurements

Col-0 control and mite-infested plants were supervised throughout the whole plant development to verify floral button, first flower and first silique appearance. The number of primary and secondary inflorescences, flowers and siliques were counted daily, and plants were maintained until they could not produce more mature siliques.

To determine the effect of the mite on the plant host fitness, seeds from each plant were collected, and total seed weight, individual seed weight, seed number, number of seeds per silique, seed size and seed germination were analysed. Plants were covered with a paper bag when 70% of the siliques were dried. After seed harvesting, total seed weight was measured with a precision scale by weighting all the collected seeds from each plant. For seed weight, 200 seeds were counted and weighted in the precision scale. Seed number was estimated from total seed weight and individual seed weight. Seeds per silique data was extracted by dividing the total seed number by the total number of siliques produced by each plant. For the analysis of the seed size, 200 seeds were scanned using a size reference on a HP Scanjet (HP Scanjet 5590 Digital Flatbed Scanner series) with a white background employing the same scanning conditions used by Ojeda-Martinez *et al.* (2020). Images were then processed in ImageJ to extract the size of each of the seeds.

3.4.2.2. Germination assays

To characterize how mite infestation can affect seed longevity, germination assays were performed with two sets of seeds: three-week-old seeds and eighteen-month-old seeds, using the term 'aged' to refer to the older ones. Seeds were stored in vented Eppendorf tubes. Fifty seeds from each Col-0 control and mite-infested plant were directly plated onto Petri dishes containing 0.6% agarose medium. After stratification at 4 °C for 3 d, seeds were grown under controlled conditions (22 °C, > 60% relative humidity and a 16 h/8 h day/night photoperiod). Seeds were considered to have germinated when the seed coat was broken and the radicle was visible (Park *et al.*, 2009). Final germination proportion for each plant was calculated as the number of germinated seeds divided by the total number of seeds sown. Finally, the method used by Pagán *et al.* (2007) to estimate virulence has been adapted to calculate the negative effect of the mite infestation on plant fitness (NMF) using the following equation: $NMF = 1 - (SN_{mite} \times SG_{mite}) / (SN_{control} \times SG_{control})$. Data was obtained from 11 and 16 plants for control and infested conditions, respectively.

3.4.3. Trichome quantification

Trichomes were automatically quantified following Garcia *et al.* (2022) protocol. The same three leaves of each rosette were individually analysed with Ilastik v.1.3.3 (Sommer *et al.*, 2011). The leaf area of these three leaves was quantified with Adobe Photoshop CC 2018 v20.0 (Chen & Williams, 2006). The trichome density of a rosette was obtained by dividing the

number of trichomes of the three leaves by its area. Nine replicates coming from three different experiments were used. In addition, evaluation of the trichomes on *B. napus* and *S. tuberosum* was performed on four-week-old leaves, during the vegetative stage of the plant.

3.4.3.1. Image acquisition, processing and area estimation

Arabidopsis entire rosettes from three-week-old plants were collected and scanned (HP Scanjet 5590 Digital Flatbed Scanner series). A millimetre paper was used as a size reference (**Figure 3.2A**). The conditions optimized by Ojeda-Martinez *et al.* (2020) were used for plant scanning (black background, -10 brightness and 50 contrast). Black background was generated with the employment of a black cloth during the scanning process. All images were taken in Adobe RGB colour mode, with 1,200 dpi of resolution and saved as tiff files. Individual rosettes and/or leaves were separated from the original images (duplicated/renamed) using Adobe Photoshop program, retaining the size and resolution, and were saved as tiff files (**Figure 3.2B**). As adaxial epidermis contains more trichomes than the abaxial part in *Arabidopsis* (Bownman, 2012), for these analyses, only the adaxial epidermis of *Arabidopsis* was scanned for trichome identification (Pomeranz *et al.*, 2013). In addition, due to the close spatial distribution of the trichomes on the central part of the rosettes, trichome identification and quantification was evaluated exclusively on detached leaves. Concretely, upper surface of leaves 5, 6 and 7 from each rosette were selected (Handley *et al.*, 2005). The area of either rosettes or leaves was estimated using Adobe Photoshop CC 2021 v. 22.1.1.138 (Ojeda-Martinez *et al.*, 2020).

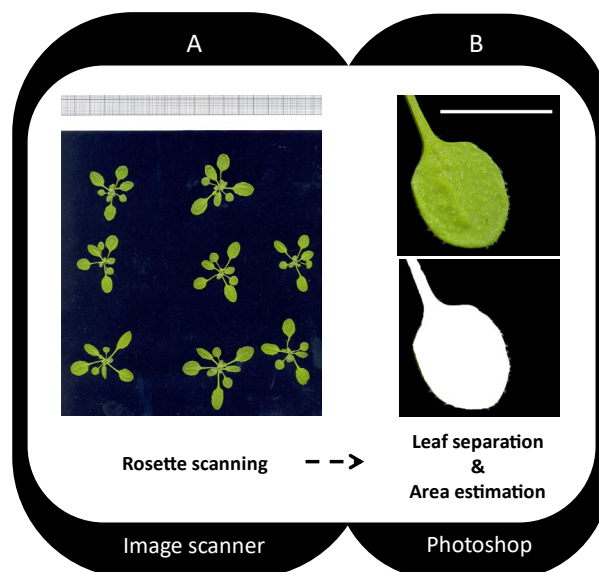


Figure 3.2. Scanning process and area estimation. A total of 24 rosettes were scanned in three different rounds of $n=8$ (A). Three leaves from each rosette were individualized and their area was calculated using Photoshop (B). White scale bars indicate 1 cm.

3.4.3.2. Trichome counting methods

For evaluation of the trichome counting methods, trichome number estimation was determined by both manual and automated approaches.

3.4.3.2.1. Manual counting

Manual counting was based on the visual identification of trichomes from each image. All rosette and leaf images were analysed using the “Cell Counter” plugin from Fiji (Schindelin *et al.*, 2012). Briefly, trichomes were identified by manually covering them using dots. Each dot corresponded to a unique trichome. The final number of dots represents the total number of trichomes in the image.

3.4.3.2.2. Automated counting

Automated trichome counting was performed using the Ilastik software v1.3.3 (Sommer *et al.*, 2011). Ilastik uses automated learning approaches to segment images and extract information from them. In all the methods selected, an Ilastik “pixel classification” approach was used as a first step to identify trichome structures. This approach allows the program to classify the pixels of the images in clusters of pixels identified as “trichome”, “leaf” or “background” areas, based on pixel features and user annotations. The training process was performed on each plant genotype and condition with the use of 44 different leaf 2D images to ensure a robust and trustful image analysis. During the training phase, pixels were manually labelled on each image as “trichome”, “leaf” or “background” areas. The main advantage of this software lies on its capacity of instantly model the unlabelled pixels of the images based on the information provided. This allows a high-speed evaluation of the classification processing of the program, using the unlabelled regions of the images as testing datasets, and a reliable correction of the incorrectly classified areas by a constant re-training process based on the increased amount of training labels where inaccurate classification is solved. These processes of correction and training were performed until no further improvement in performance was detected.

3.4.3.2.2.1 Automated approaches

Three fully automated approaches were considered based on the different nature of the initial images used and on the processing method selected.

Ilastik – Noisy background: Once the training process was accomplished, the images were opened in Ilastik (**Figure 3.3A**), batch segmented (**Figure 3.3B**) and exported to Fiji as jpeg images (**Figure 3.3C**). Consecutively, these new images were converted to black and white

images (**Figure 3.3D**) in Fiji. A noise filtering step was included to remove those residual pixels on the images that do not correspond to trichome structures (**Figure 3.3E**). The macros used were adapted from Ojeda-Martinez *et al.* (2020) and customized for the purpose of trichome detection and quantification.

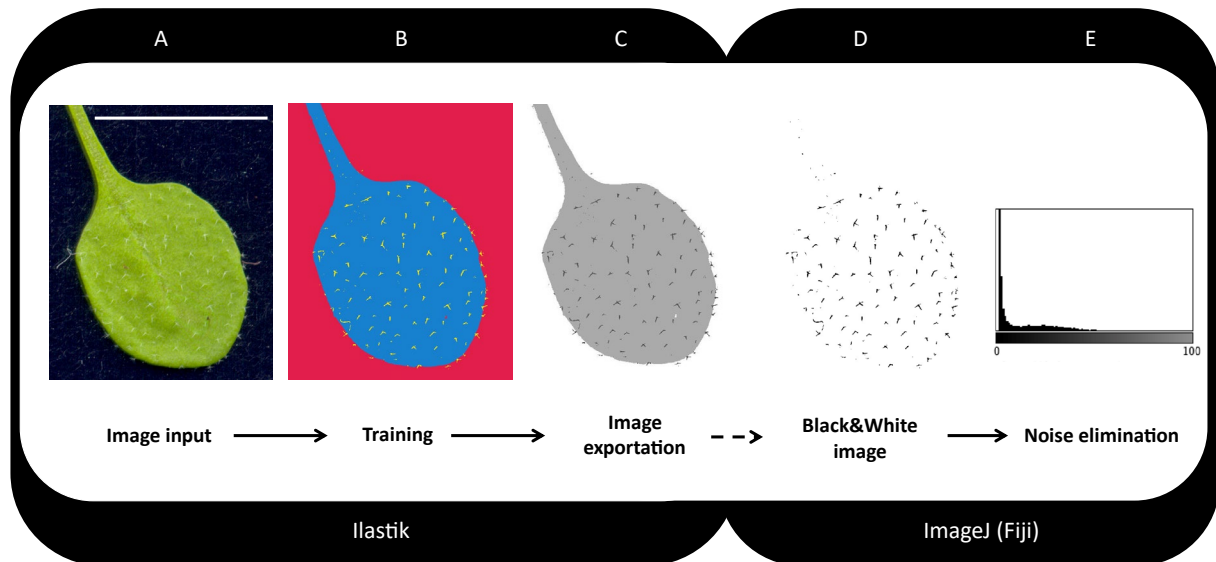


Figure 3.3. Flow chart of the trichome quantification procedure using the Ilastik (pixel classification) + Fiji approach with noisy background. A total of 24 rosettes were scanned in three different rounds of $n=8$ and a randomized sample of individual leaves ($n=44$) were imported to Ilastik (A) and used to train the program (B) to differentiate trichomes (yellow) from leaf tissue (blue) and background (red). All the images were processed, renormalized from [0,3] to [0,255] and exported (C). Then, they were imported to Fiji where trichomes are extracted and exported as black and white images (D). The number of trichomes was estimated for each leaf after filtering by a specific noise threshold (each group of pixels with a size above a specific threshold was considered a trichome) (E). White scale bars indicate 1 cm.

Ilastik – Black background: To avoid the presence of dust and background noise that could interfere with the Ilastik classification and Fiji analysis and quantification, scanned images were submitted to an additional pre-processing step. In this step, the background was manually cleaned by filling it with a black background using Adobe Photoshop. For this method, Ilastik “pixel classification” was trained using these pre-processed images. The rest of the quantification process and analysis was performed in an analogous way as in the *Noisy background* method. Same steps, workflow and macros were used to extract the final trichome number as they were specifically designed and customized for trichome number extraction from Ilastik and Fiji images.

Ilastik – Pixel + Object: In this method, classification was performed by adding an additional Ilastik “Object classification” step. Using the information from the training procedure of the “pixel classification”, images are opened in Ilastik in the same way as in the other methods, but after segmentation, they are exported as “probability maps” in jpeg format. A probability map

is a multichannel image containing the probability of each pixel to belong to a specific class. In this case, the probability of a pixel to belong to a “trichome”, “leaf” or “background” area. These new images are reopened in Ilastik besides the raw images and used as inputs in the “Object classification” procedure for batch processing. This additional step allows to perform a classification of the set of pixels previously classified as trichomes in the “pixel classification” process and discriminate between “trichome” and “non-trichome” objects based on size and shape features. Therefore, in this case, size filtering is directly performed in Ilastik. The training process was also performed with the use of 44 different leaf images, as performed in the other approaches, for the extraction of robust results. However, after this second classification, the information is exported as a table for each of the images analysed. In this table, each object is classified as “trichome” or “non-trichome” object. To avoid the manual counting of the number of times of “trichome” objects, a script for Windows users was designed to extract the total number of trichomes for each image.

A detailed protocol of the steps performed on each automated method for the trichome quantification in *Arabidopsis* is included in **Annex A**. The macros and scripts used for each method are also available as **Annex B**.

In all the tested methods, trichome densities were obtained by dividing the total number of trichomes on each leaf by the area of the leaf. Trichome densities of plants were compared by merging the trichomes and areas of the three different leaves from the same rosette as a unique value. Trichome densities obtained manually were used as standard values to validate the accuracy/reliability of the automated methods.

3.4.3.3. Method reliability

To test the method reliability, a comparison of their results with the manual method was performed on leaves of the Col-0 accession of *A. thaliana*. Lin’s Concordance Correlation Coefficients (CCC) were calculated to check the level of concordance and bias between different methods. Furthermore, to validate the performance of the final automated method selected, an evaluation of its accuracy, precision, concordance, agreement and bias was conducted with the comparison of the results obtained with the manual method through six different *A. thaliana* accessions with a diverse spectrum of trichome densities: *Ler*-0, *Gr*-1, *Col*-0, *Sha*, *Can*-0 and *Rub*-1 (Hauser *et al.*, 2001; Hilscher *et al.*, 2009). Agreement between the manual and the automated method was evaluated through Spearman or Pearson correlation coefficients depending on their data distribution. Bland-Altman (B&A) plots were used to

evaluate the concordance and bias between methods. Evaluation of the performance of the automated method was also individually performed on each *Arabidopsis* accession to demonstrate the reproducibility of the method on leaves with different trichome densities. Method reliability was also evaluated by comparing our results with those obtained by Hauser *et al.* (2001) and Hilscher *et al.* (2009).

3.4.4. Photosynthetic performance and anthocyanin determination

To check whether plants reconfigure their primary metabolism during plant development in response to mite feeding, the photosynthetic performance of the plants was subjected to evaluation. Quantum yield (Qy) and the continuous fluorescence (Ft) were estimated as indicators of the photosynthesis efficiency using a portable fluorometer (FluorPen FP 100, Photon Systems Instruments). Both parameters were measured twice on each plant. Eighteen plants from three independent experiments were used for each infestation time and treatment.

Anthocyanin content of rosette leaves was measured using Nakata *et al.* (2013) approach. Fifty milligrams of rosette leaves were grounded in a mortar with liquid nitrogen and resuspended in 1 ml of extraction buffer (45% methanol and 5% acetic acid) using photo-protected tubes. After removing cell debris by centrifugation at 12,000 g for 5 min twice (L344 Eppendorf 5810R centrifuge), the absorbance of the supernatant was measured at 530 and 637 nm using a UV-vis spectrophotometer (UltroSpec™ 3300pro, Amersham Bioscience). Nine replicates were used for each genotype and treatment. The anthocyanin content was determined using the equations of Laby *et al.* (2000) and Vieira *et al.* (2018).

3.4.5. Cuticle evaluation

Evaluation of the plant cuticles was performed on three-week-old plants from the genotypes Col-0, *Ler-0*, *rgl3-5* and *p35S:RGL3-GFP*. Plants were infested with 20 female mites for 4 d, and after that time, plant cuticle permeability and visualization under confocal microscope were performed.

3.4.5.1. Cuticle permeability test

For the evaluation of cuticle permeability, the toluidine test was carried out by placing a droplet of 10 µL of a 0.025% toluidine blue solution in ¼ PDB placed on the leaf surface. After 2 h of incubation leaves were washed gently with distilled water to remove excess of toluidine blue

solution from leaves. The same two leaves from each plant were used. Twelve replicates for each genotype were used.

3.4.5.2. Confocal imaging

From each genotype, the same leave of each plant was excised and subjected to subsequent washes with EtOH 100% and EtOH 70% for 1 h each to remove the chlorophyll from their tissues. Once removed, leaves were washed and stored in ClearSee solution. Twelve replicates for each genotype were used. Staining procedures were performed according to Ursache *et al.* (2018). Auramine O was used for staining cuticles and Calcofluor White for cell wall staining according to Buda *et al.* (2009). Plant leaves were stained in a 0.5% Auramine O solution for 12-16 h. After that, Auramine O solution was removed, and leaves were washed with ClearSee Solution until excess of staining solution is not observed. Then, the specimens were mounted in ClearSee solutions and observed using a Confocal Zeiss LSM 880 confocal microscope. Auramine O was excited under an Argon laser line of 458 nm, with the emission collected in the range 490–560 nm. 2D images and z-stacks were obtained using a scan rate of 400 Hz. Stacks of xyz and xzy series were analysed using the programs LAS X and Fiji. For the xzy series, xz planes (perpendicular to the leaf surface) were taken along the y-axis.

3.5. Hormone analysis

Quantification of jasmonates, salicylates, abscisates, cytokinins, auxins, giberrellins (GAs) and brassinosteroids (BRs) was performed according to previously published protocols (Urbanová *et al.*, 2013; Oklestkova *et al.*, 2017; Šimura *et al.*, 2018) with some modifications. First, samples (15 mg FW) were extracted in 1 ml of 60% (v/v) acetonitrile supplemented with a stable isotope internal standard mixture (Šimura *et al.*, 2018) using a Retsch MM400 bead mill (Retsch, Haan, Germany), sonicated for 3 min, incubated for 30 min at 4 °C and the centrifuged at 36,670 g for 10 min at 4 °C (Allegra 64R benchtop centrifuge, Beckman Coulter, Brea, CA, USA). After loading the supernatant onto an Oasis[®] HLB column (30 mg/1 ml, Waters, Milford, CT, USA), analytes were eluted with 0.5 ml of 60% (v/v) acetonitrile and 0.5 ml of 30% (v/v) acetonitrile. All combined fractions were then evaporated in a SpeedVac concentrator (RC1010 Centrivap Jouan, ThermoFisher, Waltham, MA, USA) and dissolved in 40 µl of 25% (v/v) acetonitrile. Second, for GA quantification, samples (10 mg FW) were extracted with 1 ml of ice-cold 80% acetonitrile containing 5% formic acid and a mixture of internal standards (purchased from OlChemIm, Olomouc, Czech Republic). Homogenates were centrifuged at

36,670 g and 4 °C for 10 min, and the corresponding supernatants were further purified using an Oasis[®] MAX SPE column (60 mg/3 ml; Waters). GAs were eluted with 0.2 M formic acid in acetonitrile (3 ml), which was evaporated to dryness in vacuo and reconstituted in 50 µl of 10% (v/v) methanol. Third, 10 mg FW used for BR profiling were extracted in ice-cold 60% acetonitrile and 25 pmol of deuterium-labelled internal standards of BRs (Olchemim s.r.o. Olomouc, Czech Republic) was added. After 12 h incubation, all extracts were centrifuged and supernatants were passed through 50 mg Discovery[™] DPA-6S cartridges (Supelco[®], Bellefonte, PA, USA), evaporated to dryness and then reconstructed in 40 µl of methanol.

All LC-MS/MS analyses was performed using an Acquity UPLC I-Class System coupled to a triple quadrupole mass spectrometer Xevo TQ-XS MS (Waters). Details for LC-MS/MS conditions were described by Šimura *et al.* (2018) for plant hormonomics, Urbanová *et al.* (2013) for GA quantification, and Oklestkova *et al.* (2017) and Tarkowská *et al.* (2016) for BR profiling. In all cases, the MassLynx[™] v4.2 software (Waters) was used for MS data acquisition and processing.

3.6. Gene expression analysis

3.6.1. Real-time qPCR

For real-time qPCR analysis, total RNA was extracted by the phenol/chloroform method, followed by precipitation with 8 M lithium chloride LiCl (Oñate-Sánchez & Vicente-Carbajosa, 2008). Complementary DNAs (cDNAs) were synthesized from 2 µg of RNA using the Revert Aid[™] H Minus First Strand cDNA Synthesis Kit (Fermentas) following manufacturer's instructions. RT-qPCR was performed using a LightCycler[®] 480 SYBR[®] Green I Master (Roche), a SYBR Green Detection System (Roche) and the LightCycler[®]480 Software release 1.5.0 SP4 (Roche). RT-qPCR was performed for three samples coming from three independent experiments as previously described (Santamaría *et al.*, 2017). mRNA quantification was expressed as relative expression levels ($2^{-\text{dCt}}$) (Livak & Schmittgen, 2001) using ubiquitin as a housekeeping control. Primer sequences used for these analyses are indicated in **Supplementary Table D.1**.

3.6.2. RNA-sequencing

For RNA-seq analysis, collection of plant samples was performed following the same experimental design for plant phenotyping experiments (**Figure 3.1**). Plant aerial parts were sampled and frozen and four biological replicates composed of pools of four plants were used for each time point and condition. Frozen material was grinded in liquid nitrogen and total RNA was isolated and purified using the RNeasy Qiagen Plant Mini Kit (Qiagen), including the DNase treatment (Qiagen), following manufacturer's instructions. Quality and integrity of RNA samples was checked and validated through Bioanalyzer 2100 system (Agilent Technologies) and electrophoresis on 1% agarose gels. Total RNA was sent to Novogene Europe. RNA libraries were generated using NEBNext Ultra RNA Library Prep Kit for Illumina according to manufactures's instructions. Libraries were sequenced on an Illumina platform generating paired-end reads fragments of 150 bp.

Raw data (reads) obtained as FASTQ files were processed to remove adapters, poly-N sequences and reads with low quality from raw data. Quality of the resulting data was assessed by the evaluation of Q20, Q30 and GC content (**Supplementary Table D.2**). Paired-end clean reads were mapped against the TAIR10 reference genome of *A. thaliana* using HISAT2 software (Kim *et al.*, 2015). Quality of the mapping can also be checked in **Supplementary Table D.3**. FeatureCounts was used for the generation of the count data files from sorted BAM files (Liao *et al.*, 2014). FPKM values were obtained, and Principal Component Analysis (PCA) was performed as a visualization tool for clustering the different experiment samples using the 'factoextra' R package (Kassambara & Mundt, 2017). Confidence ellipses with a confidence level of 0.95 were calculated to reveal distribution of overlapping or nonoverlapping groups in the PCA analysis. Differential expression analysis comparing treated against control samples at each time point was performed using the DESeq2 R package (Love *et al.*, 2014). *P*-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate (FDR). Differentially expressed genes (DEGs) were considered when *p*-value adjusted < 0.05 and $\log_2FC > |1|$ were present. Hierarchical clustering of the DEGs and the samples were conducted using the heatmap.2 function of the 'gplots' R package (Warnes *et al.*, 2016). UpSet plots were generated using the 'ComplexHeatmap' R package (Gu, 2018). Gene ontology (GO) enrichment analysis of the DEGs of the different clusters was implemented employing Metascape (Zhou *et al.*, 2019). A list of DEGs expression can be found in **Supplementary Dataset S3.1** (Garcia *et al.*, 2024 – DatasetS2 - <https://onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2Fppl.14479&file>

[=ppl14479-sup-0003-DatasetS2.xlsx](#)) . For DEG differentiation between biosynthetic and signalling processes within hormonal pathways, genes were classified according to its GO term and its involvement in the hormone metabolic process and signalling responses. The RNA-seq data that support the findings of the study are openly available in Gene Expression Omnibus (GEO) at <https://www.ncbi.nlm.nih.gov/geo/>, reference number GSE255811.

3.7. *In silico* analysis

3.7.1. Meta-analysis

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; Moher *et al.*, 2009) was used to design a systematic review protocol to compile information on the following questions: i) What are the effects of arthropod herbivory on plant growth, photosynthesis or reproduction?, ii) Are these effects dependent on the parameters used to estimate plant fitness? and iii) How important are the variables mediating plant-pest interaction in the final effects?

3.7.1.1. Compilation of the databases

A literature search was conducted to collect all relevant published data with no restriction of publication date related to the effect of arthropod herbivory on plant fitness. Selection of the experiments was performed via online databases such as Google Scholar or ScienceDirect by a combination of keywords searches including “plant defence”, “herbivory attack”, “herbivory impact”, “plant fitness”, “plant insect herbivore interactions”, “tolerance”, “growth-defence trade-off”, “growth impact”, “photosynthesis impact”, “reproduction costs” or “fitness costs”. In this first step, a title and abstract screening procedure was followed, excluding studies not contemplating outcomes related to fitness and surrogate parameters, studies with data related to other pathogens rather than herbivores, with specified artificially defoliation treatments instead of herbivore attack, and with a lack of proper control data. Additional studies were also retrieved by examining the bibliographies of the selected papers. Once selected, to be included in the analysis, a study had to satisfy the following criteria: (1) Experiments include an arthropod herbivory treatment affecting plant growth, photosynthesis, reproduction or other parameters related to plant fitness; (2) The herbivory treatment has an appropriate control, not being altered their response by the application of herbicides, insecticides or previous herbivory damage; (3) If additional treatments were present in the experiment, data were selected from

the control and herbivory treatment groups only; (4) The effects on plant fitness were measured immediately after herbivory removal with no long times after infestation measurements, not allowing the plants to recover from the stress; (5) The data required for the calculation of effect sizes (sample sizes, means, standard deviations, standard errors, *F*-test statistics or independent Student's *t*-tests) can be extracted from the article in either numerical or graphical form; (6) The study provides information about both control and treated plants, including the study design and their characteristics. Measurements from different parameters, plant or arthropod species, plant stage, levels of infestation or experimental durations within a single study were considered as distinct observations. Based on these criteria, experiments were excluded from the analysis if: (1) Fitness parameters were not included in the experimental set-up; (2) Herbivores included in the experiments were not arthropods but mammals or slugs; (3) Experiments were performed with artificial defoliation simulating herbivore damage instead of true direct phytophagous damage; (4) No control treatment was present in the experiment to compare the effect of the herbivore attack or control treatments were specified as plants with lower level of herbivory damage; (5) The effect of the fitness was measured weeks later after the herbivore feeding had taken place, being possible a recovery of the host plant; (6) Data available lack information for the extraction of the different effect sizes. In addition, one article was removed from the search because it was not possible to retrieve the full text of the paper.

3.7.1.2. Variable categorization

The retrieved studies reported measurements of plant responses that are directly related to plant fitness: plant growth, photosynthesis, reproduction and carbohydrate content. Plant fitness-related parameters were grouped for the analyses due to the low number of total studies retrieved. The final conformed groups and their individual variables in the database were: plant growth (leaf area, leaf length, number of leaves, plant height, plant biomass, relative growth rate (RGR), water content and branch production), photosynthesis (photosynthetic rate, stomatal conductance, transpiration, pigment content, chlorophyll fluorescence, efficiency of PSII and quantum yield), reproduction (days to first flower, number of flowers, flowering period, fruit production, fruit weight, fruit quality, pollen production and size, seed weight, seed production, yield) and carbohydrate content (glucose, fructose, sucrose, starch, sugar content).

We also collected several variables that might affect the plant responses to herbivory and could explain the differences in the plant fitness. These variables were: 1) Feeding guild. Arthropod

species were classified into different groups based on their feeding mode. This classification includes chewing, phloem-feeding, cell-content feeder, gall-forming and leafminer insects. 2) Plant host. Plants were classified into crops, herbaceous and woody plants. The division between crops and herbaceous was performed according to the main use of the different species. Plants commonly used in crop management in fields in which production is usually quantified were termed as crops, while wild plants commonly found in nature were termed as herbaceous plants. 3) Plant stage. Based on the literature and the life cycle of each plant, plants were also classified based on their ontogenetic stage. The classification includes the early vegetative stage (from the seedling to the middle phase of vegetative stage), late vegetative stage (from the middle phase of the vegetative stage to the first reproductive event) and reproductive stage (after first reproductive event). 4) Infestation length. The time of infestation was categorized into short- and long-term infestation time. Those experiments with an infestation length ranged between 0 and 10 d were classified as “short term”, while infestation lengths larger than 10 d were classified as “long term”. 5) Magnitude of infestation. Based on the type and amount of arthropod used in each experiment, division of the variable include light, medium or heavy infestation levels. This classification was based on the literature included in the experiment. In those papers where no specification of the infestation levels was included, classification was performed according to the information of related papers. A summary of the experiments with their effect size and classification in the different explanatory variables is provided in **Supplementary Dataset S3.2** (<https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2020.615122/full#supplementary-material> – Supplemental Data Sheet 2, Garcia *et al.*, 2021).

3.7.1.3. Data extraction

The meta-analysis was conducted using R 4.0.0 (R Core Team, 2020) and RStudio 1.1.463 software (RStudio Team, 2016). Effect sizes were calculated as Hedge’s g , the standardized mean difference (Gurevitch and Hedges, 2001) between the herbivore and control treatments by using the “esc” package (Lüdtke, 2018). Hedge’s g is a similar measure than Cohen’s d to calculate standardized mean differences, but it follows a different formula to calculate the pooled variance, controlling the slight bias in the small studies present in the Cohen’s d (Hedges, 1981). If means and standard deviations or errors needed for the calculation of effect sizes were only present in graphs, the plugin “Figure Calibration” in ImageJ, available at: http://www.astro.physik.uni-goettingen.de/~hessman/ImageJ/Figure_Calibration/, was used to

obtain data from plots (Hessman, 2009; Schneider *et al.*, 2012). In some studies, means and standard deviations were unavailable. In those cases, univariate statistics such as *F*-test statistics or independent *t*-tests were transformed into Hedge's *g* estimates when present. When samples sizes were specified as a range of possible sizes, the lowest number was employed.

The individual measurements previously described to be related to plant fitness were extracted in agreement with the following rules. If more than one cultivar from the same plant species were analysed in a single study, the most common host in nature for the herbivore was selected. If unspecified in the study, the susceptible one was selected over the resistant to estimate the real scope of the herbivory damage. However, in those cases where this resistance to the herbivore was not indicated, the data from all cultivars were included in the database, and aggregation of the effect into a single effect was calculated according to the "BHHR" procedure (Del Re, 2015). In those papers where varied length or magnitude of infestation were analysed, one item per category (short/long term or light/medium/heavy) was selected. If a variable was measured in different tissues, foliage measurements were selected. In the case of photosynthesis, measurements including systemic responses were preferred over local responses as the data are more comparable to those studies where whole plant responses are analysed. Finally, if the experiment was performed independently in two different years, data were selected randomly from one of them if the results were similar. When different, data was aggregated to include only one single effect. In the particular cases of the reproduction parameters of "days to first flower" and "flowering period", a negative effect was considered when the time period increases. In these cases, longer periods to reach the first reproductive stage or longer flowering periods were considered to have a negative effect on reproduction.

All the analyses were performed following the random-effects model for pooling the different effect sizes using the 'metagen' function of the 'meta' package (Balduzzi *et al.*, 2019). The random effects model assumes that, in addition to the sample error associated with each study, the true effect in each experiment will be influenced by several factors, including their characteristics, design and execution. Therefore, it is assumed that effects of individual studies deviate from the true intervention effect not only by sampling error, but also random variation. Assuming that in this meta-analysis sampling and random errors are likely to be important sources of variation, it was decided to follow this model. Once effect sizes were calculated, the experiments were divided into plant response parameters related to growth, photosynthesis, reproduction or carbohydrate content based on the individual variables analysed. The magnitude of the treatment was considered to be statistically significant when the 95%

confidence interval (CI) of the effect size did not overlap with 0 (Gurevitch and Hedges, 1993). Throughout the manuscript, the effect size and their confidence intervals as the mean effect size (Hedge's g) $\pm$ value to the 95% confidence interval limit (Hedge's $g \pm X.XX$) was reported. In the cases where mean effect sizes were significantly different from 0, fail-safe number (n_{fs}) was calculated using the weighted method of Rosenberg (2005). This number indicates the number of supplementary studies of null effect and mean weight needed to eliminate the significant effect. In addition, it was examined publication bias by performing the Egger's test of the intercept (Egger *et al.*, 1997) for testing funnel plot asymmetry. Publication bias was considered if Egger's test was significant. Finally, Duval & Tweedie's trim-and-fill procedure (Duval and Tweedie, 2000) was followed in the cases where fail-safe number and Egger's test fail to reject the presence of publication bias. This method is also based on the funnel plot symmetry/asymmetry and is used to estimate the actual effect size that would be present in an asymmetric funnel plot by imputing "missing" studies until symmetry is reached. Egger's tests were conducted by using the 'egger.test' function of the 'dmetar' package (Harrer *et al.*, 2019) while the trim-and-fill method was performed using the 'trimfill' function of the 'meta' package in R (Balduzzi *et al.*, 2019).

To detect the presence of low-quality studies of small sample sizes, outlier detection was performed using the 'dmetar' package (Harrer *et al.*, 2019). Several potential outliers were identified in the data related to the general parameters of growth, photosynthesis and reproduction. Their effects on the results were tested by removing them from the data and re-running analyses. However, obtained results were very similar for analyses conducted with and without the potential outliers. For this reason, these studies were included in the final dataset. Subgroups of explanatory variables mediating plant-pest interactions were also analysed individually to see if the presence of outliers altered their individual performance, showing high heterogeneity in each subgroup. Because of this reason, outliers on each subgroup were detected and removed to obtain more reliable effect sizes for the explanatory variables. To test whether effects on plant fitness in response to phytophagous differed among the explanatory variables discussed above (feeding guild, plant host, plant stage, infestation length and magnitude of infestation), studies were subdivided into corresponding groups, and between-group heterogeneity was examined using the χ^2 statistic Q_b (Gurevitch and Hedges, 2001).

3.7.2. Transcriptomic analysis

3.7.2.1. Database collection

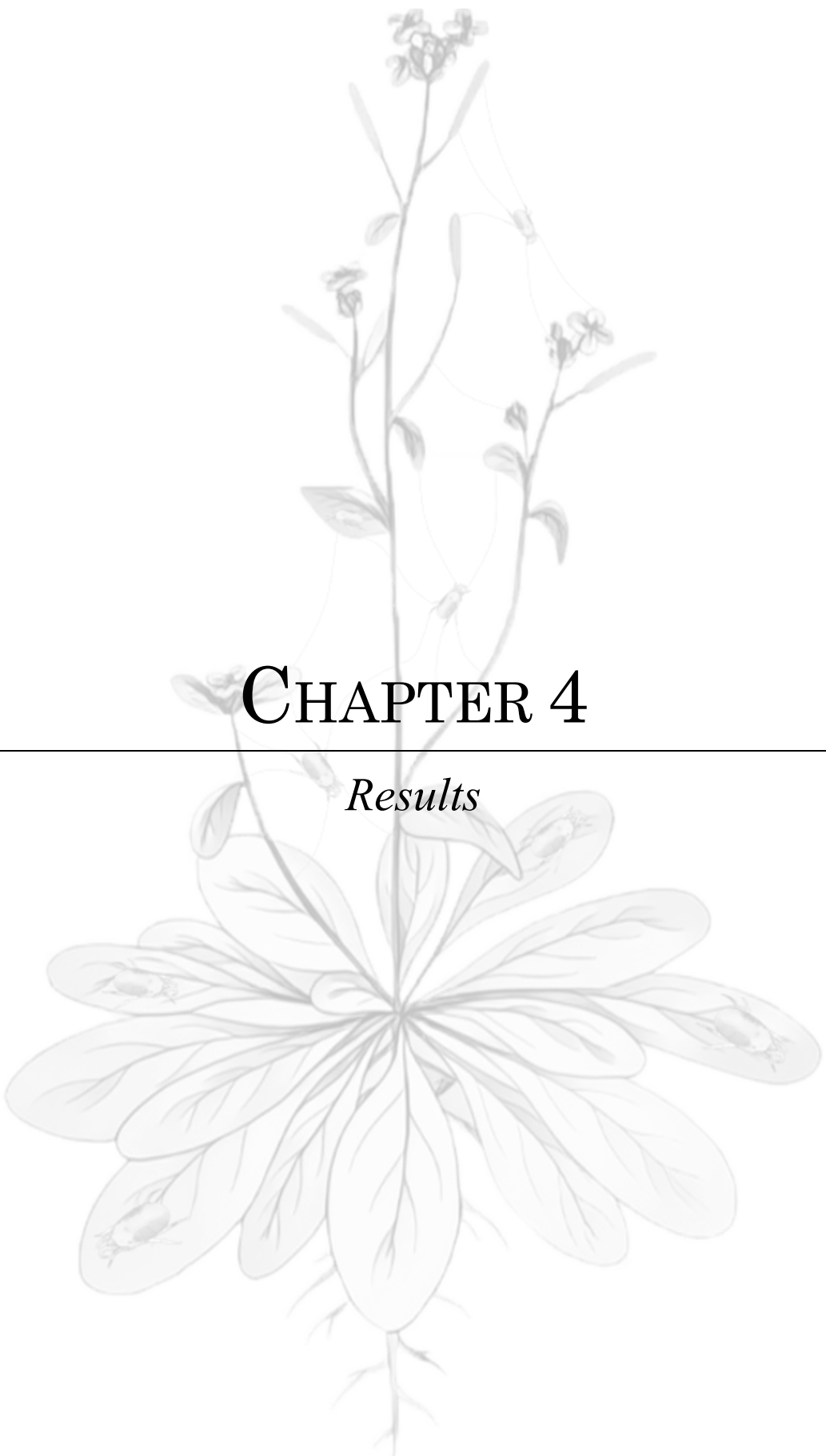
For the evaluation of the GA biosynthetic and signalling pathways, transcriptomic data from plant infestation assays with *T. urticae* were extracted from different public repositories such as ArrayExpress (<https://www.ebi.ac.uk/arrayexpress>), ExpressionAtlas (<http://www.ebi.ac.uk/gxa>) or National Center for Biotechnology Information Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) using “transcriptomics”, “*Tetranychus urticae*” or “plant stress” as keywords with no restrictions in the date of publication. Four additional studies were also included by searching in the literature using Google Scholar (<https://scholar.google.es/>) and Springer database (<https://link.springer.com/>). Transgenic genotypes were excluded from the analysis. Differentially expressed gene (DEG) lists from each experiment were extracted from processed data files without changes in the parameters used for publication.

To analyse the effect of the mite infestation on the GA biosynthetic pathway, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (Kanehisa and Goto, 2000) was used as reference to extract the different genes involved in this pathway across different plant species. Using *A. thaliana* as plant model species, orthologous genes from the different plant species were directly extracted from KEGG. To fully characterize the GA biosynthetic pathway across plant species, an additional gene search was performed on the different plant genome annotations of all the plant species included in the analysis. Genome annotated files from all plant species were downloaded from Phytozome genome database (Goodstein *et al.*, 2012). For *H. vulgare* at 7 d, *Manihot esculenta* at 5 d, and *Solanum lycopersicum* at 7 dpi. (Nachappa *et al.*, 2013; Santamaría *et al.*, 2018; Schimmel *et al.*, 2018; Yang *et al.*, 2019b), no data regarding GA biosynthetic and signalling pathways were recovered.

When gene annotation between KEGG and the published studies did not match, the corresponding gene annotation in a previous version of the genome was found using basic local alignment search tool (BLAST) (Altschul *et al.*, 1990). Protein sequences from the selected genes were extracted and aligned against all the protein sequences from the genome version used in the papers. Pheatmap R package was used for comparison of DEGs across experiments (Kolde, 2019). R software v.4.1.1 and RStudio v2022.02.0 + 433.pro2 software were used for the analyses (R Core Team, 2021; RStudio Team, 2022).

3.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8, the R software v4.1.1 (R Core Team, 2021) and RStudio v2023.9.0.463 software (Posit Team, 2023). Prior statistical analysis, data normality and homoscedasticity were tested, applying data transformations when needed. Shapiro tests were conducted to check the normality of data. Levene's tests were used to assess the presence of homogeneity of variance. For comparisons between two groups or between control and infested conditions at a specific time point, statistical analyses were performed using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. For comparison including more than two groups and one independent variable, non-normally distributed data were analysed using Kruskal Wallis followed by Dunn's tests with Benjamini-Hochberg *p*-value adjustment, while normally distributed data were analysed using One-way ANOVA followed by specific *post hoc* comparison depending of the characteristics of the data: Tukey's multiple comparison test for equal sample sizes, Bonferroni test for unequal samples sizes and Dunnett T3 tests for unequal variances and sample sizes. For comparisons including two independent variables, Two-way ANOVA followed by Tukey's multiple comparison test was performed. In the cases where data did not follow a normal distribution, R software v4.1.1 (R Core Team, 2021) was used to develop generalized linear models (GLM). A significance threshold of 0.05 was applied in all tests. A Chi-Square distribution test was used to analyse all the GLM models and differences were identified by the application of a pair-wise comparison analysis with Bonferroni correction. In addition, to analyse data correlation, Spearman or Pearson correlation tests were calculated according to the distribution of the data. In the case of the RNA-seq validation, correlation of the RT-qPCR data with RNA-seq results was performed by using the non-parametric Spearman rank correlation test. Finally, to assess the magnitude of the effect between two conditions, effect sizes were calculated using Hedge's *g* method (Gurevitch & Hedges, 2001).

A detailed botanical illustration of a plant, likely a species of Ranunculus. The plant features a basal rosette of numerous elongated, lanceolate leaves with prominent venation. A single, slender, upright stem rises from the center of the rosette, bearing several small, five-petaled flowers at the top. The illustration is rendered in a light, sketchy style with fine lines and subtle shading.

CHAPTER 4

Results

4. RESULTS

4.1. The price of the induced defences against pests: a meta-analysis

The evaluation of plant defence-growth trade-off process implies a complex interaction that depends on the parameters used to estimate the plant performance and, on the factors surrounding the interaction at the time of the infestation. All these factors modulate the final plant response and adaptability to the biotic stress and determine the resource allocation and final cost of the establishment of defences in the plant fitness.

To get a general perspective of the parameters used to analyse the cost of defences in the final plant fitness, a meta-analysis was performed with the compilation of a robust set of studies covering plant-arthropod interactions. A meta-analysis is a suitable option to combine and synthesize the results of multiple studies, and helps to identify patterns, differences and overall trends, providing a clearer and more comprehensive understanding of the research area. Due to the interest on the evaluation of defence-growth trade-off and their consequences on the final plant fitness, we conducted a search focusing on studies analysing the effect of arthropod pest infestations on plant fitness and surrogate parameters.

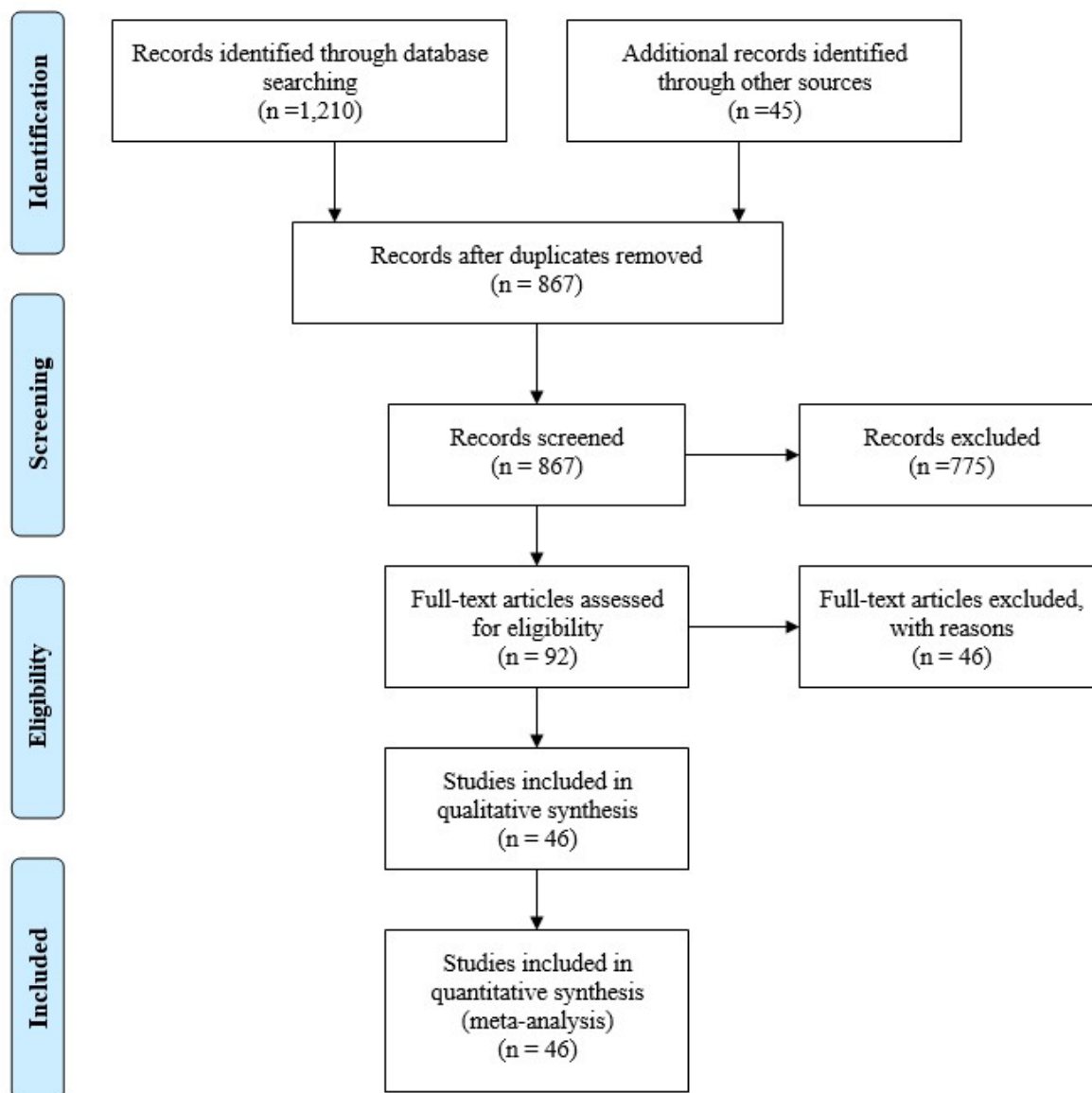
4.1.1. Meta-analysis data

After an initial collection process, 1,255 papers were subjected to evaluation, with 1,210 coming from searches in ScienceDirect or Google Scholar, and other 45 from cited literature of these papers. A PRISMA flow diagram with the screening process followed in this meta-analysis is depicted in **Figure 4.1**. After duplicate removal, 867 papers remained for abstract screening. However, only 92 studies of these 867 were identified as relevant articles to the meta-analysis, which after being carefully examined, a final number of 46 studies fitted our criteria and were included in the final meta-analysis. Within the excluded studies, 40% of them showed low quality data for the extraction of effect sizes or lacked the information required for its inclusion, 21% included artificial defoliations rather than arthropod infestations, 15% lacked an appropriate control for the analysis, 10% reported data measurements from plants weeks after the removal of arthropods from them, 8% did not include fitness related parameters on their data, and 6% were performed with mammalian herbivores rather than arthropod species. The final 46 studies contain a total number of 209 measurements of fitness and surrogate

parameters caused by direct herbivore damage, compiling observations of 46 different arthropod herbivores on 44 plant species. A list with the 46 studies used in the study is provided in **Supplementary Table D.4**.



PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses:

The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Figure 4.1. PRISMA flow diagram detailing screening process of articles included in analysis.

Among all studies, growth, photosynthesis and reproduction were the most prevalent processes analysed to estimate fitness costs, with 62, 88 and 42 measurements, respectively. Evaluation of parameters related to plant carbohydrate content were additionally included in the analysis, with a final number of 17 measurements. Regarding growth parameters, 34 of the 62 measurements evaluated plant biomass and relative growth rate, 18 included leaf parameters such as leaf number, area or size, and the other 10 quantified plant height, stem size, branch production or water content. On the other hand, evaluation of photosynthesis included a more balanced number of measurements among different parameters. Of the 88 measurements, 26 were related to quantification of the quantum yield (Qy) or the efficiency of the PSII, 24 measured the stomatal conductance, other 24 the photosynthetic rate, 11 included pigment and chlorophyll contents, and 3 measured the carbon exchange rate and the assimilation of CO₂. In the case of the reproduction, its evaluation showed a very heterogeneous group of parameters, including 18 measurements of seed weight or yield, 13 regarding the number and quality of the fruits, 7 quantifying the flower production or flowering time, and finally 2 evaluating pollen size and production.

However, in addition to these sub-group of parameters (growth, photosynthesis, reproduction and carbohydrate content related parameters), a deeper evaluation of the selected studies allowed us to determine possible explanatory variables modulating plant responses. The different variables mediating plant-pest interactions included in the analysis were: (1) the arthropod feeding guild (comprised by chewers, cell-content and phloem-feeders, gall-formers and leafminers), (2) the plant host (crops, herbaceous or woody plants), (3) the plant ontogenetic stage (composed by early and late vegetative stage, and reproductive stage), the length (short-term and long-term infestations) and (5) magnitude of the infestation (light, medium or heavy densities of arthropod infestation). These variables affected to the final plant response, enhancing or reducing the effect of the arthropod infestation on plant fitness, modifying the final effect size observed when evaluating growth, photosynthesis, reproduction or carbohydrate content related parameters (**Supplementary Dataset S3.2** - Supplemental Data Sheet 2, Garcia *et al.*, 2021b). In **Figure 4.2**, a summary of the main parameters and variables used to estimate the trade-off between the physiological processes implicated in the allocation of resources upon plant herbivore infestation is provided.

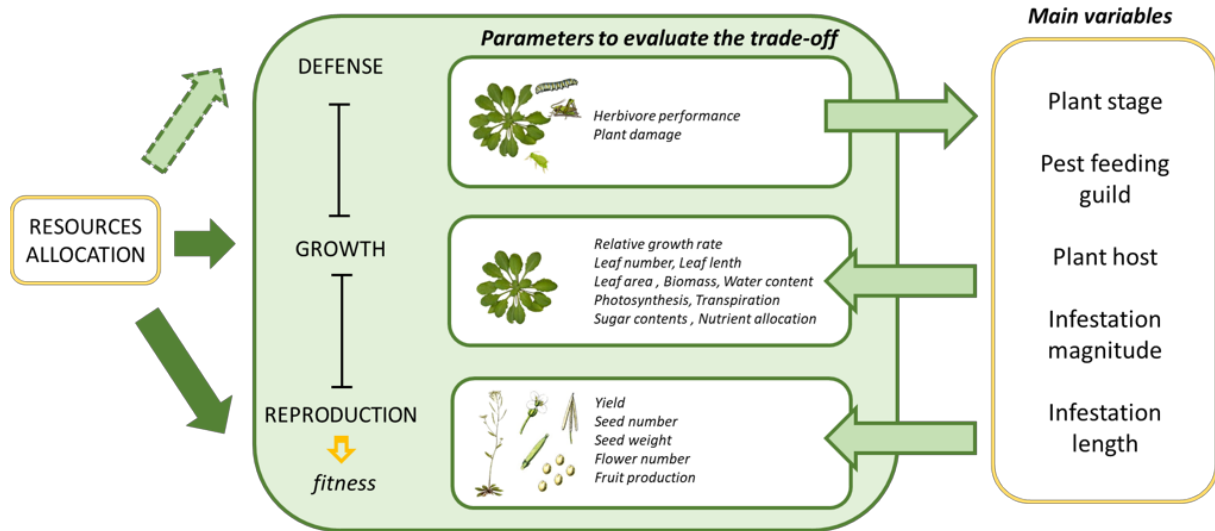


Figure 4.2. A diagram depicting the concept of growth defence trade-offs, the parameters to evaluate the fitness and the main variables involved. Resource allocation is related to different processes by arrows. Solid arrows refer to natural processes occurring in plants, while arrows with dashed lines refer to events in which resource allocation is altered by herbivore infestation.

4.1.2. Evaluation of growth, photosynthesis and reproduction parameters exerts negative effects on plant fitness upon arthropod infestation conditions

Evaluation of all the selected studies allowed us to calculate effect sizes and estimate the effect of pest infestation on plant fitness. According to this, a variable effect size on plant fitness was observed depending on the groups of parameters used to estimate it ($p < 0.05$). Negative effect sizes were detected when growth (Hedge's $g = -0.88 \pm 0.29$, $N = 62$), photosynthesis (Hedge's $g = -1.029 \pm 0.30$, $N = 88$) or reproduction (Hedge's $g = -0.83 \pm 0.30$, $N = 42$) parameters were evaluated (**Figure 4.3**). However, no significant effect sizes were observed with the evaluation of carbohydrate content parameters (Hedge's $g = -0.01 \pm 0.29$, $N = 17$), suggesting a higher variability in the data that is more dependent on the specific plant-pest interaction.

4.1.3. Effects on plant growth of the plant-pest interaction variables

Although general negative effects were reported on the evaluation of plant growth parameters upon pest infestations, differences on the negative effect on plant growth were observed in infestation with arthropods with different feeding guild ($p < 0.05$). While negative effects on plant growth were detected in infestations with phloem- (Hedge's $g = -1.36 \pm 0.34$, $N = 16$), chewing (Hedge's $g = -0.38 \pm 0.24$, $N = 17$) and cell-content feeders (Hedge's $g = -1.87 \pm$

0.67, $N = 11$), gall formers did not show a significant effect size on plant growth (Hedge's $g = 0.05 \pm 0.20$, $N = 7$) (**Figure 4.4A**).

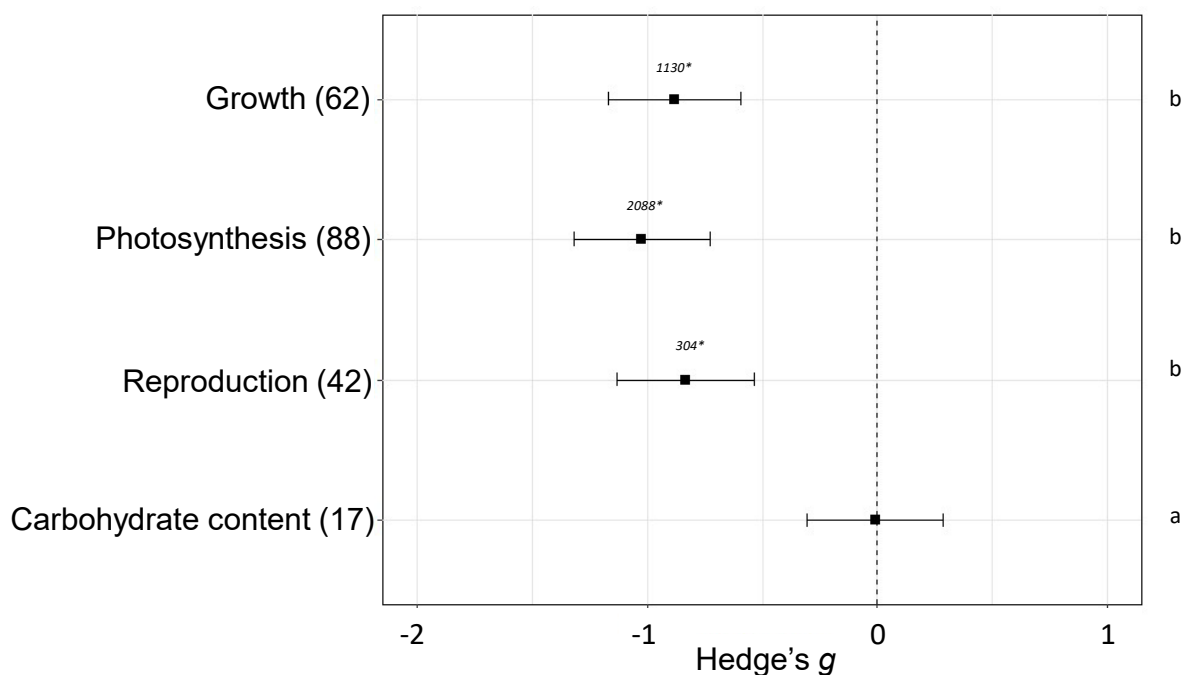


Figure 4.3. Effect sizes on general parameters related to fitness. Sample sizes are provided in brackets. Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's t -test. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number.

Interestingly, negative effects on plant growth were greater in those infestations performed with phloem and cell-content feeders rather than with chewing insects, probably suggesting that the deviation of resources from growth to other processes is greater in infestations with these types of arthropods than the one originated by chewing insects. In the case of the infestations on different plant hosts, although a general negative effect size was observed in all cases, a significant difference was found between the infestations on crops and on herbaceous plants ($p < 0.05$). The most negative effects were identified in crop infestations (Hedge's $g = -1.13 \pm 0.43$, $N = 19$), followed by those in woody (Hedge's $g = -0.60 \pm 0.47$, $N = 10$) and in herbaceous plants herbaceous (Hedge's $g = -0.38 \pm 0.25$, $N = 18$) (**Figure 4.4B**). On the other hand, only negative effects were observed on infestations performed during both the early (Hedge's $g = -0.87 \pm 0.24$, $N = 28$) and late vegetative stages of the plant (Hedge's $g = -1.54 \pm 0.71$, $N = 10$). Conversely, no effects were detected on infestations performed at the plant reproductive stage (Hedge's $g = -0.18 \pm 0.38$, $N = 9$) (**Figure 4.4C**). Finally, variations in the length and

magnitude of the infestations also impacted the final plant growth. In these cases, while no significant effects were identified for short-term infestations (Hedge's $g = -0.24 \pm 0.25$, $N = 10$) and light infestation levels (Hedge's $g = -0.24 \pm 0.35$, $N = 15$), significant negative effects were observed during long-term (Hedge's $g = -0.90 \pm 0.22$, $N = 33$) and medium (Hedge's $g = -0.55 \pm 0.24$, $N = 19$) and heavy infestations (Hedge's $g = -2.38 \pm 0.77$, $N = 11$) (**Figure 4.4D-E**).

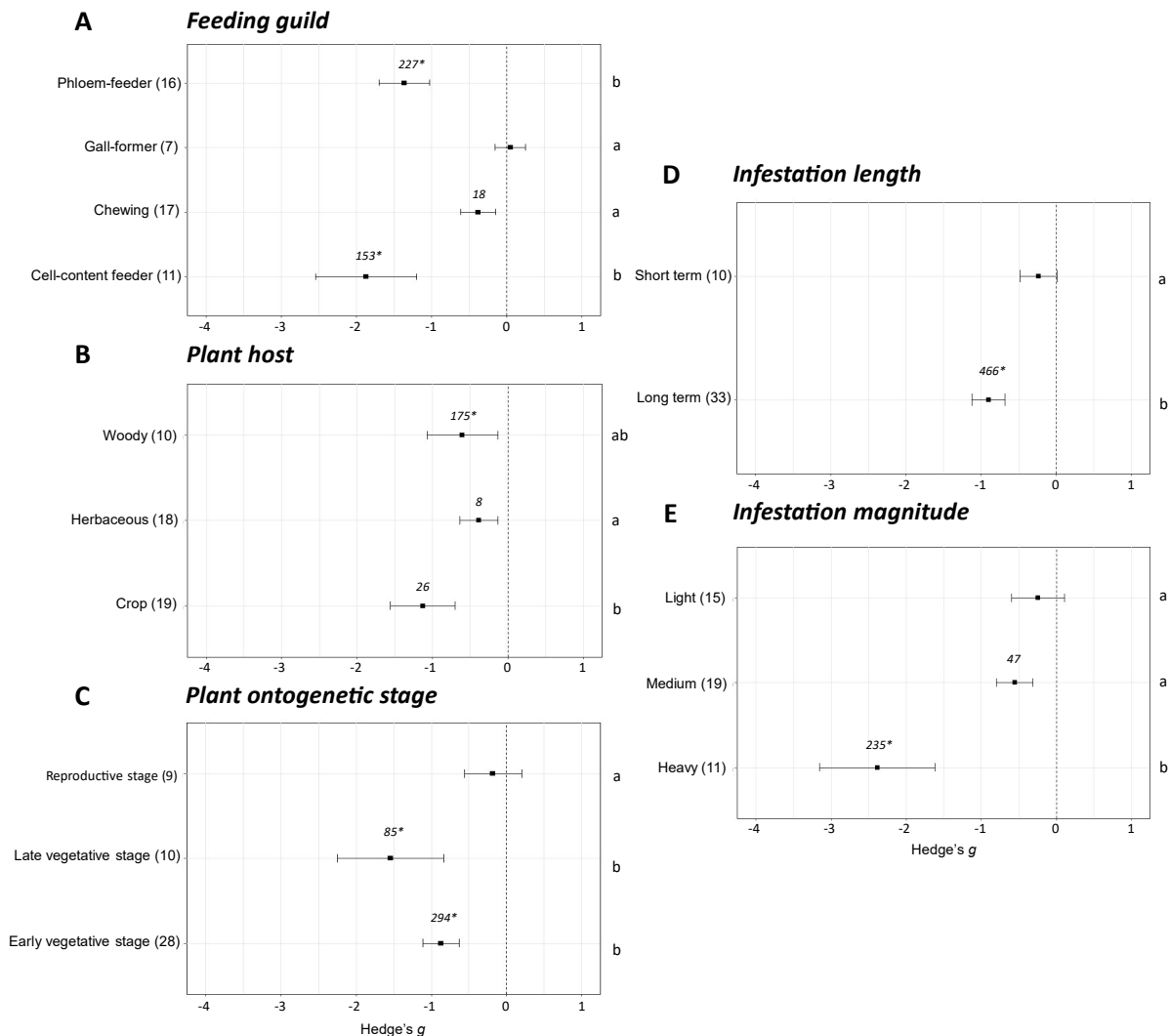


Figure 4.4. Growth effect sizes classified by subgroups. Fitness was analysed based in different parameters related to growth. Subgroups included feeding guild (**A**), type of plant host (**B**), plant ontogenetic stage (**C**), infestation length (**D**) and magnitude of infestation (**E**). Sample sizes are provided in brackets. Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's t -test. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenthal's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number.

4.1.4. Effects on plant photosynthesis of the plant-pest interaction variables

Photosynthetic parameters were also subjected to a thorough evaluation concerning the different subgroups of variables modifying the plant response. Using photosynthetic parameters, differences in plant fitness were found depending on the different arthropod feeding mode ($p > 0.05$). Interestingly, although a positive impact was observed in experiments performed with gall-formers (Hedge's $g = 0.65 \pm 0.58$, $N = 5$), its fail-safe number is 0, indicating that this effect cannot be distinguished from the null effect. In the rest of the arthropod feeding styles, a negative effect was found on plant photosynthesis, with leafminers (Hedge's $g = -3.81 \pm 1.10$, $N = 3$) and cell-content feeders (Hedge's $g = -1.64 \pm 0.52$, $N = 20$) showing the highest negative effects, followed by phloem feeders (Hedge's $g = -0.79 \pm 0.41$, $N = 28$) and chewing arthropods (Hedge's $g = -0.69 \pm 0.42$, $N = 14$) (**Figure 4.5A**). Concerning the different plant hosts, significantly different negative effects were observed in herbaceous (Hedge's $g = -1.87 \pm 0.64$, $N = 9$) and crop plants crops (Hedge's $g = -0.79 \pm 0.24$, $N = 49$), being the effects on herbaceous plants the most negative of all plant hosts ($p > 0.05$). Interestingly, no significant effects on photosynthesis were reported in infestations on woody plants (Hedge's $g = 0.14 \pm 0.65$, $N = 11$) (**Figure 4.5B**). In addition, plant photosynthesis was not affected in infestations performed at the plant reproductive stage (Hedge's $g = -0.88 \pm 0.91$, $N = 6$), showing a clear negative impact only when plants were infested at early (Hedge's $g = -1.13 \pm 0.30$, $N = 29$) and late vegetative stages (Hedge's $g = -0.97 \pm 0.48$, $N = 23$) (**Figure 4.5C**). Regarding the length of infestations, negative effects on photosynthesis were detected for both short-term (Hedge's $g = -0.87 \pm 0.27$, $N = 39$) and long-term infestations (Hedge's $g = -1.56 \pm 0.43$, $N = 23$), showing a higher negative effect in the last case ($p < 0.05$) (**Figure 4.5D**). Finally, the magnitude of the infestation also affected the final plant response ($p < 0.05$). While medium (Hedge's $g = -1.88 \pm 0.52$, $N = 17$) and heavy infestations (Hedge's $g = -1.14 \pm 0.44$, $N = 18$) induce a negative effect on photosynthesis, light infestations did not show any negative effects on photosynthesis (Hedge's $g = -0.26 \pm 0.24$, $N = 33$) (**Figure 4.5E**).

4.1.5. Effects on plant reproduction of the plant-pest interaction variables

In the case of plant reproduction, despite the number of studies was lower than for growth and photosynthesis evaluation, phytophagous infestations also induced a general negative effect

size in most of the variables evaluated (**Figure 4.6**). Regarding the different feeding guild, cell-content feeders (Hedge's $g = -1.23 \pm 0.54$, $N = 3$) and chewing insects (Hedge's $g = -0.72 \pm 0.25$, $N = 14$) showed a negative effect on plant reproduction (**Figure 4.6A**). This negative impact was also observed independently of the plant host infested, not founding significant differences among woody (Hedge's $g = -0.64 \pm 0.37$, $N = 7$), herbaceous (Hedge's $g = -0.57 \pm 0.32$, $N = 8$) and crop plants (Hedge's $g = -1.10 \pm 0.49$, $N = 15$) (**Figure 4.6B**).

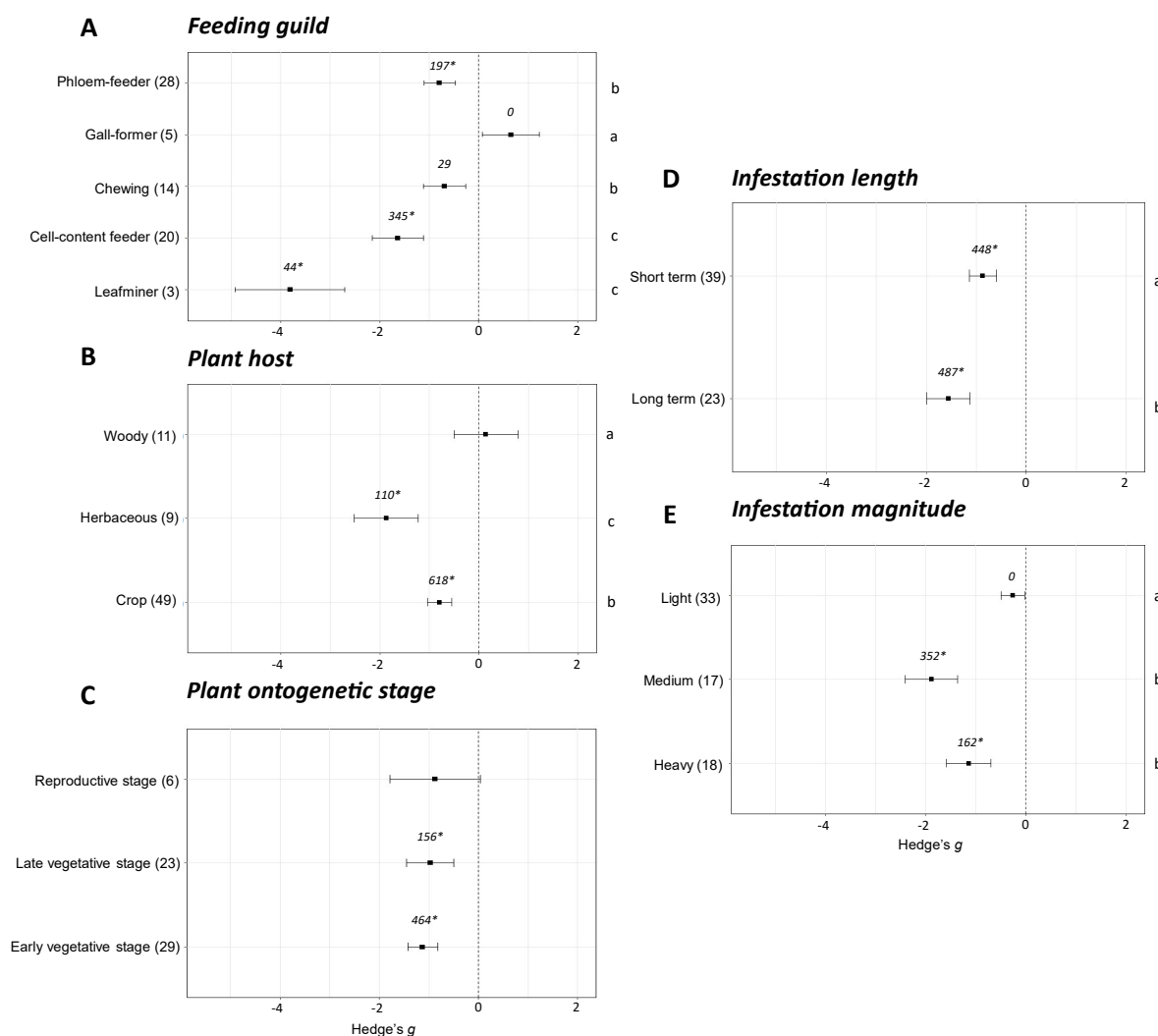


Figure 4.5. Photosynthesis effect sizes classified by subgroups. Fitness was analysed based in different parameters related to photosynthesis. Subgroups included feeding guild (**A**), type of plant host (**B**), plant ontogenetic stage (**C**), infestation length (**D**) and magnitude of infestation (**E**). Sample sizes are provided in brackets. Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. Different letters indicate significant differences between subgroups ($p < 0.05$). When comparing two groups, statistical analyses were performed using the non-parametric Mann-Whitney U test. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data followed by Bonferroni test, and Kruskal Wallis tests for non-normally distributed data followed by post-hoc Dunn's tests. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number.

Additionally, negative effects on reproduction were also found when evaluating the plant ontological stage and the infestation length. However, although negative, early vegetative (Hedge's $g = -0.74 \pm 0.31$, $N = 13$), late vegetative (Hedge's $g = -0.68 \pm 0.27$, $N = 16$) and reproductive stages (Hedge's $g = -2.32 \pm 1.39$, $N = 1$) did not show significant differences among them (**Figure 4.6C**). Nevertheless, the reproductive stage data cannot be used to infer effect sizes as it was composed by a unique experiment. Similarly, short-term (Hedge's $g = -0.65 \pm 0.42$, $N = 4$) and long-term infestations (Hedge's $g = -0.84 \pm 0.25$, $N = 22$) reported negative effects without significant difference between them (**Figure 4.6D**). Finally, concordantly with the analysis of the growth and photosynthetic parameters, while no effects were found in experiments with light infestation conditions (Hedge's $g = -0.40 \pm 0.58$, $N = 6$), a negative effect was observed when medium (Hedge's $g = -0.74 \pm 0.23$, $N = 17$) and heavy infestations conditions (Hedge's $g = -1.63 \pm 0.90$, $N = 11$) were present (**Figure 4.6E**).

4.1.6. Risk bias assessment

Publication bias is a critical issue in meta-analysis that needs to be addressed to extract trustful and robust conclusions. As significant effects are more likely to be published than non-significant ones (Borenstein *et al.*, 2021), there is a probable scenario of overestimating effects. For this reason, the generation of a final database with high quality data is essential for the extraction of robust and valid conclusions.

In this study, the risk of including overestimating effects in the analysis was determined by the calculation of Rosenberg's fail-safe numbers and Egger's tests. Significant Egger's test suggests the presence of publication bias. However, fail-safe numbers allowed to reject his assumption when their value was higher than $5n + 10$, being n the number of studies included in the analysis, which is considered a robust value against publication bias (Rosenthal, 1991; Rosenberg, 2005). Additionally, in the cases where sample size was small ($n < 10$), p -values from Egger's tests were not considered as it may lack the statistical power to detect bias. Evaluation of the data allowed to reject the presence of publication bias in most cases, suggesting a non-overestimation of the effects. Nevertheless, when publication bias was not rejected through these methods, Duval & Tweedie's trim-and-fill procedure was performed, in which effect sizes are recalculated for estimating the true effect size without the presence of publication bias (Duval & Tweedie, 2000). The fail-safe numbers, Egger's test values and groups where effect sizes were recalculated by the trim-and-fill method are provided in **Supplementary Table D.5**.

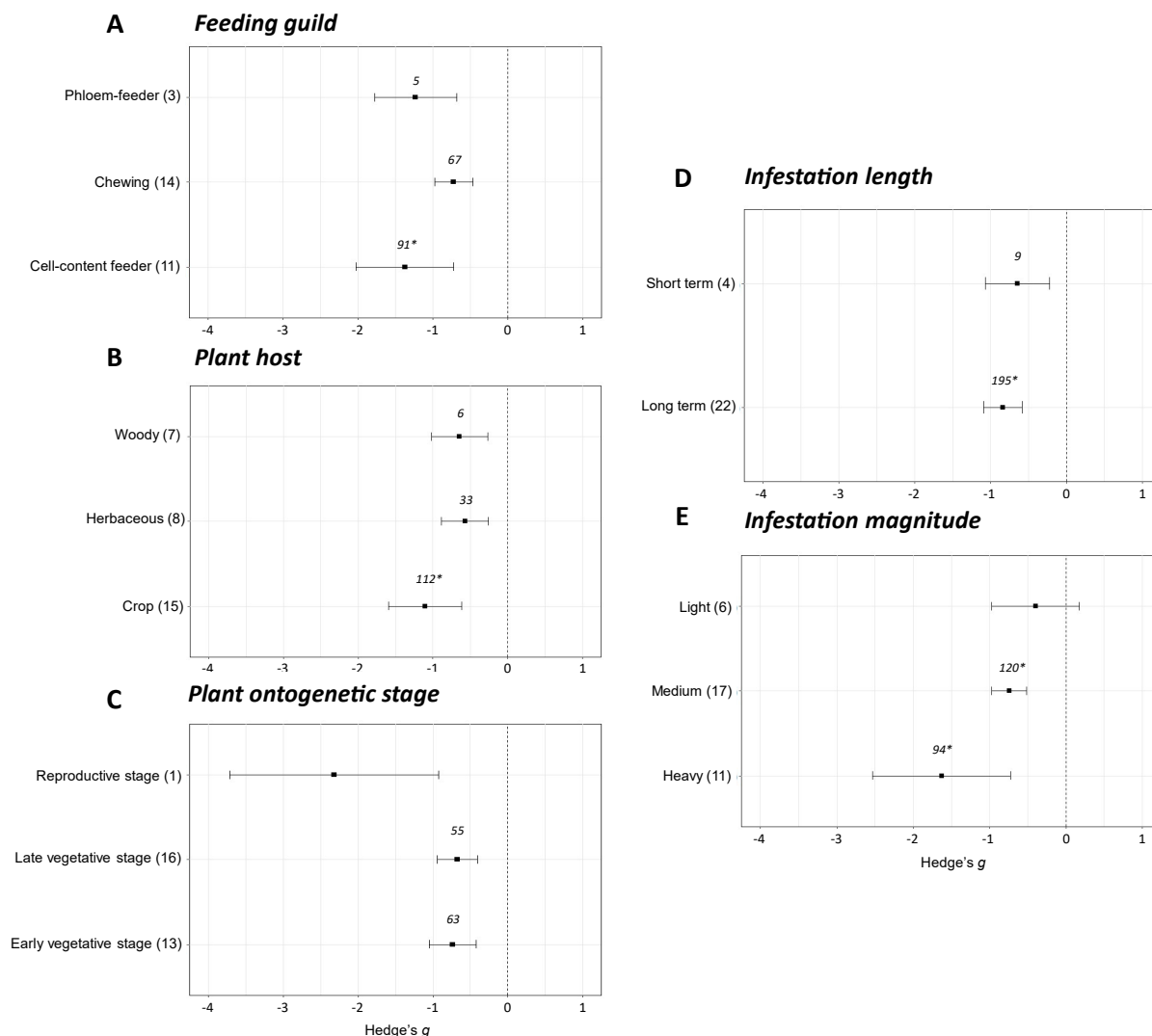


Figure 4.6. Reproduction effect sizes classified by subgroups. Fitness was analysed based in different parameters related to reproduction. Subgroups included feeding guild (**A**), type of plant host (**B**), plant ontogenetic stage (**C**), infestation length (**D**) and magnitude of infestation (**E**). Symbols specify mean values of Hedge's g with their 95% CI. Negative values indicate a higher negative effect in fitness on attacked plants than control plants. No statistical differences are present between subgroups ($p > 0.05$). When comparing two groups, statistical analyses were performed using the parametric Student's t -test with Welch's correction for unequal variances. If more than two groups were compared, statistical analysis were performed using One-way ANOVA for normally distributed data. Rosenberg's fail-safe numbers are reported in italics. An asterisk indicates a significant fail-safe number.

4.2. An automatic method to quantify trichomes in *A. thaliana*

As part of the analysis of the plant growth and development, a phenotypic evaluation of the *Arabidopsis* rosettes under control and spider mite infestation conditions was performed. Particularly, the analysis of defensive traits such as trichomes provided information about the establishment of plant defences in response to the spider mite. With this aim, a new method for

trichome quantification was developed, utilizing a basic scanner (HP Scanjet 5590 Digital Flatbed Scanner series) and machine learning to automate the process in an efficient and accurate manner. The method aimed to be fast, user-friendly, automated and accessible without the need for advanced equipment or pre-treatment of samples.

4.2.1. Scanner trichome imaging and selection of experimental conditions

Being a model organism of study, *A. thaliana* plants were employed for the development of the trichome quantification method, requiring only a basic scanner (HP Scanjet 5590 Digital Flatbed Scanner series) to generate 2D images. The unicellular non-glandular trichomes in *Arabidopsis* serve as a perfect model for studying the trichome development in plants. Trichome quantification was exclusively performed on the adaxial side of the leaves, where natural variation in trichome distribution is observed.

Using Ojeda-Martínez *et al.* (2020) scanning conditions, whole *Arabidopsis* rosettes were processed, and generated images were analysed both manually (**Figure 4.7A-B**) and through machine learning approaches (**Figure 4.7C-D**). Nevertheless, due to the dense spatial distribution of trichomes in the centre of the rosette, an accurate trichome quantification was not achievable. When performing manual quantification, there were inconsistencies across repeated trichome counts of the same rosette with the inclusion of this central part of the rosette, even when these counts were performed by the same researcher (**Figure 4.7A**). Automated quantification was also not able to solve this problem, producing images where overlapping trichomes cannot be distinguishable and differentiated in separated trichomes (**Figure 4.7C**). For these reasons, to be able to obtain robust results, the central part of the rosettes was removed for the trichome analysis, performing the evaluation on detached leaves of the main rosette. Based on the plant ontogenetic stage in the moment of the analysis, the same three leaves of each rosette (leaves 5, 6 and 7) were subjected to trichome quantification (Handley *et al.*, 2005). This evaluation of the same three leaves allows a more robust comparison of the results obtained, avoiding heterogeneities of the density of trichomes inherent to each specific leaf. As observed in **Figures 4.7B** and **D**, the analysis of the detached leaves provided to a clearer and cleaner trichome identification on the leaf surface in both manual and automated quantification methods.

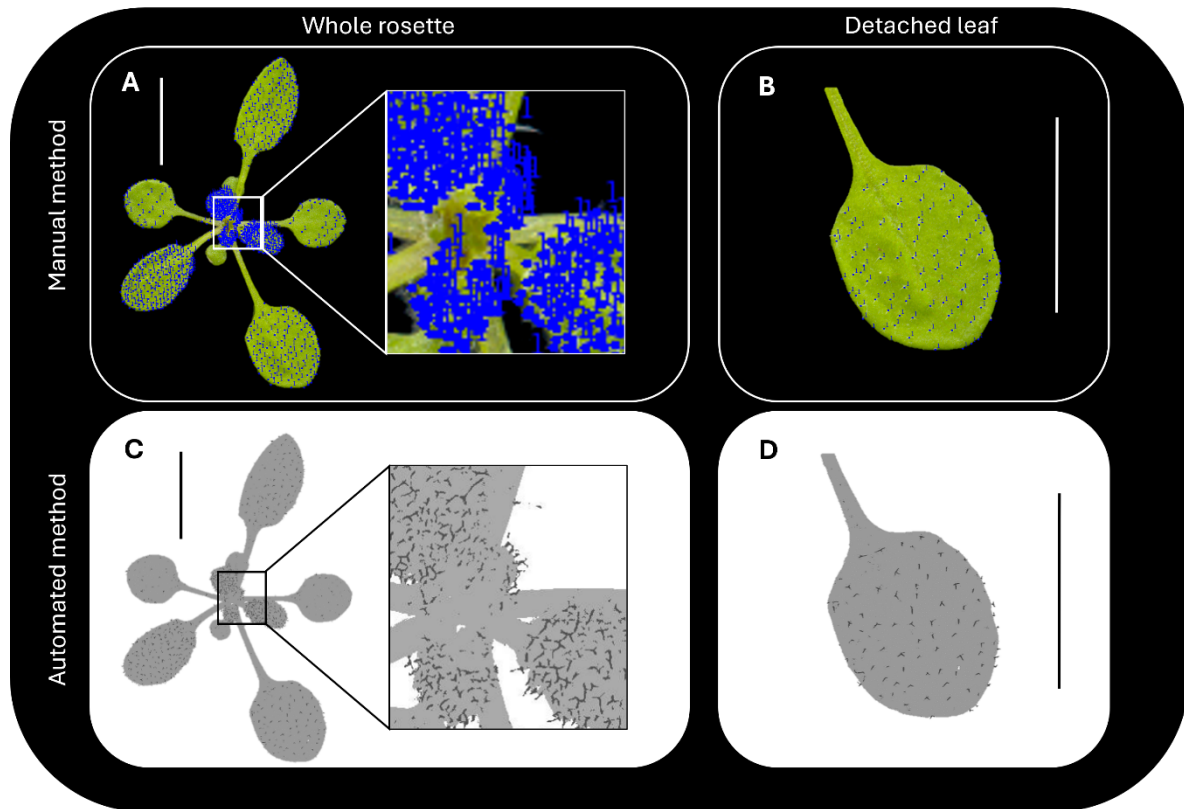


Figure 4.7. Manual and automated trichome quantification on Arabidopsis rosettes and leaves. Visualization of trichome quantification performed on a whole Arabidopsis rosette (**A**, **C**) or a detached leaf (**B**, **D**) with both manual and automated methods. Blue marks indicate the presence of a trichome by manually annotation (**A**, **B**). The automated quantification method (**C**, **D**) produces a grayscale image where the trichomes are identified by a darker grey region. Black and white scale bars indicate 1 cm.

However, once determined the use of detached leaves for the quantification process, it was necessary to determine the specific method to use for a proper and robust trichome quantification. To achieve this, three different automated approaches were tested for the extraction of trichomes from the leaf images, using in all of them Ilastik software as a machine learning tool. Particularly, this tool was selected based on the solid and robust results previously obtained for the damage area identification in Arabidopsis rosettes by Ojeda-Martinez *et al.* (2020) in comparison to other machine learning tools, being an ideal tool for the recognition of trichome structures on our images. The first step included in the three automated methods was based on a “pixel classification” approach trained for the identification of trichome structures. The entire workflow followed for the “Ilastik – noisy background” is provided **Figures 3.2** and **3.3**, and thoroughly described in **Annex A**. Once extracted the original images and having quantified the leaf area (**Figure 3.2**), images are segmented containing the information reflecting the areas of the images identified as trichomes (**Figure 3.3C**). After this step, segmented images are analysed in Fiji to extract the regions recognized as trichome structures

(**Figure 3.3D**) (**Annex A**) and processed using a size filtering step to remove those regions containing noise that interferes with the analysis (**Figure 3.3E**).

Nevertheless, considering the results obtained by Pomeranz *et al.* (2013) in which the presence of dust particles in the image background could interfere with the proper trichome identification process, an “Ilastik – black background” approach was also performed. In this approach, prior to the image analysis on Ilastik software, the background was manually cleaned with Adobe Photoshop to remove the presence of interferences and dust particles. However, after this pre-processing step, the images were equally analysed than in the “Ilastik- noisy background” approach.

Finally, a third approach termed as “Ilastik – Pixel + Object” in which the image analysis and noise filtering step is directly performed on Ilastik was also tested. The peculiarity of this approach relies on the fact that after the “Pixel classification” performed on Ilastik, images are also classified based on their size and shape, being able to separate overlapping trichomes as different individuals.

For testing the different approaches, a training process was performed with 44 images on each case. In **Figure 4.8**, a summary of the steps used for each approach is provided. In addition, a detailed explanation of the parameters and steps followed for each of the approaches is also included in **Annex A**.

4.2.2. Comparison of manual and automated approaches

To evaluate the performance of the three automated methods, a concordance analysis was performed by comparing the trichome density values obtained by each method. The manual quantification method was used as the reference point for all comparisons. The results from all tested methods were consistent, showing no significant differences, suggesting that all the tested automated methods have comparable accuracy to the manual method (**Figure 4.9A**). Furthermore, no significant differences were found among the automated methods ($p = 0.42$), confirming that all of them are suitable for trichome quantification and highlighting the effectiveness of Ilastik as an ideal tool for this task. To further evaluate the agreement between the four selected methods, Pearson correlation coefficients (r_p) and Lin’s concordance correlation coefficient (CCC) were calculated. While Pearson correlation coefficients identify relationships between two methods, Lin’s CCCs can additionally detect the presence of constant

bias and penalize them. According to this, significant correlations were observed in all cases ($p < 0.001$), showing a relationship nearing perfect agreement (**Figure 4.9B**).

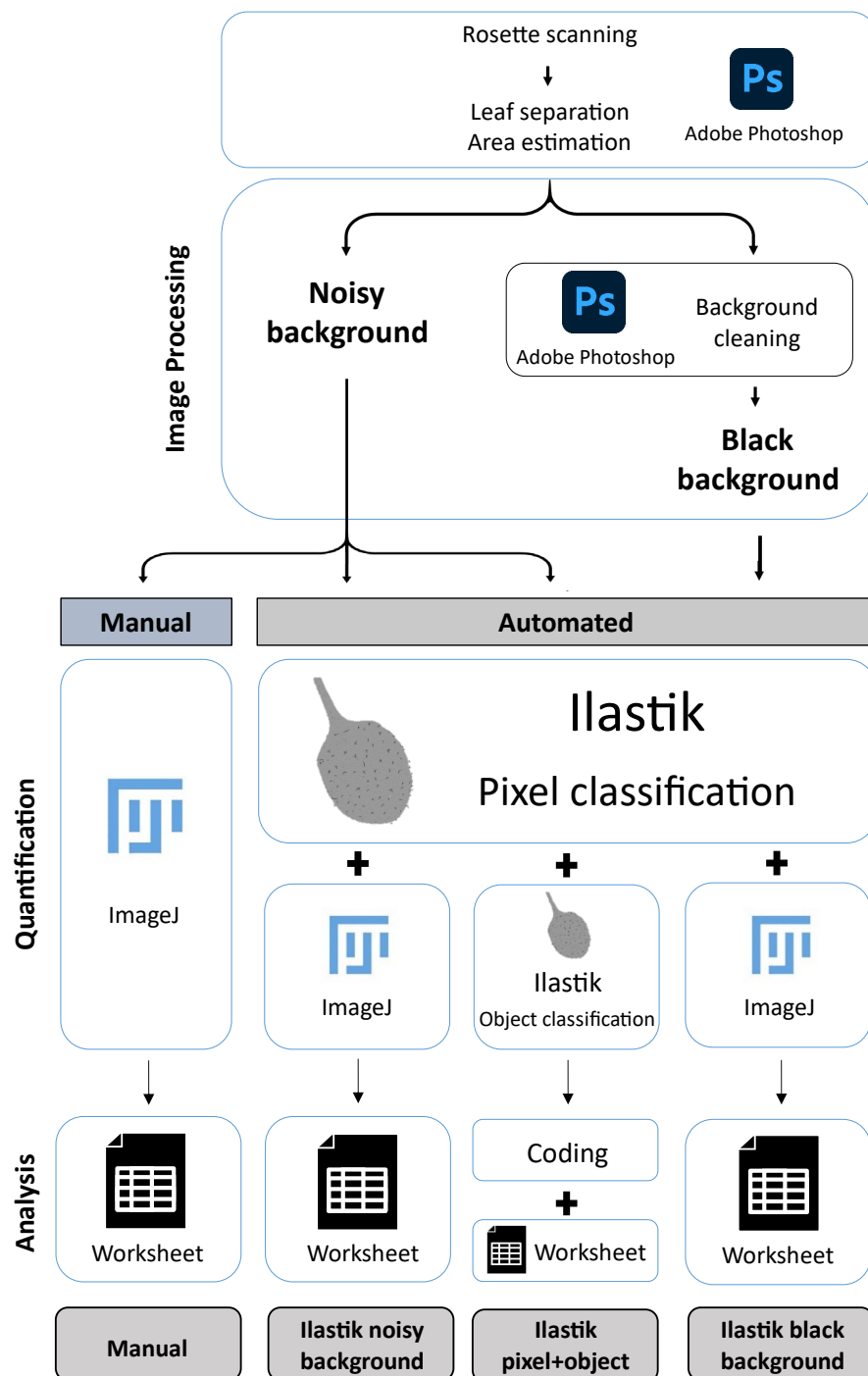


Figure 4.8. Flow chart depicting the general steps and tools for both manual and automated approaches tested. Image acquisition initial step using Photoshop is common for all the methods. After image processing, all automated methods start with the use of the machine learning tool Ilastik. Each square represents a specific step performed with a specific software inside each approach.

In addition, the analysis further illustrates the accuracy and precision of the methods, with data points clustering near the line of perfect agreement, as evidenced by the high Lin's CCC values. Interestingly, although the "Ilastik pixel+object" methods showed a light tendency to overestimate the values compared to the manual method, it also yielded the highest CCC, indicating a stronger agreement. However, the CCCs are very similar across all methods and close to 1. Since biased results would be reflected by lower Lin's CCCs, these values indicate that bias is minimal in the results. Moreover, when the automated methods were compared with each other, none showed a tendency to under- or overestimate the results related to another, with all CCCs consistently close to 1.

Nevertheless, while the methods were similar in terms of accuracy, precision and concordance, they differed significantly in the time required to perform the trichome quantification (**Figure 4.9C**). As expected, the manual method was the most time-consuming method, taking 1 min and 2 seconds per image, whereas the three automated methods were considerably faster. The automation of the trichome identification and counting significantly sped up the process, underscoring the effectiveness of machine learning in reducing the time needed for manual labour-intensive tasks. Of the three automated methods, the "Ilastik noisy background" approach was the fastest, taking only 10 seconds per image and making the analysis 6.10 times faster than the manual method. The "Ilastik pixel + object" method was the second fastest, taking 27 seconds per image, 2.26 times faster than the manual approach, while the "Ilastik blackbackground" method required 39 seconds per image, making the process 1.65 times faster than the manual method.

Consequently, considering its simplicity, speed in image analysis and processing, and comparable performance to the manual reference, the "Ilastik noisy background" approach was the final method selected for trichome quantification.

4.2.3. Reliability assessment of the selected automated method in *Arabidopsis*

To verify the reliability and sensitivity of our selected method, a trichome density analysis was conducted across different *A. thaliana* accessions. According to Hausser *et al.* (2001) and Hilscher *et al.* (2009), who manually quantified trichomes in 75 *Arabidopsis* accessions, 6 accessions with trichome densities ranging from the lowest to the highest spectrum were selected: *Ler-0*, *Gr-1*, *Col-0*, *Sha*, *Can-0*, and *Rub-1*.

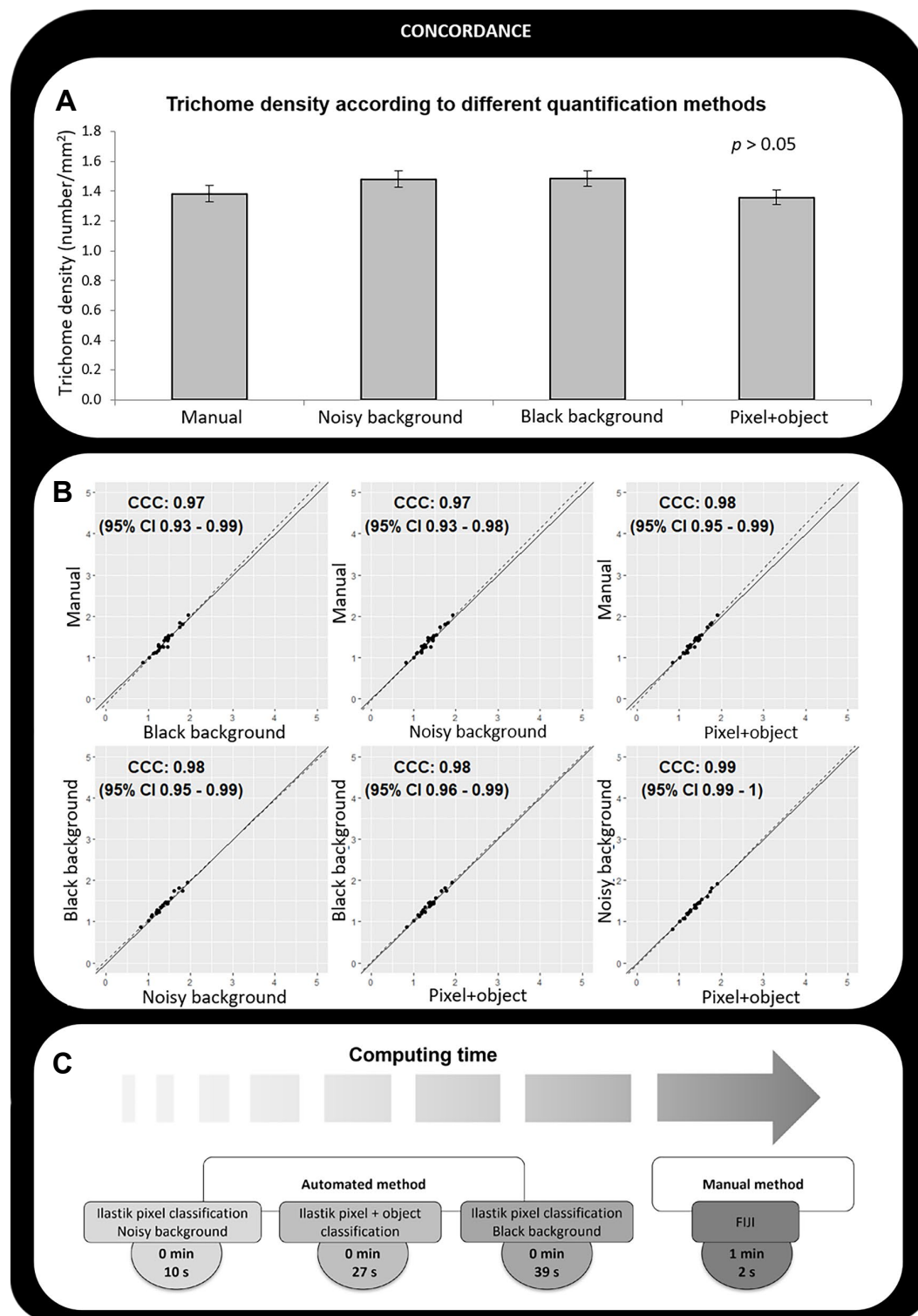


Figure 4.9. Concordance analysis of trichome densities manually estimated against all three automated approaches. Bars plot (A) represents absolute density values for each method. No significant differences were found using One-Way ANOVA test. Lin's concordance correlations ($n=24$). (B) show the agreement between all different combinations of methods. The solid lines are the lines of concordance which indicate perfect agreement. The dashed lines indicate the best fit to the values. The coefficient (CCC) and its respective 95% confidence interval (CI) are represented for each case. Pearson correlation coefficients (r_p) describing the relationship between the trichome density estimation by manual and automated methods are also depicted ($n=24$). The computing time (C) for each method is expressed as the time taken to process one image from a whole-rosette-image to the number of trichomes identified in a single leaf.

Our method demonstrated a high level of reproducibility when compared to the manual measurements (**Figure 4.10A**). In fact, no significant differences were found between the manual and automated methods, yielding both similar results for each accession. Moreover, the precision displayed by the methods appeared to be quite similar in all cases (**Supplementary Table D.6**). Particularly, the automated method consistently produced lower standard errors compared to the manual methods. However, there was a smooth gradual increase in errors from accessions with lower trichome densities to those with higher densities.

Nonetheless, this increase in variability was observed in both manual and automated processes, underscoring the similarity between the two approaches in trichome quantification. Correlation with data from other sources (Hauser *et al.*, 2001; Hilscher *et al.*, 2009) was additionally observed (**Figure 4.10B**), and even though experimental conditions such as the number of leaves evaluated and the plant age varied, the pattern of trichome density variation among *Arabidopsis* accessions was consistently maintained ($r_p = 0.8984$). To determine whether this variation resulted from errors or unspecific measurements in the automated image processing, a correlation with the manual measurements for each leaf of each accession was additionally performed (**Figure 4.10C**). In this case, the correlation was also significant ($p > 0.001$) with a high correlation coefficient ($r_s = 0.982$), indicating that the automated measurements provide results that closely match to the reference ones. This suggests that despite the correlation with previous studies (Hauser *et al.*, 2001; Hilscher *et al.*, 2009) was slightly lower than the one between the manual and automated methods, these differences can be related to variations in experimental conditions rather than inaccuracies or errors in our method. To further confirm the reliability of our automated approach, we performed a concordance analysis using a Bland-Altman (B&A) plot to evaluate the assess and precision of the method (**Figure 4.10D**). Our automated method showed a mean difference from manual measurements that was nearly zero (-0.0224). Additionally, regarding the data point distribution, most data points cluster around the zero value, showing a low standard error of 0.279 and indicating a low data dispersion in our analysis. Nevertheless, as trichome densities increase, a slight increase in data dispersion is also observed, with Rub-1 accession showing the largest discrepancies between manual and automated quantification and a lower precision of the data points.

Due to this result, an individual evaluation of the method's reliability on each of the *Arabidopsis* accession tested was carried out (**Figure 4.11**). Accessions were grouped into “low”, “medium” and “high” categories based on their trichome densities (Hilscher *et al.*, 2009). Concretely, Ler-0 and Gr-1 were categorized as low-density, Col-0 and Sha as medium-density, and Can-0 and

Rub-1 as high-density accessions. B&A plots were used to determine the agreement between the automated and manual trichome quantification for each accession.

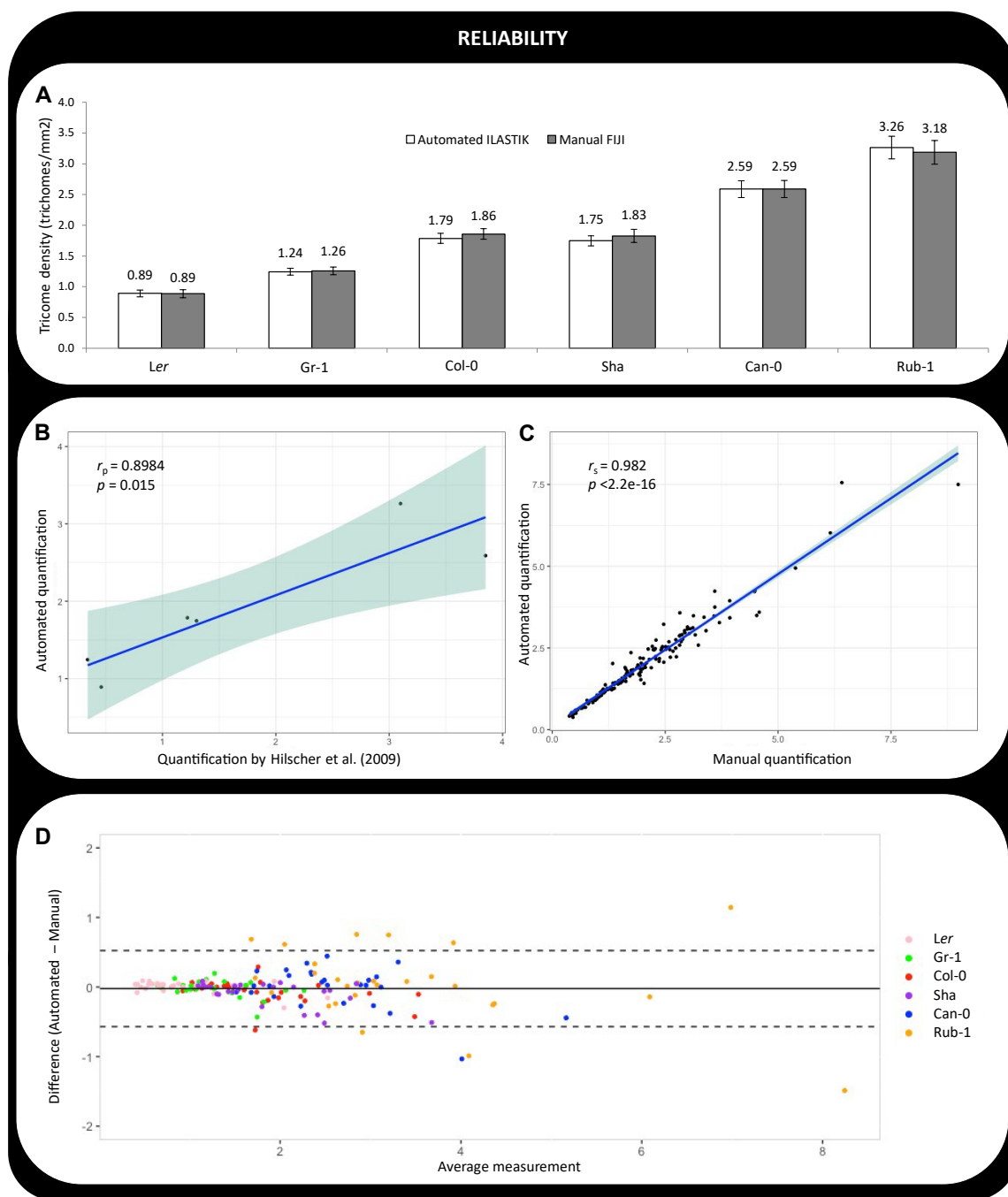


Figure 4.10. Reliability of trichome densities obtained by our automated quantification method. Absolute density values estimated manually and automatically are represented as bar plots (A) Data are means $\pm$ SE of nine independent replicates. Statistical analysis for differences between values obtained by manual and automated methods were performed following a Student's *t*-test for each accession ($p > 0.05$). The Pearson correlation coefficient r_p (B) describes the relationship between trichome densities estimated by Hilscher *et al.* (2009) and our automated method ($n = 6$). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability. The Spearman correlation coefficient r_s (C) describes their relationship ($n = 27$). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability. The Bland-Altman plot (D) studies differences between both manual and automated methods. The central solid line indicates the mean difference, the two outer dashed lines indicate ± 1.96 standard deviations. Different colours indicate values for different *A. thaliana* ecotypes ($n = 27$).

For accessions with “low” trichome density, our method showed no bias. The accuracy of the automated method was nearly identical to the standard, displaying minimal differences between manual and automated counting approaches for both *Ler-0* and *Gr-1*, with values of 0.002 and -0.019, respectively (**Figure 4.11, A and G**). Additionally, these accessions exhibited the narrowest agreement limits of all accessions, resulting from the low standard errors obtained in their analysis, 0.083 for *Ler-0* and 0.117 for *Gr-1*.

Regarding the trichome evaluation of “medium” trichome density accessions, the automated method showed a slight bias towards identifying fewer trichomes than the manual methods, with mean differences of -0.068 for *Col-0* and -0.09 for *Sha* (**Figure 4.11, B and H**). Nonetheless, the standard errors for *Col-0* and *Sha* were 0.169 and 0.175, respectively, showing narrow agreement limits for these accessions.

Finally, for accessions with “high” trichome densities, our method showed a slight bias towards the identification of a higher number of trichomes in comparison to the manual approach, particularly observed in the evaluation of the *Rub-1* accession, which showed a mean difference of 0.049 (**Figure 4.11, C and I**). Both high-density accessions displayed the widest agreement limits across all tested accessions, accompanied by higher standard errors, which reported values of 0.301 and 0.547 for *Can-0* and *Rub-1*, respectively. However, as previously shown in **Figure 4.10A**, this high variability affects both manual and automated methods, with the manual method exhibiting higher standard errors.

Furthermore, to additionally validate the method’s reliability, a correlation analysis was conducted for each accession. Correlations were significant in all cases ($p > 0.05$), displaying very high correlation coefficients ($r > 0.9$) (**Figures 4.11D-F and J-L**). As suggested by the B&A plots, the correlation with the manual reference method was stronger for accessions with “low” and “medium” trichome densities and slightly lower for “high” densities. Nevertheless, the strong correlation observed across all accessions demonstrates that our automated method provides consistent and robust results that accurately reflect the trichome densities in each accession. Therefore, our method has shown to be reliable for trichome quantification, regardless of the trichome density present in the leaves.



Figure 4.11. Bland-Altman plots (A, B, C, G, H, I) and correlation coefficients (D, E, F, J, K, L) of estimated trichome densities describing the relationship between trichome densities estimated manually (Fiji) and by the automatic method developed. For the Bland-Altman plots the central solid line indicates the mean difference, the two outer dashed lines indicate ± 1.96 standard deviation. For the correlations, Pearson (r_p) or Spearman (r_s) coefficients were calculated according to the data distribution ($n = 27$). The blue line represents the best fit and the light blue areas show a confidence interval with 95% probability.

4.2.4. Method adaptability to other plant species

Originally developed for *A. thaliana*, a model species for studying unicellular non-glandular trichomes, our method faced the challenge of adapting to the diversity of trichome structures present in the nature and was tested for broader applicability to other plant species. We have confirmed that our proposed method can effectively identify and quantify trichomes in other

species, such as *S. tuberosum* and *B. napus*, without the need for pre-treatments and including an adequate training of the Ilastik program for each species (**Figure 4.12**).

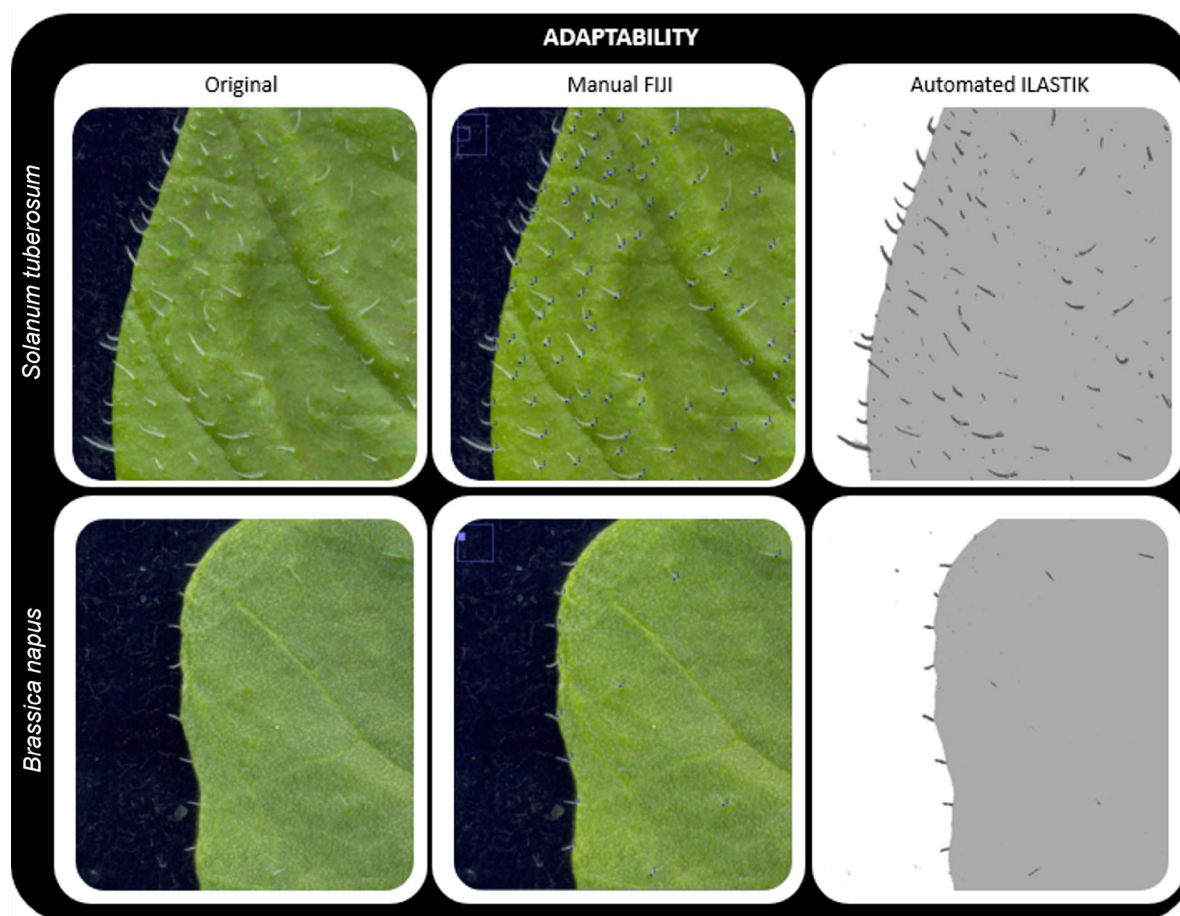


Figure 4.12. Trichome identification on leaves of different plant species. Original images are compared against manual quantification in Fiji and our automated method with Ilastik. Leaves from *Solanum tuberosum* and *Brassica napus* are good candidates for trichome quantification with our automated method.

4.3. Spider mite infestation triggers coordinated hormonal trade-offs enabling plant survival with a fitness cost

In plant-herbivore interactions, the balance between defence and growth is a crucial aspect of plant survival that determines its reproductive success. These two main processes are intricately regulated by hormone signalling pathways that can either synergize or antagonize one another, in a process commonly known as defence-growth trade-off. While previous studies have primarily focused on the immediate costs of induced, such as their impact on growth or photosynthesis at specific points of herbivore infestation, there has been limited exploration of these effects throughout the entire plant life cycle, including their influence on seed production and offspring establishment, parameters directly related to plant fitness. Using the *A. thaliana*-

T. urticae system, this research tries to fill this gap by comprehensively assessing the selective pressures exerted by *T. urticae* across the life cycle of *A. thaliana*. This includes detailed physiological, transcriptional, and hormonal analyses to uncover the molecular mechanisms underlying the defence-growth trade-off. By understanding these interactions, we can develop new crop management strategies that enhance plant resistance while minimizing negative impacts on fitness and crop production.

4.3.1. Phenotypic traits reveal plant morphological and physiological changes upon mite infestation

To investigate the dynamics of the interaction between *A. thaliana* and *T. urticae* and its impact on plant growth, development, and fitness, we analysed the phenotypic variations present in plants in both control and mite infestation conditions throughout their life cycle (**Figure 3.1**). Concretely, physiological traits related to growth, photosynthesis and reproduction were recorder to identify the critical points in which biotic stress impacts plant behaviour and performance.

Spider mite infestation triggered changes in growth, rosette architecture, and a subtle change in leaf colour (**Figure 4.13, A and B**). Additionally, mite-infested plants exhibited a higher trichome number after just 24 hours post-infestation (hpi), followed by an increase in trichome density at 4 dpi (**Figure 4.13C**). At this stage, significant reductions in rosette area and petiole length were also observed, persisting until the end of the plant's life cycle (**Figure 4.13, D and E**). These changes in rosette area significantly influenced trichome evaluation, particularly amplifying the impact of the trichome number on the final trichome density (**Figure 4.13C**). Moreover, mite infestation led to an increased production of rosette leaves, as plants showed a higher number of leaves at 12 and 15 dpi (**Figure 4.13F**).

Further phenotypic analysis of the plant aerial parts showed a higher fresh weight on control plants in comparison with the infested ones from 4 dpi to 15 dpi, time in which no significant differences were observed (**Figure 4.14A**). Similarly, variations in water content were also noted, with control plants having higher water content at 4 and 7 dpi, no significant differences at 12 dpi, and lower water content than mite-infested plants at 15 dpi, coinciding with the onset of senescence in control plants (**Figure 4.14B**). In addition, mite infestation resulted in the generation of shorter plants since 7 dpi, showing a fewer number of branches of the main inflorescence at 15 dpi (**Figure 4.13, G and H**).

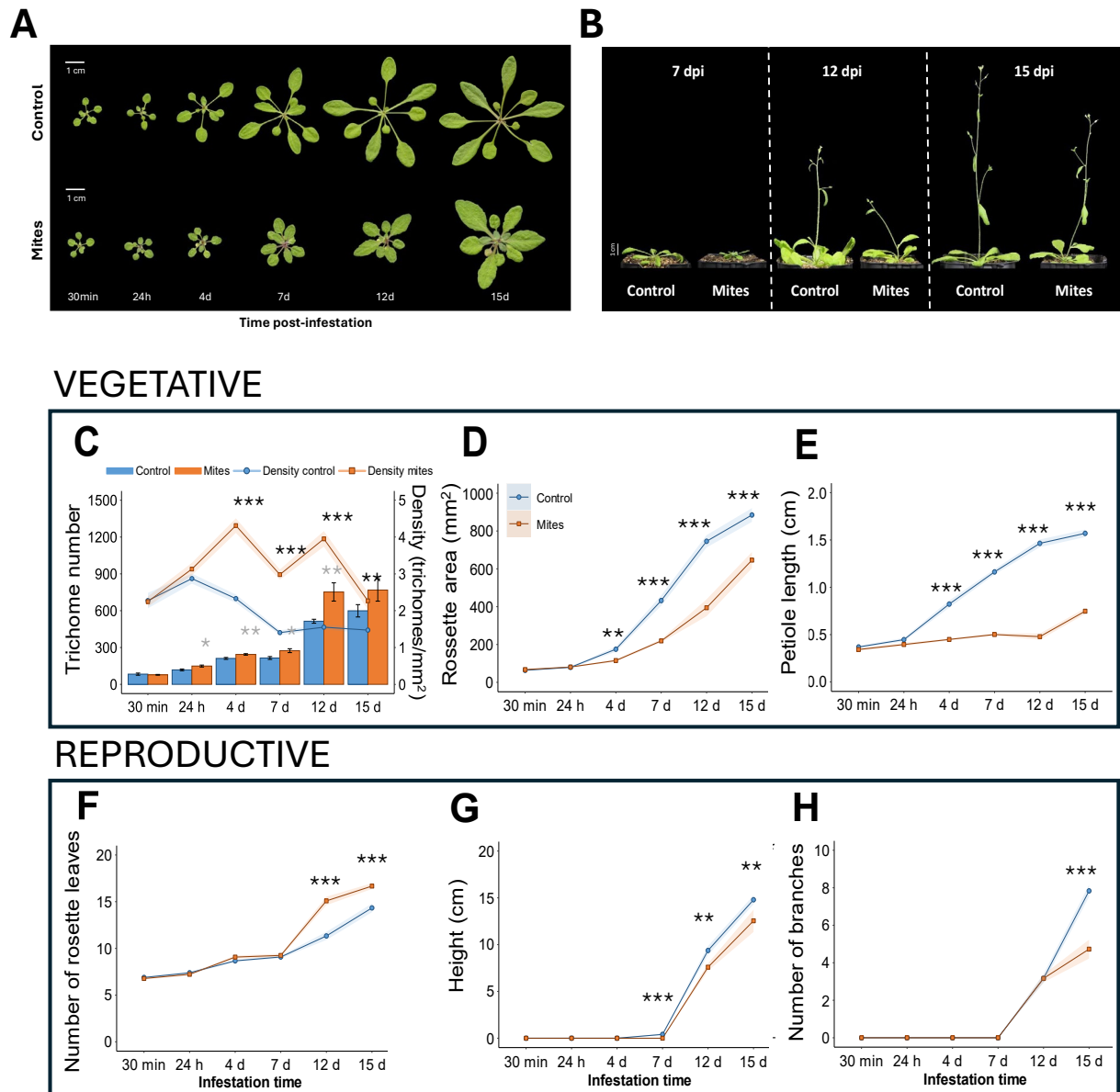


Figure 4.13. Infestation of *A. thaliana* plants by *T. urticae* leads to a delay in plant aerial development with rosette phenotype changes related to plant growth and defence. Three-week old Col-0 plants were infested with 20 mites of *T. urticae*. (A) Rosette phenotype of the control and infested Arabidopsis Col-0 rosettes along plant development. (B) Aerial phenotypes of the control and infested Arabidopsis Col-0 plant after 7 d, 12 d and 15 d of infestation. Control and infested plants were evaluated for (C) trichome number and density (n=9), (D) rosette area (n=9), (E) petiole length (n=9), (F) number of rosette leaves (n=12), (G) plant height (n=12), (H) number of branches (n=12) at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of mite infestation. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. Significant differences in trichome number and trichome density are represented by grey and black asterisks, respectively.

Regarding the photosynthetic parameter evaluation, mite infestation reduced the photosynthetic performance of the plants, showing a decreased instantaneous fluorescence and quantum yield

in comparison to non-attacked plants (**Figure 4.14, C and D**). Furthermore, infestation also induces anthocyanin accumulation, presenting mite-infested plants higher anthocyanin levels than the control ones at 24 h, 4 d, and 7 dpi (**Figure 4.14E**).

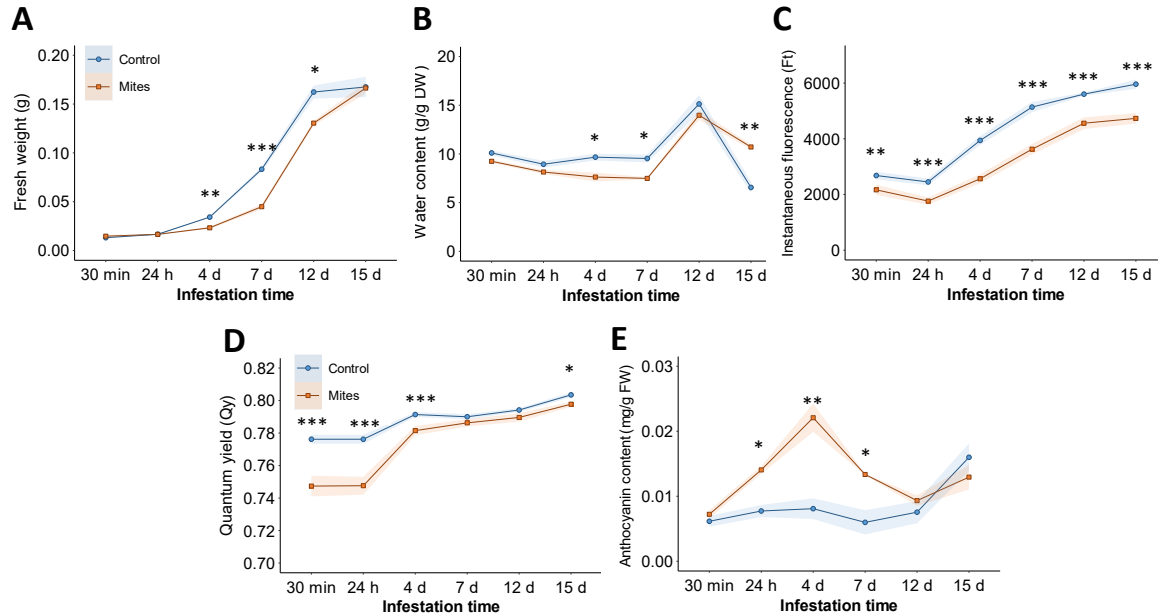


Figure 4.14. *T. urticae* infestation reduces plant growth, photosynthetic performance and increases the anthocyanin levels in *Arabidopsis*. Three-week old plants were collected after 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. (A) Fresh weight (n=5), (B) water content (n=5), (C) instantaneous fluorescence (n=12), (D) quantum yield (n=12) and (E) anthocyanin content (n=9), were evaluated at the different time points of *T. urticae* infestation in *A. thaliana* Col-0 plants. Coloured shadows represent the standard error of the samples. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$.

4.3.2. Transcriptomics analysis reveals a defence-growth contrasting interplay in the plant response to mite infestation

The PCA analysis revealed a sample distribution in the PCA plot in which samples were clustered according to their specific time point and treatment, showing a discernible trajectory from the shortest to the longest infestation times, highlighting the influence of the plant development on the transcriptional profiles (**Figure 4.15A**). Interestingly, along this trajectory, control samples were clustered ahead of mite-infested samples at each of the time points of evaluation, correlating with the phenotypical developmental delay previously observed. In addition, a deeper evaluation of the of three first PCA components is provided in **Supplementary Figure C.1A**, in which those samples showing any overlapping in the two first dimensions appeared independently clustered after including the third PCA dimension.

In addition to the PCA analysis, the differential expression analysis revealed a main gene up-regulation during the three first time point of evaluation, at the plant vegetative stage, being most pronounced at 24 hpi. Conversely, there was a predominant gene downregulation during the three last time points, at the reproductive stage of the plant, notably at 7 dpi (**Figure 4.15B**). UpSet plots further illustrated these transcriptional responses, showcasing both the similarities and specificities across different time points within the vegetative and reproductive stages (**Supplementary Figure C.1B**). Shorter time points consistently showed a higher DEG number. Concretely, at 30 min, 24 h and 4 d, there were 1096, 1537 and 1178 DEGs, respectively, while 823, 623 and 592 DEGs were observed at 7, 12 and 15 dpi. Notably, 243 DEGs were shared during the vegetative stage of the plant, with 131 of them being unique to the time points included during this plant stage. However, during the reproductive stage of the plant, 108 DEGs were shared among different time points, with 12 of them uniquely expressed at these time points. Remarkably, 32 DEGs were commonly expressed across all evaluated time points (**Supplementary Figure C.1B**).

Finally, hierarchical clustering of the samples emphasized the transcriptomic similarities in the pattern of response to mite infestation during the vegetative and reproductive stages, showing a clustering in which the samples belonging to each of the plant stages were clustered together (**Figure 4.15C**). In the case of the gene clustering, seven different clusters were identified and depicted on the left part of the heatmap. Notably, clusters 1 to 6 contained genes directly related to defence processes, showing a general gene up-regulation with a shared response across time points at both vegetative and reproductive plant stages (**Figure 4.15C**, **Supplementary Figure C.1E** and **Supplementary Dataset S3.1 - Garcia *et al.*, 2024 – DatasetS2**). However, cluster 7 was characterized by a prevalent down-regulation of genes with a more particular response for each time point. This cluster mainly involved genes linked to growth processes, with responses differing between the vegetative stage – characterized by a main hormonal response (response to auxin, regulation of hormone levels, brassinosteroid or gibberellin biosynthetic process) – and the reproductive stage, primarily associated with cell wall metabolism (plant-type secondary cell wall biogenesis, xylan metabolic process, hemicellulose metabolic process, lignin metabolic process) (**Supplementary Figure C.1E**).

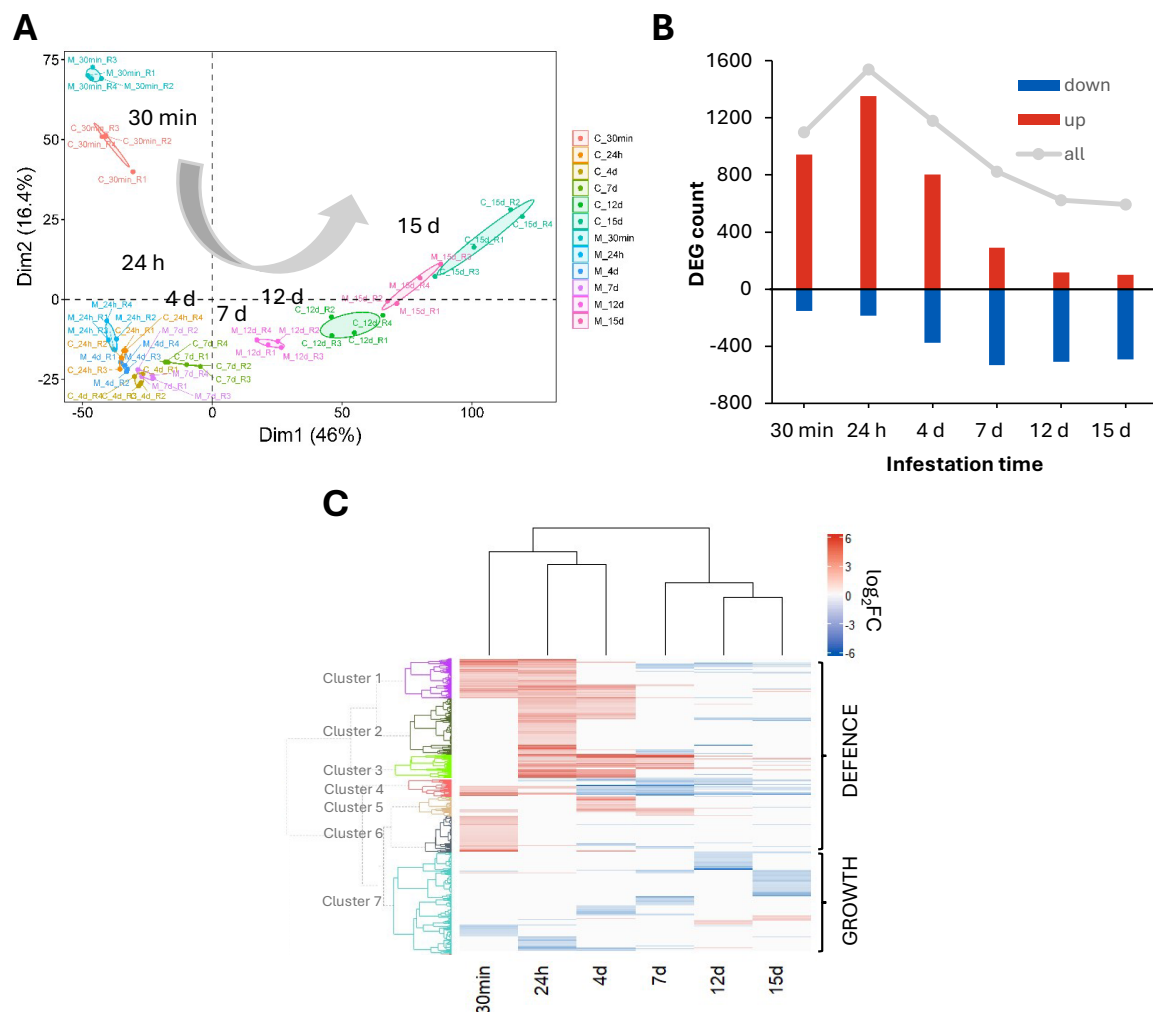


Figure 4.15. RNA-seq transcriptomic analysis evidences the presence of a defense-growth trade-off in which mites are responsible of a delay in plant development. **(A)** Principal Component Analysis (PCA) of control and mite-infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation in the two first PCA dimensions. Ellipses define confidence areas (95%) for each specific treatment and time. Each circle represents a biological replicate. Samples are identified using a specific colour code for different time and treatment. **(B)** Bar diagrams showing the number of total, up- or down-regulated genes at each infestation time point. **(C)** Heatmap showing hierarchical clustering analysis of samples and of the log₂FC values exhibited by the differentially expressed genes (DEGs) found in mite-infested plants compared to control plants through all time points. Clusters of genes are represented in the left dendrogram with a specific colour for each cluster. General biological function of the DEGs, belonging to defence or growth processes, is indicated on the right part of the heatmap.

4.3.3. Phytohormone profiling unveils dynamic interplay among defence and growth hormones in response to *T. urticae* infestation

To identify the main compounds driving the plant defence-growth trade-off upon spider mite infestation, a thorough hormone evaluation was performed. To differentiate between defence and growth processes, PCA analyses were conducted using the hormone levels, categorizing

them into defence (JA and SA), growth hormones (auxins, BRs, GAs and CKs), and ABA, which has been described to be involved in both defence and growth processes.

In the case of the defence response, the PCA biplot revealed a sample distribution with a clear separation between control and mite-infested samples, with the samples infested at 30 min and 24 h showing the highest separation from control samples along the second and first dimensions, respectively (**Figure 4.16A**). Interestingly, the levels of SA and jasmonoyl-L-isoleucine (JA-Ile) were the main responsible of the distribution of the samples at 30 min, while from 24h onward, the distribution of mite-infested samples was predominantly driven by levels of JA, its precursor 12-oxo-phytodienoic acid (*cis*-OPDA) and hydroxylated metabolites 11-hydroxy-/12-hydroxyjasmonic acids (Σ 11-OH-JA/12-OH-JA). In this last case, as infestation progresses, samples appeared to be gradually recovering from the stress, showing at 12 and 15 dpi, a sample distribution that closely matches the one of the controls (**Figure 4.16A**). In fact, although all these compounds were accumulated during the initial stages of the infestation, there were almost no significant differences at 12 and 15 dpi. Concretely, the JA and its precursors, *cis*-OPDA and dinor-oxo-phytodienoic acid (dnOPDA), showed increased accumulation during the first time points, with JA reaching its maximum at 24 hpi. This peak of JA correlates with the decrease in JA-Ile levels and the increase of hydroxylated JA metabolites (Σ 11-OH-JA/12-OH-JA), as high JA levels promote the production of these compounds (**Figure 4.16B**). Finally, SA accumulation in mite-infested plants was observed at 30 min and 24 h, coinciding its reduction with the JA accumulation. In addition, control plants also accumulated SA at 15 dpi, when plants start to senescence, a phenomenon previously documented in *Arabidopsis* (Morris *et al.*, 2000; Carella *et al.*, 2015).

The evaluation of growth hormones involved the quantification of auxin-, BR-, GA- and CK-related compounds (**Figure 4.17** and **Supplementary Figures C.2-C.5**). Analysis of the PCA biplot revealed a clear separation between control and mite-infested samples along the second dimension. This separation was mainly driven by the accumulation of indole-3-acetonitrile (IAN), isopentenyladenine-9-glucoside (iP9G) and brassinolide (BL) in the infested samples, and of teasterone (TE), *trans*-zeatin (*tZ*) and 28-homocasterone (28-homoCS) in the control plants (**Figure 4.17A**). Particularly, the treatment separation started at 24 h, as no different hormonal growth response was observed at 30 min. In the case of growth hormones, the greatest separation between control and infested samples was observed at 4 dpi, followed by a gradual recovery in which samples at 15 dpi approach the zero value in the second dimension.

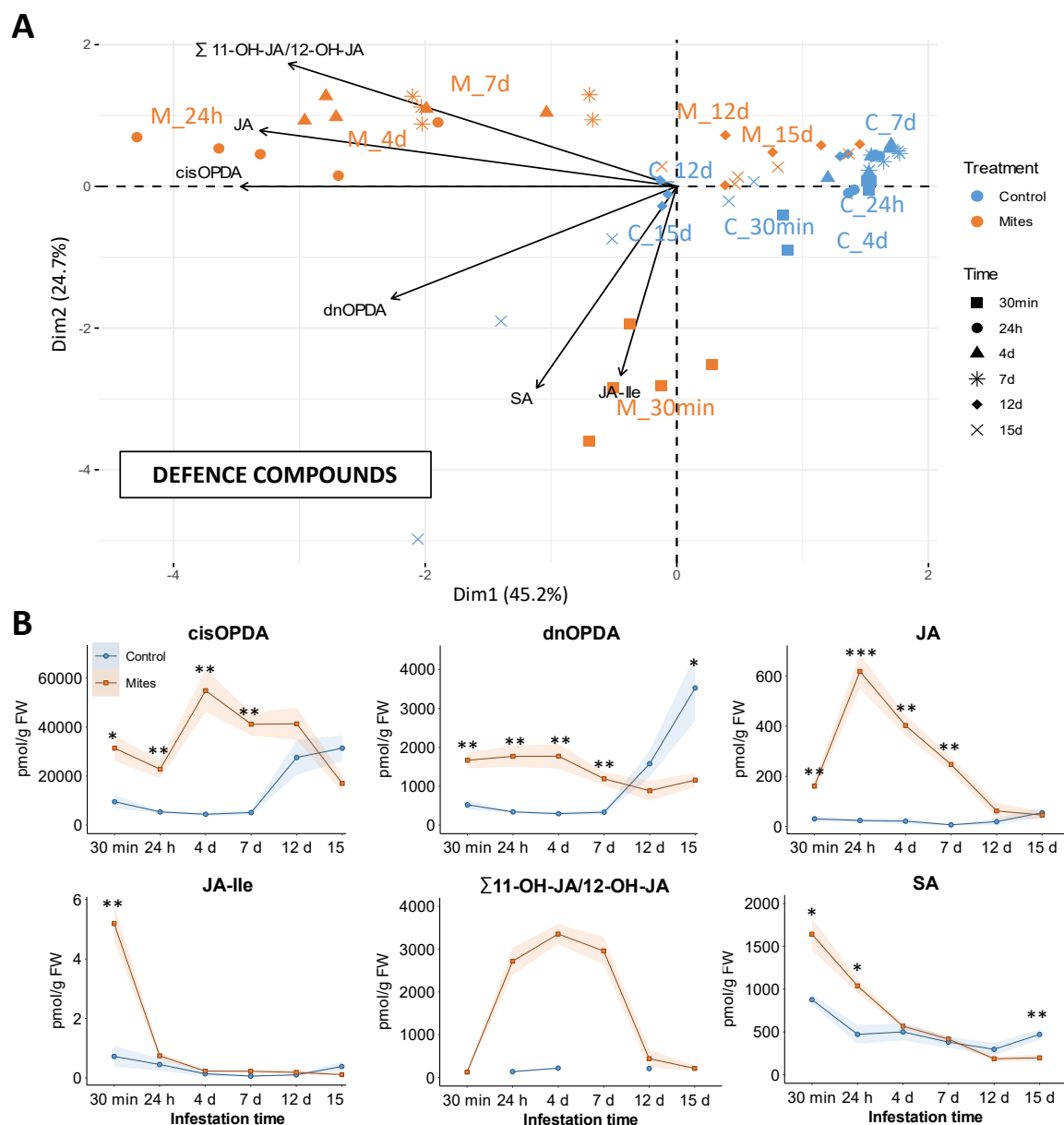


Figure 4.16. Spider mite infestation rapidly triggers the accumulation of the main hormones involved in plant defence response. **(A)** Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of biotic stress hormones. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. **(B)** Hormone levels of the different defence hormone-related compounds. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from 5 biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. cisOPDA: cis-12-oxophytodienoic acid; dnOPDA: dinor-OPDA; JA: jasmonic acid; JA-Ile: jasmonic acid-isoleucine; Σ 11/12-OH-JA: 11/12-hydroxyjasmonic acid; SA: salicylic acid.

Regarding the first PCA dimension, a clear distinction is depicted between samples belonging to short- and long-time points, being the samples at 12 and 15 dpi positioned on the right side of the plot and reflecting the relevance of the developmental stage on the plant growth response. Therefore, this separation in the first dimension was mainly driven by high variety of compounds accumulated during plant development, such as IAA and its catabolite oxIAA (2-oxindole-3-acetic acid), BL precursor typhasterol (TY), gibberellins GA₁₉ and GA₂₀, or active CK bases iP (isopentenyladenine) and *cZ* (*cis*-zeatin) with their metabolites *cZ*ROG (*cis*-zeatin riboside-*O*-glucoside) and DHZROG (dihydrozeatin riboside-*O*-glucoside) (**Figure 4.17A**).

In addition to the previous analysis, the evaluation of individual hormone groups also revealed specific response patterns to *T. urticae*. In the case of auxin-related compounds, a significant accumulation of the precursor IAN was observed from 24 h to 7 dpi, correlating with the corresponding increase in IAA and oxIAA levels (**Figure 4.17B** and **Supplementary Figure C.2**). Conversely, BR-related compounds exhibited an opposite trend. In this case, despite high levels of both active precursors of BL, TY and 28-homoCS, were observed in control samples at 30 min and maintained until 7 dpi for 28-homocCS, higher levels of BL were only observed in infested plants at 4 dpi, coinciding with the beginning of the plant recovery from the stress (**Figure 4.17C** and **Supplementary Figure C.3**). In addition, differences between treatments were also observed with the evaluation of GAs and CKs. Mite-infested samples exhibited higher levels of bioactive GA₄ and GA₁, at 30 min and 7 dpi, respectively. Nonetheless, the levels of GA₃ were reduced in these samples at 24 hpi (**Figure 4.17D** and **Supplementary Figure C.4**). In the case of the CK evaluation, a reduction in the active CK bases (CK-B) accumulation occurred during the initial stages of the infestation, mainly produced by the levels of the active *tZ* in the samples (**Supplementary Figure C.5**). However, this early decrease upon infestation was accompanied by an increase in the CK riboside (CK-R) and CK *O*-glucosides (CK-OG), involved in the transport and reversible storage of CK, respectively. Remarkably, the CK-R transport forms were the only CK metabolites significantly accumulated at 30 min upon mite infestation, being the isopentenyladenosine (iPR) the only CK accumulated at this time point, suggesting a relevant role in the plant response to spider mite infestation (**Supplementary Figure C.5B**). In addition, at 15 dpi, there was an accumulation of CK-B, CK-OG and CK *N*-glucosides (CK-NG) in control plants, except for IP9G, accumulated in mite-infested plants (**Supplementary Figure C.5, A, C and D**).

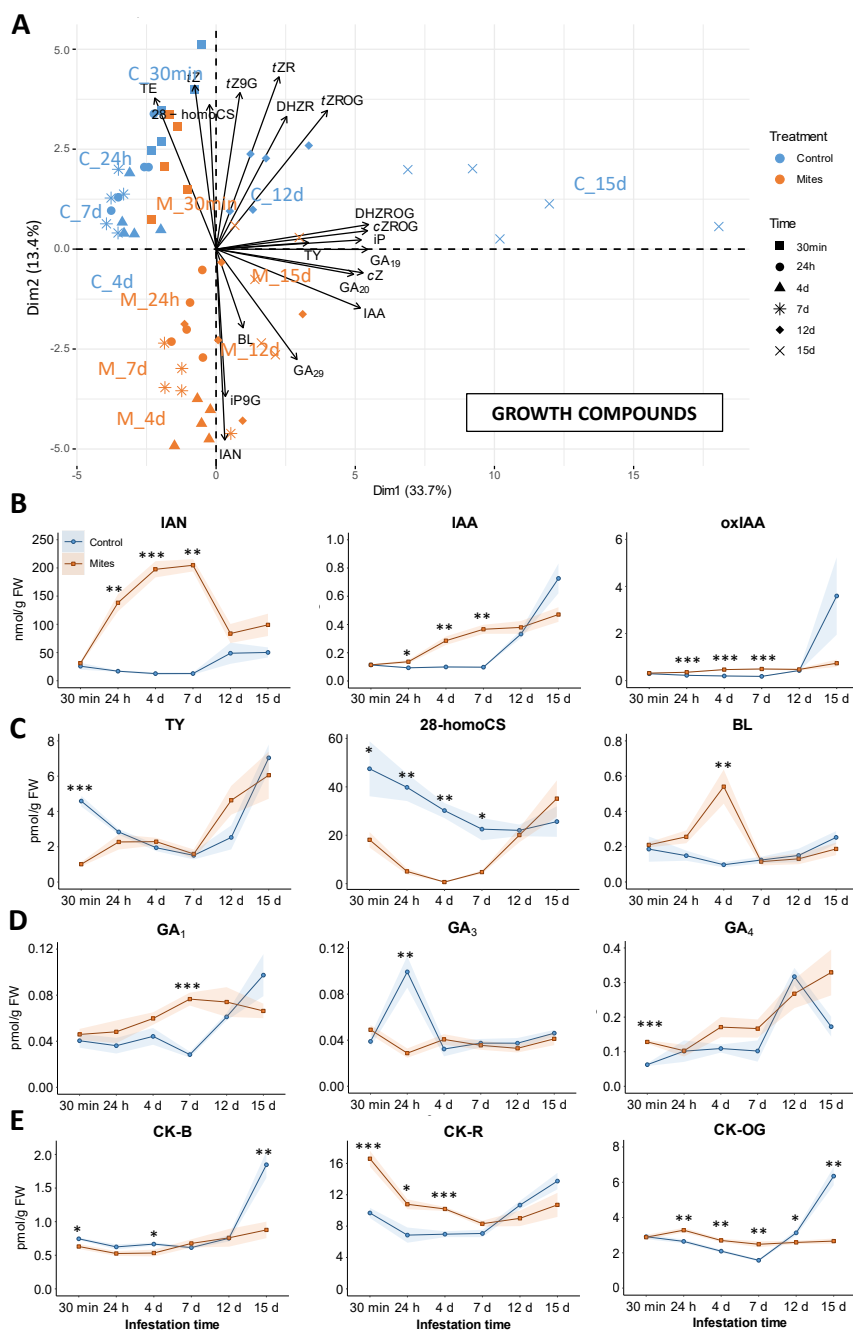


Figure 4.17. Plant growth response to spider mite infestation is orchestrated by a complex coordination of the different growth hormones. **(A)** Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of plant growth hormones. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. **(B)** Hormone levels of the different auxin-related compounds, **(C)** brassinosteroid-related compounds, **(D)** active gibberellins and **(E)** cytokinin-related compounds classified by its biological activity. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. IAN: indole-3-acetonitrile; IAA: indole-3-acetic acid; oxIAA: 2-oxindole-3-acetic acid; TY: typhasterol; 28homoCS: 28-homocastasterone; BL: brassinolide; GA: gibberellic acid; CK-B: active cytokinin free bases; CK-R: cytokinin ribosides; CK-OG: cytokinin O-glucosides.

To finish with the hormonal evaluation, ABA-related compounds were additionally analysed. The global PCA assessment for these compounds did not provide conclusive results as the ABA accumulation associated with plant aging originated a PCA plot where the impact of the plant development over short- and long-term time points was the only discernible pattern, masking the role of ABA in the plant defence during the vegetative plant stage (**Supplementary Figure C.6**). For this reason, separated PCA plots were generated for those time points in which plant material was collected during the vegetative and during the reproductive plant stages. At the vegetative stage, once mite is perceived by the plant, ABA accumulates, peaking at 24 hpi and displaying a clear separation from control samples in the PCA plot (**Figure 4.18**). No other significant differences were found until 12 and 15 dpi, when control plants, entering senescence, showed higher accumulation of ABA and related compounds than mite-infested plants. This accumulation was reflected in the PCA plot, showing a clear separation of these samples during the reproductive stage along the first PCA dimension (**Figure 4.18**).

4.3.4. Reproductive parameter evaluation reveals a negative effect of mite infestation in plant fitness at short- and long-term level

The evaluation of the number of siliques and flowers only revealed differences in the time of production of flowers and siliques. Although significant differences were observed at different time points, these differences were originated by the developmental delay previously observed at the phenotype level (**Supplementary Figure C.7**). At the end of their life cycle, both control and mite-infested plants produced a similar number of flowers and siliques, with a week of difference between them (**Figure 4.19, A and B**). To further evaluate the reproductive performance of the plants, seeds were collected, and an evaluation of different seed parameters was performed. The results showed that mite infestation led to a lower production of seeds in plants, with a lower number of seeds per silique (**Figure 4.19C**). Interestingly, seeds from mite-infested plants showed a larger seed size in comparison to those coming from control plants, but their individual seed size was similar in both conditions (**Figure 4.19C**).

To check if these variations at seed level could affect to the establishment of the offspring, an additional evaluation of the seed germination was conducted. In this experiment, the germination ability of three-week-old and eighteen-month-old seeds, the elder ones termed as ‘aged’ seeds, was analysed. The use of these two sets of seeds allows the evaluation of short- and long-term effects of the mite infestation on plant fitness. No differences in seed viability were observed when germination assays were conducted with the three-week-old seeds from

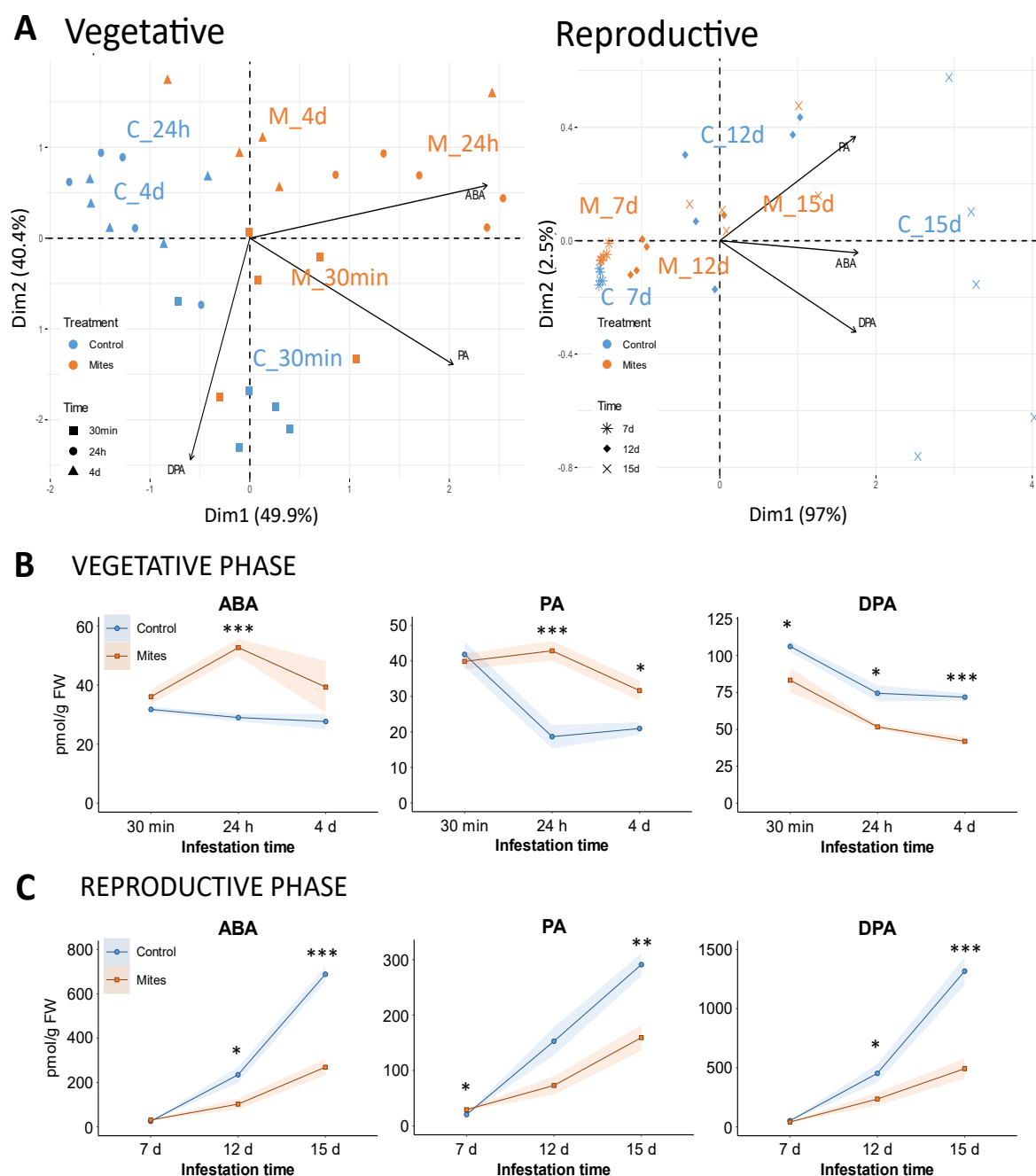


Figure 4.18. The dual role of ABA in both defence and growth responses is revealed in response to spider mite infestation. (A) Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h and 4 d (left) during the vegetative stage of the plant, and at 7 d, 12 d and 15 d of infestation, during the reproductive stage of the plant. PCA was performed on the matrix of hormonal levels of ABA-related compounds. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. (B) Hormone levels of the different ABA-related compounds at the vegetative stage of the plant (30 min, 24 h and 4 d of infestation) and (C) at the reproductive stage of the plant (7 d, 12 d and 15 d of infestation). Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. ABA: abscisic acid; PA: phaseic acid; DPA: dihydrophaseic acid.

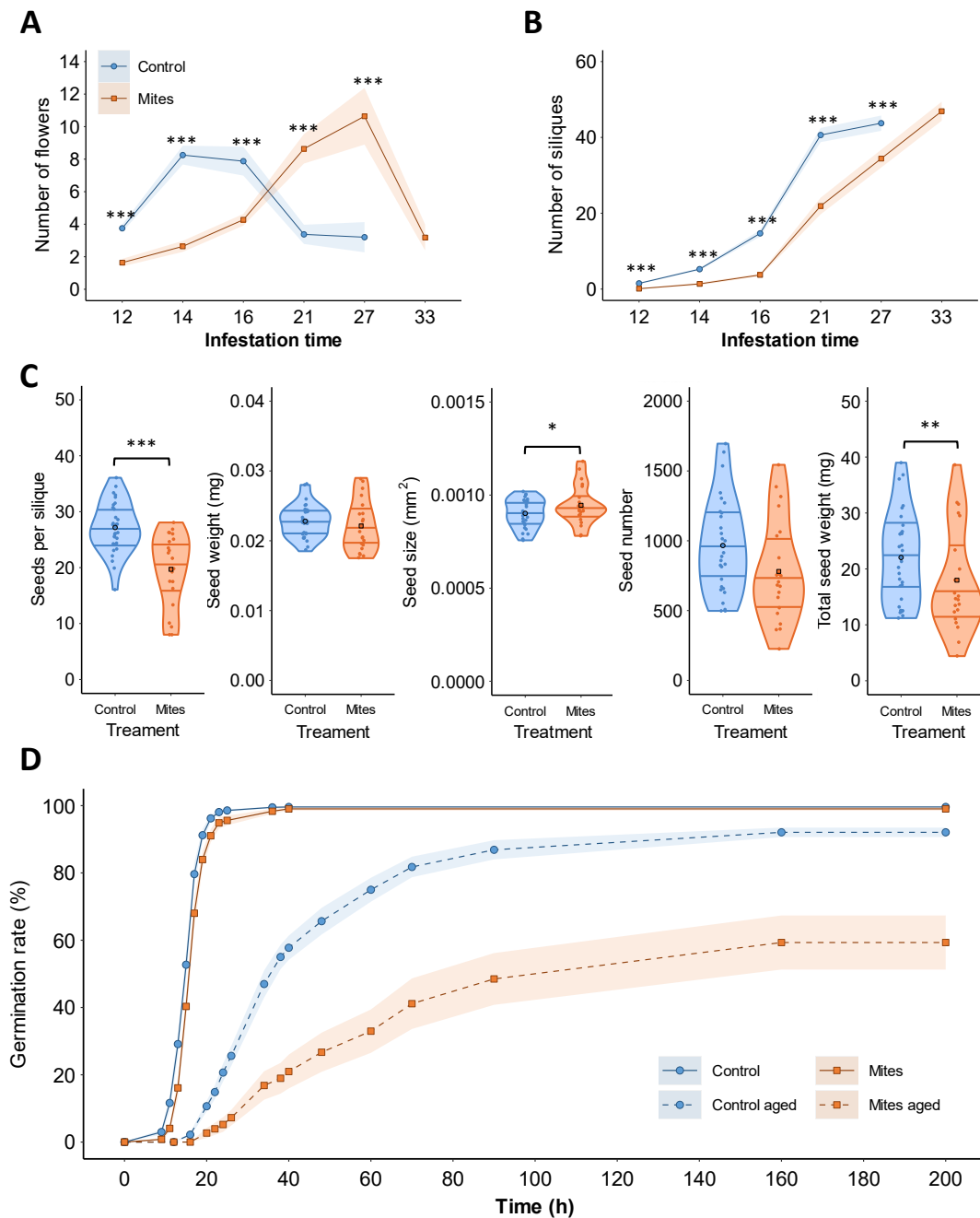


Figure 4.19. Mite infestation does not alter the final production of flowers and siliques, but it does affect the final plant fitness and germination of the offspring. Three-week-old Col-0 plants were infested with 20 adult mites of *T. urticae*. Parameters were evaluated for control and infested plants: **(A)** number of flowers, **(B)** number of siliques, **(C)** seed parameters (seed number, total seed weight, individual seed weight, number of seeds per silique, and seed size) and **(D)** the germination rate of the offspring. For **(A)** and **(B)**, parameters were measured until the end of its life cycle. In the case of **(C)**, the evaluation of seeds can only be performed at the end of the life cycle, when seeds are collected. Mean of the data is represented by a circle or a square with black border for “Control” or “Mites”, respectively. Solid lines within a violin represents Q1, Q2 (median) and Q3, from bottom to top, respectively. For **(D)**, ‘Control’ and ‘Mites’ refers to seeds of three weeks after seed collection, while ‘Control aged’ and ‘Mites aged’ refers to seeds of eighteen months after seed collection. In all cases, 11 and 16 independent replicates were evaluated for mite-infested and control plants, respectively. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student’s *t*-test for parametric data, with or without Welch’s correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$.

control and mite-infested plants, being all seeds able to fully germinate. Nevertheless, a slight delay of 2 h was observed in the germination of seeds from mite-infested plants in comparison to those from control plants, being all seeds germinated at 26 h rather than at 24 h (**Figure 4.19D**). However, seed viability was drastically affected by the aging process, and while 92% of control ‘aged’ seeds were able to germinate, only a 59% of “mite” ‘aged’ seeds germinated, reflecting the long-term effect of mite infestation on the plant fitness. Additionally, in these ‘aged’ seeds, the end of the germination was detected at 160 h, evincing the delay of germination originated by seed aging (**Figure 4.19D**). To calculate the negative effect of the mite infestation at short- and long-term level, the NMF was calculated. While at short-term level the NMF reported a value of 0.17, at long-term the NMF increased to 0.54, indicating that the negative effects of the mite infestation the final plant fitness is accentuated with the time, and manifesting the relevance of the infestation even during the seed storage.

4.4. Gibberellin homeostasis regulates plant defence-growth trade-off in response to spider mite

The evaluation of the different defence and growth hormones demonstrated the intricate regulation between the defence and growth processes, in which hormonal imbalances can affect to the global plant performance in response to specific stress. Although JA, SA and ET are the main hormones involved in the defence response triggered upon stress perception, defence-growth trade-offs suggest that hormonal imbalances in the levels of growth hormones could modulate the establishment of proper defence responses, altering the final plant performance. For this reason, a global evaluation of the defence-growth processes in *Arabidopsis* to *T. urticae* in plants with different levels of GAs and altered DELLA regulation was performed. Due to the hormonal linkage, changing levels of GAs are expected to control both growth and defence processes in response to the spider mite by modifying the hormonal balance state and potentiating or reducing the defensive plant performance, possibly associated to the plant growth response.

4.4.1. Commonalities and specificities in the GA response to *T. urticae*

To obtain a general perspective of the GA alterations produced in the GA metabolic and signalling pathways on plants after infestation with *T. urticae*, a transcriptomic meta-analysis was performed collecting data from different plant species (**Figure 4.20**). In this meta-analysis,

in addition to our RNA-seq experiment performed on *A. thaliana*, plant responses to *T. urticae* from *C. sativus*, *H. vulgare*, *S. lycopersicum*, *V. vinifera* and *Z. mays* were also included. The different experiments collected data ranging from 1-week-old plants in the case of *H. vulgare* at 7 dpi and *V. vinifera* at 24 hpi, to 5-week-old plants in the case of *S. lycopersicum* at 3 dpi. However, there was a predominant number of experiments with infestation performed at three and four weeks of plant age, such as the case of *A. thaliana*, *Z. mays*, *C. sativus* and the rest of the time point included in *S. lycopersicum*. Besides plant growth stage, infestation times also presented a high variation, ranging from 30 min to 15 d in the case of Arabidopsis. Interestingly, transcriptomic data for infestations at 24 h were found across all plant species, being an optimal point of comparison of the plant response to spider. Additionally, the number of mites required for inducing a specific plant response, varies depending on the plant species, evincing the importance of the plant host in the plant-mite interactions. A summary of the experiments included in the meta-analysis with their specific experimental conditions can be found in the **Supplementary Table D.7**.

Focusing on the GA metabolic and signalling pathways (**Figure 4.20A**), genes were classified according to the step on the pathway they are involved in. The classification included genes involved in the production of volatiles such as TMTT (4,8,12-Trimethyltrideca-1,3,7,11-tetraene), genes involved in the GA biosynthesis and catabolism, and genes involved in the recognition and regulation of the GA. Additionally, within the genes participating in the GA biosynthesis, two separate groups were also defined: those participating in the biosynthesis of the first GA compound, GA₁₂, comprising the “early biosynthesis” pathway, and those involved in the synthesis of the rest of the GA compounds since GA₁₂, the “late biosynthesis” pathway.

Once classified, the different genes were subjected to evaluation across the different plant species (**Figure 4.20B**). Commonalities and specificities in the GA responses can be extracted with the evaluation of infestation times shorter than 72 hpi, while almost no differential expression was found for none of the genes at later time points. Particularly, in response to *T. urticae*, there is an early shared induction of genes involved in the production of volatiles, except in *H. vulgare*, which showed no gene alteration in this specific route. Regarding the GA biosynthesis, some variable response with slight inductions and repressions in the genes involved in the early biosynthesis was observed in *H. vulgare*, *S. lycopersicum*, *V. vinifera* and *Z. mays*. However, there was a common general repression of the *GA20OX* and *GA3OX* biosynthetic genes accompanied by an induction of the *GA2OX* catabolic genes in the late biosynthetic pathway. Finally, focusing on the GA signalling pathway, plant responses seem to

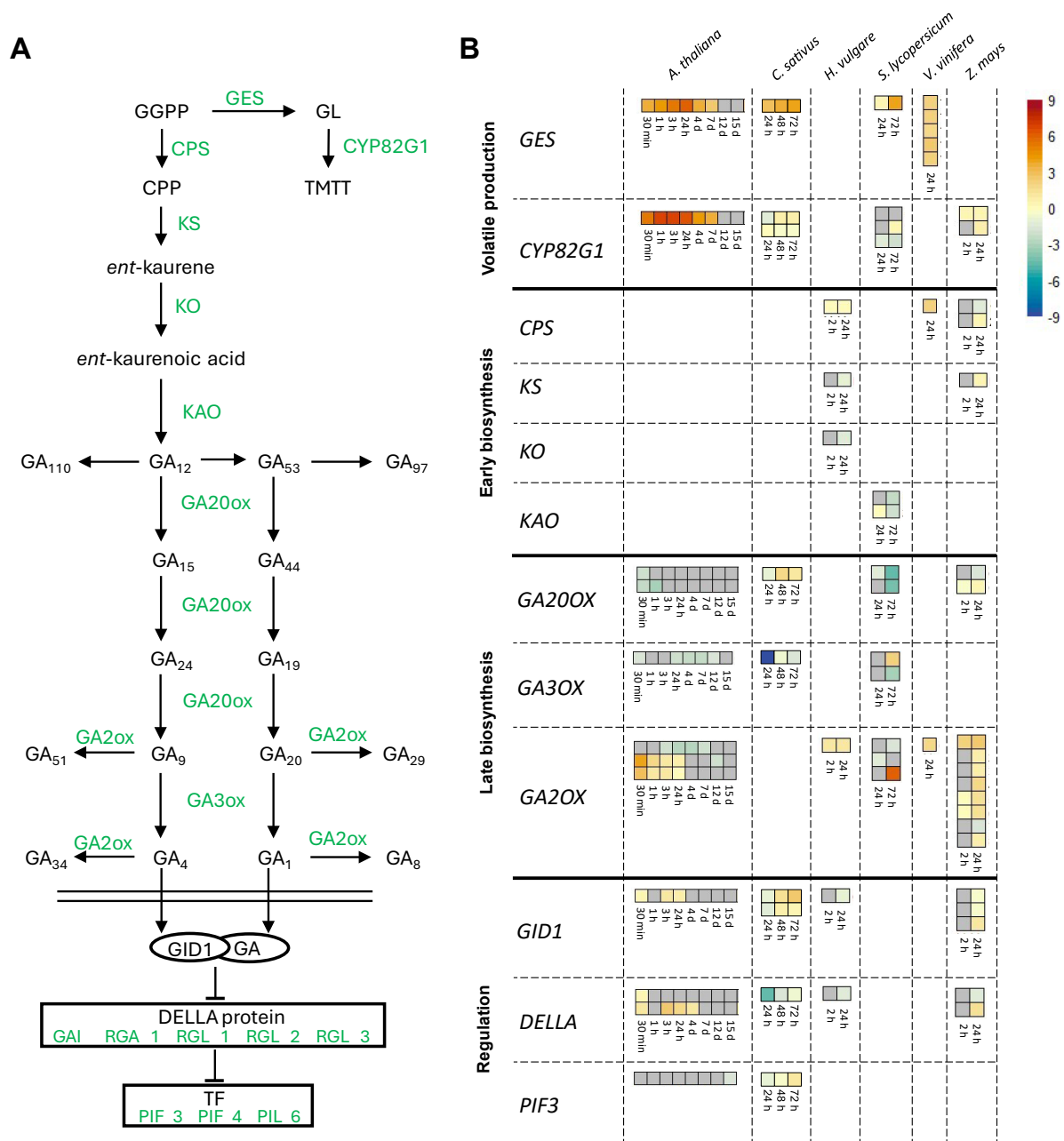


Figure 4.20. Transcriptomic profile across different plant species of all the DEGs involved in the GA biosynthetic and signalling pathways in response to spider mite infestation. **(A)** Schematic representation of the GA biosynthesis and signalling pathway with **(B)** heatmaps representing the $\log_2FC$ for DEGs at different time points of *A. thaliana*, *C. sativus*, *H. vulgare*, *S. lycopersicum*, *V. vinifera* and *Z. mays* (FDR adjusted p -value of 0.05). Upregulation or downregulation of target genes is given in red and blue, respectively (see legend). Grey colour indicates genes not differentially expressed for that time point and plant species. Genes that were not differentially expressed in none of the time points within a plant species were not plotted. GL: geranylinalool; GES: geranylinalool synthase; CYP82G1: cytochrome P450 monooxygenase; CPS: copalyl diphosphate synthetase 1; KS: ent-kaurene synthase; KO: ent-kaurene oxidase; KAO: ent-kaurenoic acid hydroxylase; GA20OX: gibberellin-20 oxidase; GA3OX: gibberellin-3 oxidase; GA2OX: gibberellin-2 oxidase; GID1: GA-insensitive dwarf 1; PIF3: phytochrome interacting factor 3.

be more specific of the plant species, showing no clear pattern of common responses. Concretely, at 24 hpi, while GID1 receptors are induced in *A. thaliana* and *Z. mays*, they are

repressed in *C. sativus* and *H. vulgare*. Similar responses are shown with *DELLA* expression at 24 hpi, in which a specific high repression of these genes and an induction are produced in *C. sativus* and *A. thaliana*, respectively. Additionally, *PIF3* response gene was only differentially expressed in Arabidopsis and *C. sativus*, being repressed in Arabidopsis at 15 dpi and in *C. sativus* at 24 hpi, showing a later induction in this last species at 48 and 72 hpi.

4.4.2. Altered plant GA levels and DELLA regulation modifies plant growth and resistance to *T. urticae*

To fully evaluate the role of GAs in the regulation of plant defence-growth responses to spider mite infestation, an evaluation of the plant performance of different mutant and transgenic lines with higher and lower levels of GA and with altered *DELLA* expression was performed. In the case of the different GA levels, the *35S:GA20ox1* and *ga2ox* lines, and the *ga20ox1/2* and *ga3ox1/2* lines, all in Col-0 background, were used as genotypes with higher and lower GA content, respectively. In the case of the altered *DELLA* expression, the *della4x* (*gai-t6 rga-t2 rgl1-1 rgl2-1*) and *rgl3* mutant plants, in combination with the *35S:RGL3* transgenic line, were used.

Being involved in the regulation of plant growth responses, a variation in the levels, distribution or signal transduction of GAs is suggested to affect the normal growth of the plants. For this reason, a phenotypical evaluation of the plant aerial parts of the different plant genotypes was performed (**Figure 4.21A**). In control conditions, *Ler* plants showed a reduced growth compared to Col-0 plants. In addition, plants with lower levels of GAs showed a reduced rosette area in comparison to the control Col-0 and *Ler* genotypes, being the *ga3ox1/2* line, the one with the smallest rosette area (**Figure 4.21B**). In addition, these plants also showed a slight change in colour, displaying a darker colour green than control plants and lines with higher GA levels (**Figure 4.21A**). In the lines with elevated GA levels, the *35S:GA20OX1* line showed the most significant growth, with a larger rosette area than the rest of the lines. However, to further characterize the importance of the GAs in the coordination of plant defence and growth responses, plant growth was additionally evaluated after mite infestations. Col-0 and *Ler* plant presented a similar rosette. Interestingly, among all tested lines, the *ga3ox1/2* was the only line displaying no growth reduction after infestation (**Figure 4.21B**). In the rest of the lines, in a smaller or larger extent, mite infestations induced a significant growth reduction in respect to the control conditions. In addition, the lines with high levels of GAs showed a similar growth as the Col-0 plants after mite infestation, losing the enhanced growth observed in the

35S:GA20OX1 line in control conditions. In lines with low GA levels, the *ga20ox1/2* plants also showed reduced growth after infestations, resulting in a rosette size similar to that of *ga3ox1/2* plants. Regarding the *della4x* plants, no differential behaviour was observed in comparison to the control *Ler* in both control and infestation conditions, exhibiting the same growth reduction as *Ler* after mite infestations. Finally, although rosette reduction was observed in both *35S:RGL3* and *rgl3* plants after mite infestation, both genotypes maintained their differences in growth respecting to their control plants, being smaller in the *35S:RGL3* and larger in the *rgl3* plants (**Figure 4.21B**). These results suggest that alteration in the GA regulation by DELLA expression is not able to suppress the growth reduction provoked by mite infestation, being only able to suppress it in the line with lower levels of GAs, the *ga3ox1/2*.

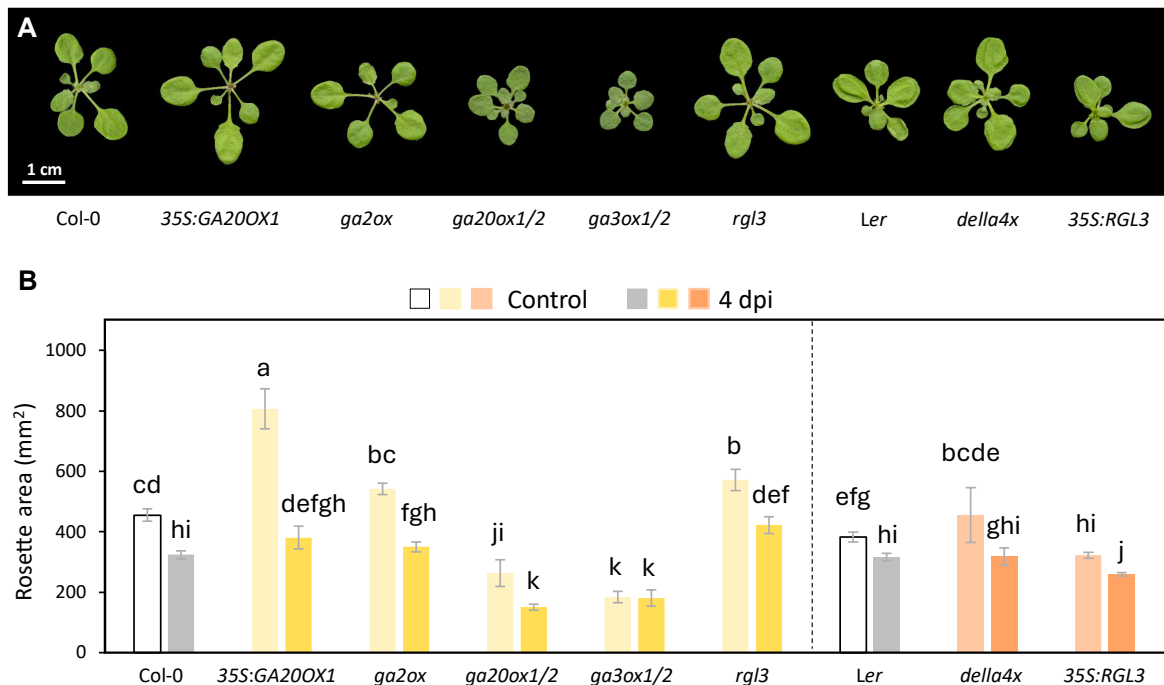


Figure 4.21. Rosette area of control and mutant plants with altered GA levels and regulation in control and mite infested conditions. Three-week-old plants of the different GA mutant and transgenic Col-0 (*35S:GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and *35S:RGL3*) lines were infested with 20 mites of *T. urticae*. (A) Rosette phenotype of the control and infested three-week-old Arabidopsis rosettes. (B) Rosette area of control and infested plants after 4 dpi (n=9). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Foliar areas were compared by means of a GLM, using a gamma distribution and a logarithm link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

In addition to the plant growth evaluation, plant tolerance or susceptibility to spider mites was assessed through the analysis of the plant damaged area and mite oviposition on the different plant lines (**Figure 4.22**). Plant damage was significantly lower in the lines with lower GA content (*ga20ox1/2* and *ga3ox1/2*), while no significant differences were observed in the lines

with higher GA content (*35S:GA20OX1*, *ga2ox*). Additionally, plant damage was also reduced in the *RGL3* overexpressing line (*35S:RGL3*), not observing any significant differences with the control in the *della4x* and *rgl3* mutant lines (**Figure 4.22A**). Moreover, the evaluation of *T. urticae* fecundity also revealed significant differences among different lines. In correlation with the plant damage, the lines with lower levels of GA (*ga20ox1/2* and *ga3ox1/2*) and the *RGL3* overexpressing line (*35S:RGL3*) were the ones that showed a reduced mite egg oviposition in comparison to their control, indicating in both damage and oviposition, a higher resistance to *T. urticae* in these three lines. Conversely, in the high GA content line *35S:GA20OX1* and the *rgl3* mutant line, it was observed a higher number of eggs oviposited in their leaves, suggesting in this case a higher susceptibility to the spider mite (**Figure 4.22B**). Finally, in the case of the *ga2ox* line and the *della4x*, no significant differences were found in respect to their control, presenting a similar resistance or susceptibility as their control plants, Col-0 and Ler, respectively.

4.4.3. Transcriptomic evaluation of the GA and defence pathways across the different GA mutants and transgenic lines

Given the observed differences in plant performance across the different plant genotypes under spider mite infestation, we sought to understand the potential role of GA in regulating plant defences. To this end, we conducted a transcriptomic analysis of genes associated with the metabolism and signalling pathways of GA, JA, and SA under both control and mite-infested conditions across the different plant genotypes.

The evaluation of the GA-related genes provided an idea of the GA status and variations in the gene expression across the different genotypes at the basal level and upon spider mite infestation. Concretely, for the analysis of the GA biosynthesis, the expression of the *GA20OX1* gene was performed (**Figure 4.23A**). As expected, the *35S:GA20OX1* line overexpressed this gene, while the *ga20ox1/2* mutant line was the one with the lowest expression. No differential expression was observed in the lines between control and mite infestation conditions except for the *35S:GA20OX1*, in which a higher expression was observed after mite infestation. Additionally, the *ga2ox* and the *della4x* lines, those with reduced GA catabolism and DELLA expression, the *GA20OX1* expression was reduced compared to their control, while in the *ga3ox1/2* and *35S:RGL3*, the expression was higher than in their control (**Figure 4.23A**). For the analysis of the GA catabolism, the *GA2OX6* gene was selected. While the expression of this gene is depleted in the *ga2ox* mutant line for both treatments, the RT-qPCR analysis revealed

that the induction of *GA2OX6* is a common effect observed after mite infestation (**Figure 4.23B**). Remarkably, the *35S:GA2OX1* presented the most elevated levels of expression upon mite infestation. Furthermore, in control conditions, in addition to the *ga2ox* mutant line, only the *ga3ox1/2* and the *35S:RGL3* showed lower expression of *GA2OX6* than their control Col-0 and *Ler* plants.

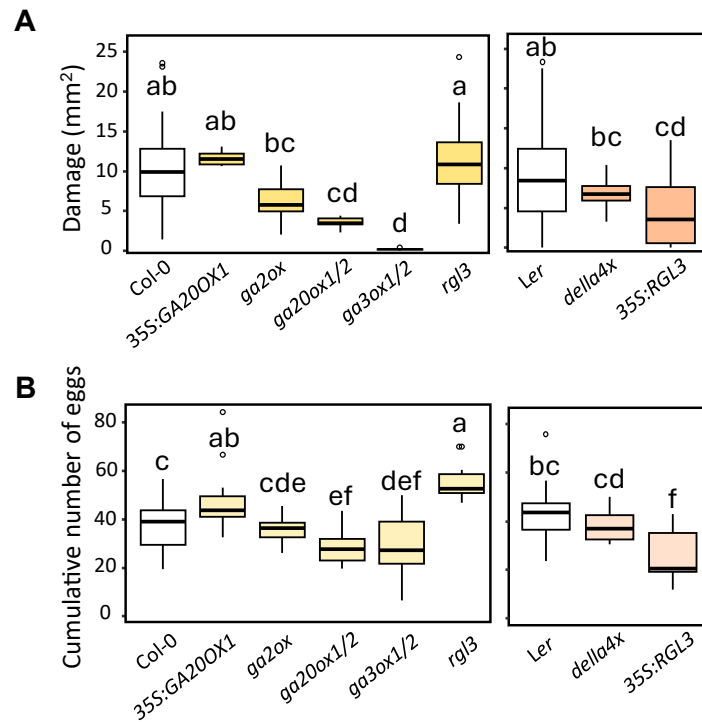


Figure 4.22. Mite performance on mutant and transgenic plants with different levels of GA and altered *DELLA* expression. **(A)** Foliar damage of rosettes after 4 dpi in Col-0 (*35S:GA2OX1*, *ga2ox*, *ga2ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and *35S:RGL3*) genotypes (n=9). **(B)** Effects on *T. urticae* fecundity measured 36 h after the infestation with synchronized mite females (n=12). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Boxplots represents the Q1, Q2 (median) and Q3, from bottom to top, respectively, and whiskers above and below the box plot indicate 1.5 times the interquartile range. Foliar damaged areas and egg counting data were compared by means of a GLM, using normal distribution and an identity link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

Moreover, to evaluate the GA regulation, we analysed the expression of the *RGL3* gene, a DELLA protein (**Figure 4.23C**). As expected, the *35S:RGL3* and *rgl3* lines, showed higher expression and repression of this gene, respectively, with no differential expression between control and infestation conditions. Interestingly, in the rest of the lines, an increase in the *RGL3* expression was observed after mite infestation, reflecting the role of *RGL3* in the plant defence response to mites (**Figure 4.23C**). In addition to this effect, in control conditions, the lines with lower levels of GA, *ga2ox1/2* and *ga3ox1/2* showed lower expression of this gene than Col-0, while the *35S:GA2OX1* showed an increased expression. In the case of the *della4x* plants,

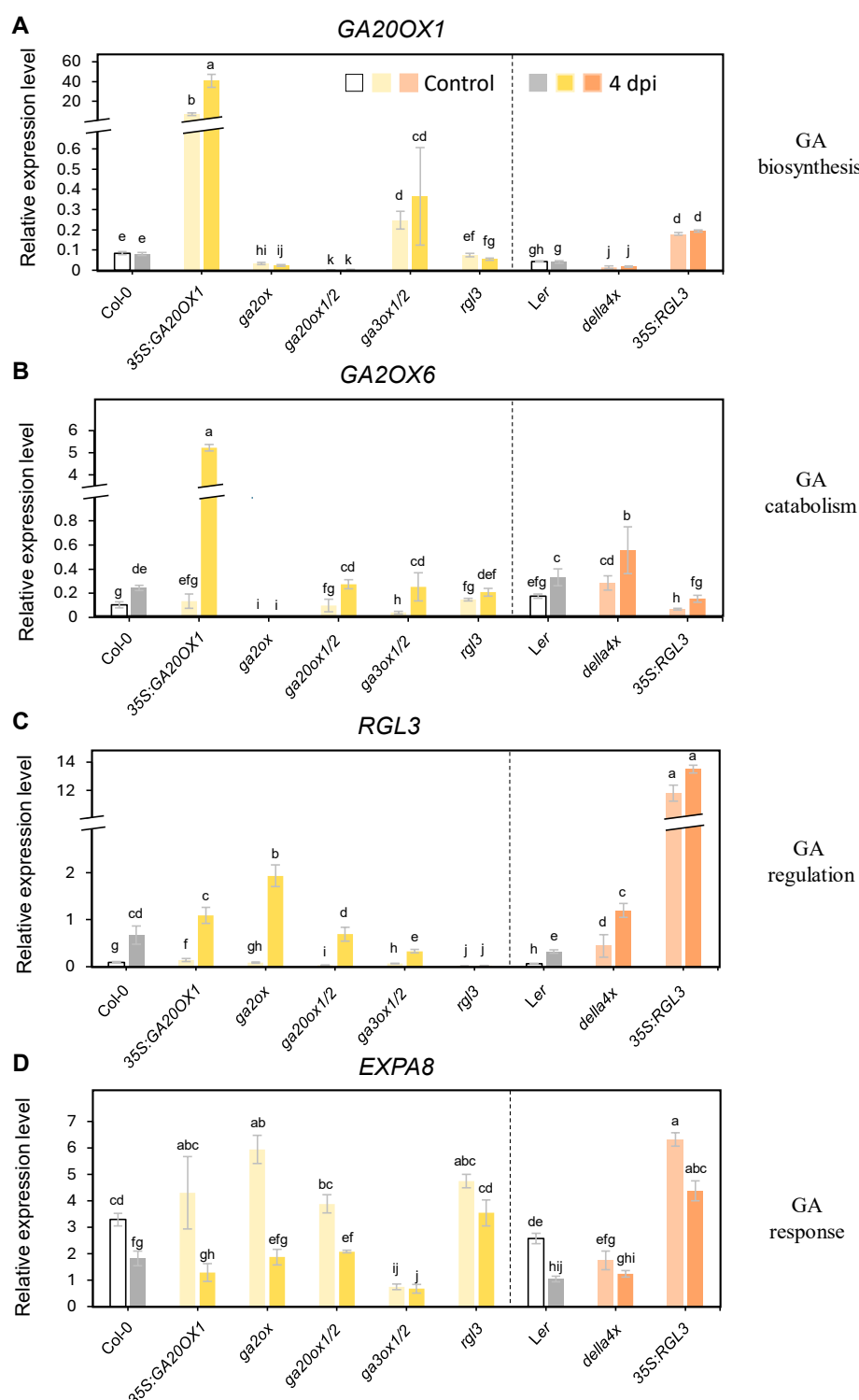


Figure 4.23. Gene expression evaluation of different genes involved in the GA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels (2^{-dCt}) of (A) *GA20OX1*, (B) *GA20X6*, (C) *RGL3* and (D) *EXPA8* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:*GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and Ler (*della4x* and 35S:*RGL3*) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a Ler background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a weibull distribution for *GA20OX1*, *GA20X6* and *RGL3*, and a gamma distribution with a logarithm function for *EXPA8*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

they also presented increased levels of expression of *RGL3* in both conditions, probably due to compensation effects with the rest of DELLA genes. Finally, to evaluate the GA responses, an analysis of the expression of the *EXPANSIN 8 (EXPA8)* was conducted (**Figure 4.23D**). The expression of this gene somehow correlated with the previously observed rosette area, showing a reduced expression tendency of this gene in all the lines evaluated except for the *ga3ox1/2* line, which presented the lowest levels of expression (**Figure 4.23D**). The *ga20ox1/2* line showed similar expression of *EXPA8* than Col-0 in both conditions, while the lines with higher GA levels showed the highest levels of expression in control conditions, being reduced to the ones observed in Col-0 after mite infestation. In the case of *rgl3* line, the expression of *EXPA8* was higher than the Col-0 in both control and infested conditions, while in the case of *della4x*, no differences were observed in respect to the *Ler* genotype. Finally, the *35S:RGL3* presented the highest expression levels of this gene in both conditions and showed a similar expression than its mutant line, suggesting that the expression of *EXPA8* is not directly regulated by *RGL3*.

Once analysed the GA pathways, an evaluation of the JA and SA defence pathways was also performed to check the variation in the defence pathway regulation and how different GA levels and altered regulation could modify these responses. In the case of the JA pathway, the genes subjected to evaluation were the *AOS*, *MYC2*, *VSP2* and *PDF1.2*. In the case of the *AOS* gene, involved in JA biosynthesis, an induction of its expression was observed after mite infestation in all lines (**Figure 4.24A**). Only higher levels of *AOS* expression were observed in the *della4x* mutant plants. In a similar way, the evaluation of the *MYC2* gene, involved in the JA regulation, showed an increased expression in all lines after mite infestation (**Figure 4.24B**). However, the *della4x* mutant line did not show any significant induction of this gene. In the rest of the lines, the *MYC2* expression was similar to that observed in their control lines, Col-0 and *Ler*, with a greater induction only observed in the *ga2ox* line after mite infestation. Finally, the expression of *VSP2* and *PDF1.2* provided an idea of the JA responses in the different lines (**Figure 4.24, C and D**). In both cases, an induction of these genes was observed after mite infestation in all lines. In the case of the *VSP2* expression, those lines with different levels of GAs and the control Col-0 showed a high level of *VSP2* expression after mite infestation. Nevertheless, although induction of *VSP2* was also observed in the DELLA mutant and overexpressing lines, the induction did not occur in the same extent, showing lower levels than in the lines with altered GA levels (**Figure 4.24C**). In the case of *PDF1.2*, slightly lower and higher levels of expression were observed in comparison to the Col-0 in control conditions for the high and low GA content lines, respectively (**Figure 4.24D**).

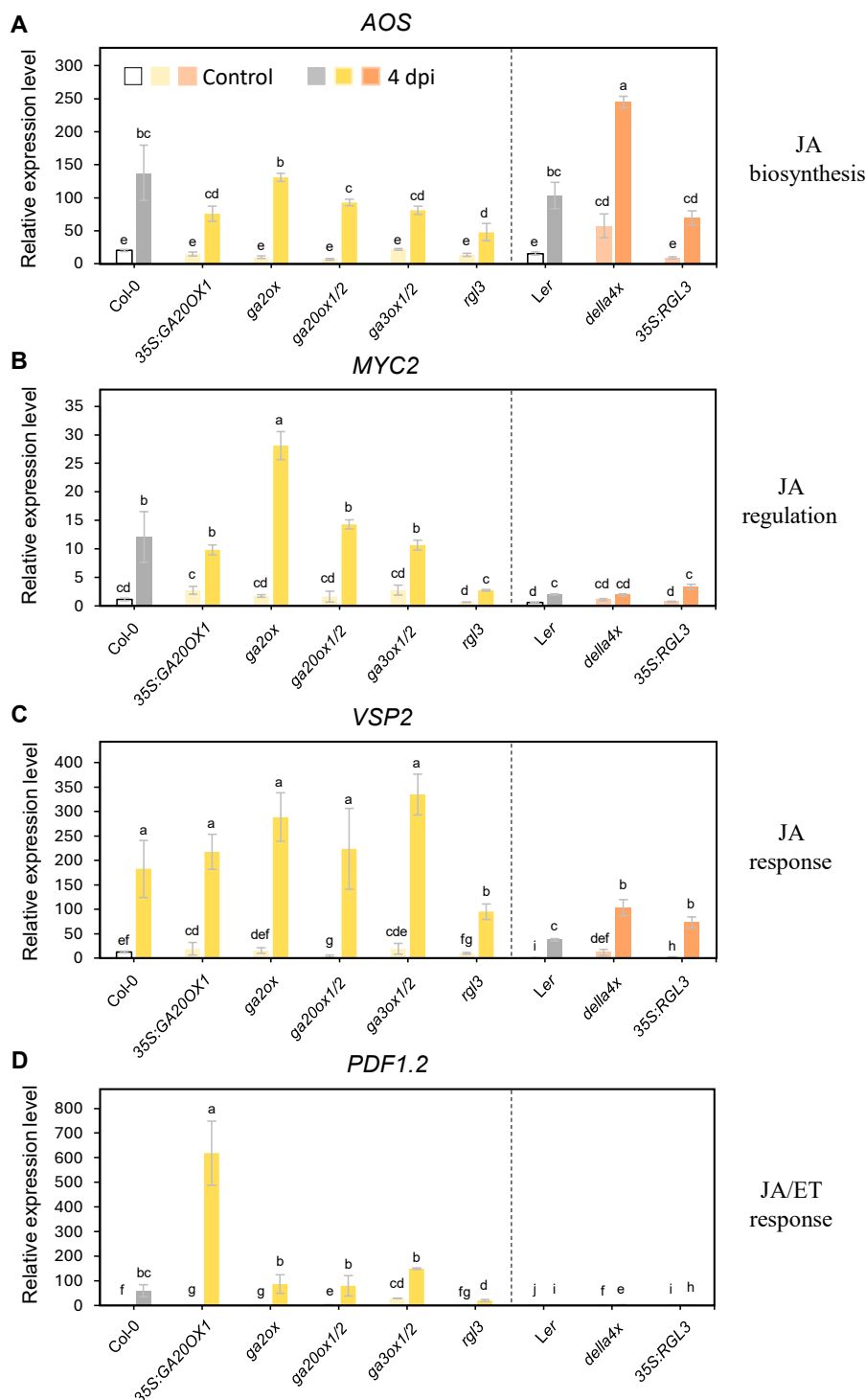


Figure 4.24. Gene expression evaluation of different genes involved in the JA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels (2^{-dCt}) of (A) *AOS*, (B) *MYC2*, (C) *VSP2* and (D) *PDF1.2* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:GA20OX1, ga2ox, ga2ox1/2, ga3ox1/2 and rgl3) and Ler (della4x and 35S:RGL3) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a Ler background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a normal distribution with logarithm function for *AOS* and *MYC2*, and a weibull distribution for *VSP2* and *PDF1.2*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

However, in infestation conditions, the induction was similar, only showing a greater induction in the *35S:GA20OX1* line. In the case of the lines with *Ler* background, the expression of *PDF1.2* was lower in all cases than those in Col-0 background, probably derived from the differences between Col-0 and *Ler* genotypes. Despite this reduced expression, *della4x* and *35S:RGL3* showed higher levels of expression than the *Ler* control, and in combination with the *VSP2* data, in general these two lines showed increased JA response than *Ler*. Conversely, in the *rgl3* showed a reduced JA response in comparison to the Col-0 control, showing reduced expression of *VSP2* and *PDF1.2* after infestation conditions. Therefore, although different GA levels do not seem to alter the JA responses in response to spider, an altered RGL3 expression lead to a differential JA response in comparison to the control plants. Finally, the evaluation of the SA pathway was performed through the analysis of the expression of the *ICS1*, *NPR1* and *PR1* genes (**Figure 4.25**). For *ICS1* gene, involved in the SA biosynthesis, no significant differences were generally found in the lines between control and infested conditions, neither with their control genotype (**Figure 4.25A**). Uniquely, the *35S:RGL3* line showed higher levels of expression of *ICS1* in control conditions, which are reduced to the levels of the control *Ler* after mite infestation. Additionally, the *ga20ox1/2* showed a significant induction of the *ICS1* after mite infestation, being the only line presenting this increase in *ICS1* expression (**Figure 4.25A**). In the case of *NPR1*, involved in the SA regulation, although a tendency to increase its expression in response to spider mite was observed, no significant differences were found between control and mite-infested plants excepting the *ga3ox1/2* and *Ler* plants (**Figure 4.25B**). In addition, the *ga20ox1/2* line presented the highest levels of expression of *NPR1*. Finally, the evaluation of the *PR1*, involved in the SA response pathway, showed a general induction in mite infestation conditions (**Figure 4.25C**). Almost no significant differences were observed between the high GA content *35S:GA20OX1* and *ga2ox* lines and the control Col-0 in control and infestation conditions. However, the *ga20ox1/2* line presented higher levels of *PR1* than Col-0 in both conditions (**Figure 4.25C**). In the case of the *ga3ox1/2* line, the basal expression of *PR1* in control conditions was higher than the one observed in Col-0. Nonetheless, after mite infestation, the expression of *PR1* is not induced, displaying a reduced expression of *PR1* than in Col-0 (**Figure 4.25C**). In the rest of the lines, *Ler* and *della4x* plants showed a similar expression of *PR1*. On the other hand, the *35S:RGL3* line presented lower *PR1* expression than *Ler* in control conditions, with a slight induction after mite infestation, while the *rgl3* mutants did not induce this gene after mite infestation (**Figure 4.25C**).

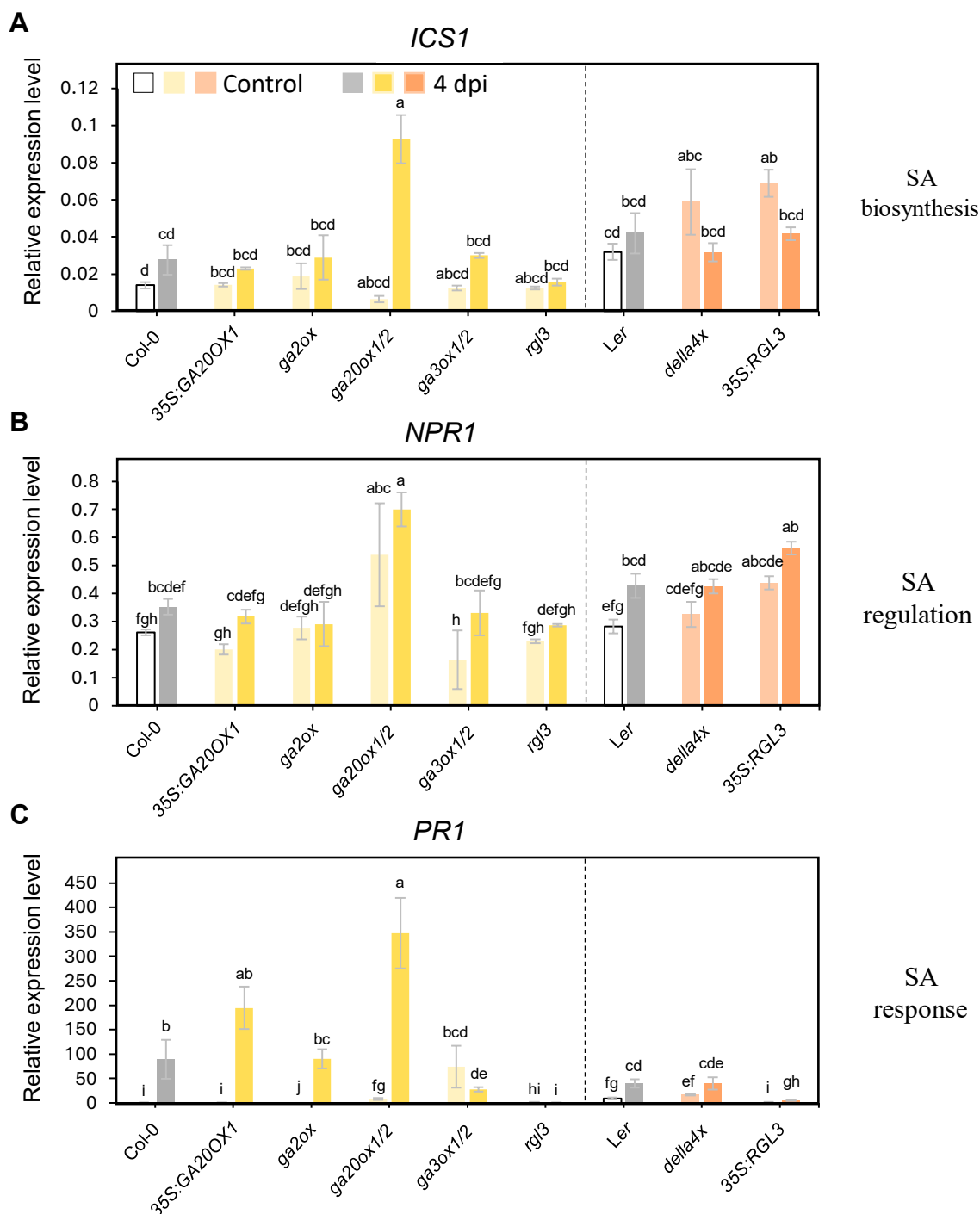


Figure 4.25. Gene expression evaluation of different genes involved in the JA metabolism and response across the different GA mutant and transgenic lines. Relative expression levels ($2^{-\Delta Ct}$) of (A) *ICS1*, (B) *NPR1* and (C) *PR1* genes at 24 hpi by RT-qPCR assays in Col-0 (35S:GA20OX1, ga2ox, ga20ox1/2, ga3ox1/2 and rgl3) and Ler (della4x and 35S:RGL3) genotypes (n=3). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a Ler background. Data are mean $\pm$ SE. Gene expression was compared by means of a GLM, using a normal distribution with logarithm function for *ICS1*, a gamma distribution with logarithm function for *NPR1*, and weibull distribution for *PR1*. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

4.4.4. Plant GA homeostasis regulates plant defence response to spider by specific metabolic routes

Based on the differences observed at the defence-growth phenotypes and due to the variable resistance and susceptibility displayed by the different lines in response to *T. urticae*, an evaluation of different defence strategies was conducted to determine the role of GA in the modulation of plant defence and growth responses. Concretely, due to the slight change in colour observed at the phenotype level in the plants with lower levels of GAs, a quantification of the anthocyanin content was performed across the lines in control and mite infestation conditions (**Figure 4.26**).

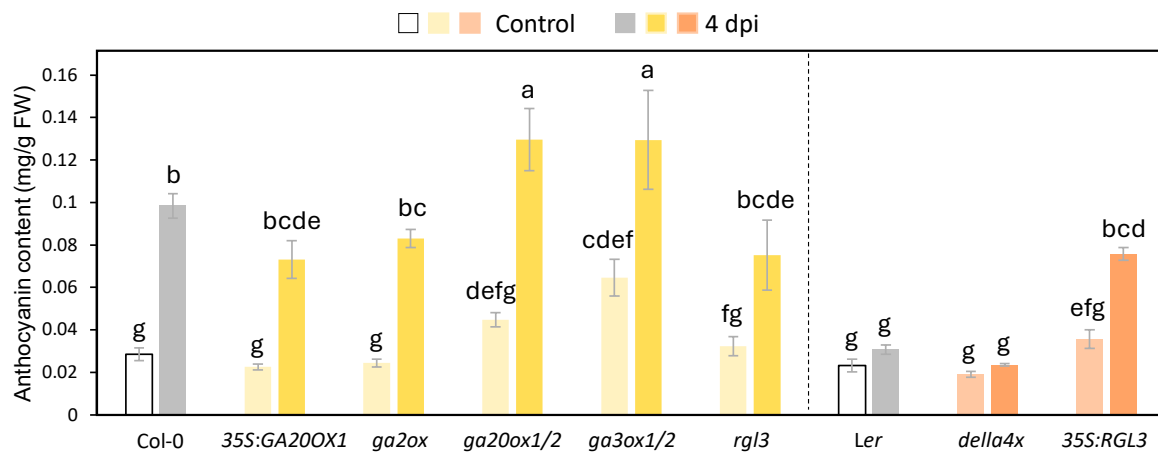


Figure 4.26. Anthocyanin determination in control and mutant plant upon infestation conditions. Quantification of anthocyanins was performed after 4 dpi in Col-0 (*35S:GA20OX1*, *ga2ox*, *ga20ox1/2*, *ga3ox1/2* and *rgl3*) and *Ler* (*della4x* and *35S:RGL3*) genotypes (n=6). Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Data are mean $\pm$ SE. Anthocyanin content was compared by means of a GLM, using a normal distribution and a logarithm link function. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. Different letters indicate significant differences ($p < 0.05$).

Interestingly, in control conditions, the lines with lower GA levels, particularly, the *ga3ox1/2* line, showed a higher basal content of anthocyanins in comparison to the rest of the lines. In the case of the *ga20ox1/2* line, although a tendency was observed, no significant differences were found. The rest of the lines showed similar anthocyanin content among them in control conditions (**Figure 4.26**). However, in all cases except for *Ler* and *della4x* plants, mite infestations induced the production of anthocyanins as a mechanism of defence, in which the lines with low GA levels, *ga20ox1/2* and *ga3ox1/2*, revealed a significantly higher content of anthocyanins than Col-0. Similarly, plants of the *35S:RGL3* genotype also showed a higher anthocyanin content than the control *Ler* after infestations, suggesting a possible correlation between the higher production of anthocyanins and the resistance to the spider mite. In contrast

to this, the high GA content lines, *35S:GA20OX1* and *ga2ox*, and the *rgl3* mutant line showed similar anthocyanin levels than the Col-0 in both control and infested conditions.

Moreover, although *35S:RGL3* plants did not show an initial change in colour in control conditions, they physically develop a toughened epidermis with a reflective waxy aspect. As these plants also showed an increased resistance to *T. urticae* and thicker cuticles have been associated with this effect, an evaluation of the plant cuticles was performed.

An analysis of the cuticle permeability was performed in control and mite infestation conditions to see whether the mite affected to this trait (**Figure 4.27, A and B**). The results revealed that in control conditions the *35S:RGL3* plants showed the lowest cuticle permeability of all the plants tested. Interestingly, *Ler* plants showed a higher cuticle permeability than Col-0 plants, being also the highest among all genotypes. However, in contrast to the lower permeability of *35S:RGL3* plants, *rgl3* mutant plants displayed an increased permeability in comparison to Col-0 plants. Remarkably, upon infestation conditions, there is an increase in the cuticle permeability for Col-0, *Ler* and *35S:RGL3* plants, not seeing this increase in the *rgl3* mutant plants (**Figure 4.27B**). However, no apparent differences in the width of epicuticular waxes and lipid content were observed in leaves with Auramine O staining (**Supplementary Figure C.8**). Interestingly, alongside the evaluation of cuticle permeability, trichome density was also assessed (**Figure 4.27C**). Unexpectedly, *35S:RGL3* plants were glabrous, lacking trichomes on their leaves. In control conditions, Col-0 plants exhibited a higher trichome density compared to the *Ler* ecotype, while *rgl3* mutants showed trichome densities similar to those of the Col-0 control. In addition, as previously observed in **Figure 4.13C**, mite infestation triggered trichome production with a reduction in plant growth, leading to increased trichome densities in mite-infested plants of Col-0, *rgl3* and *Ler*.

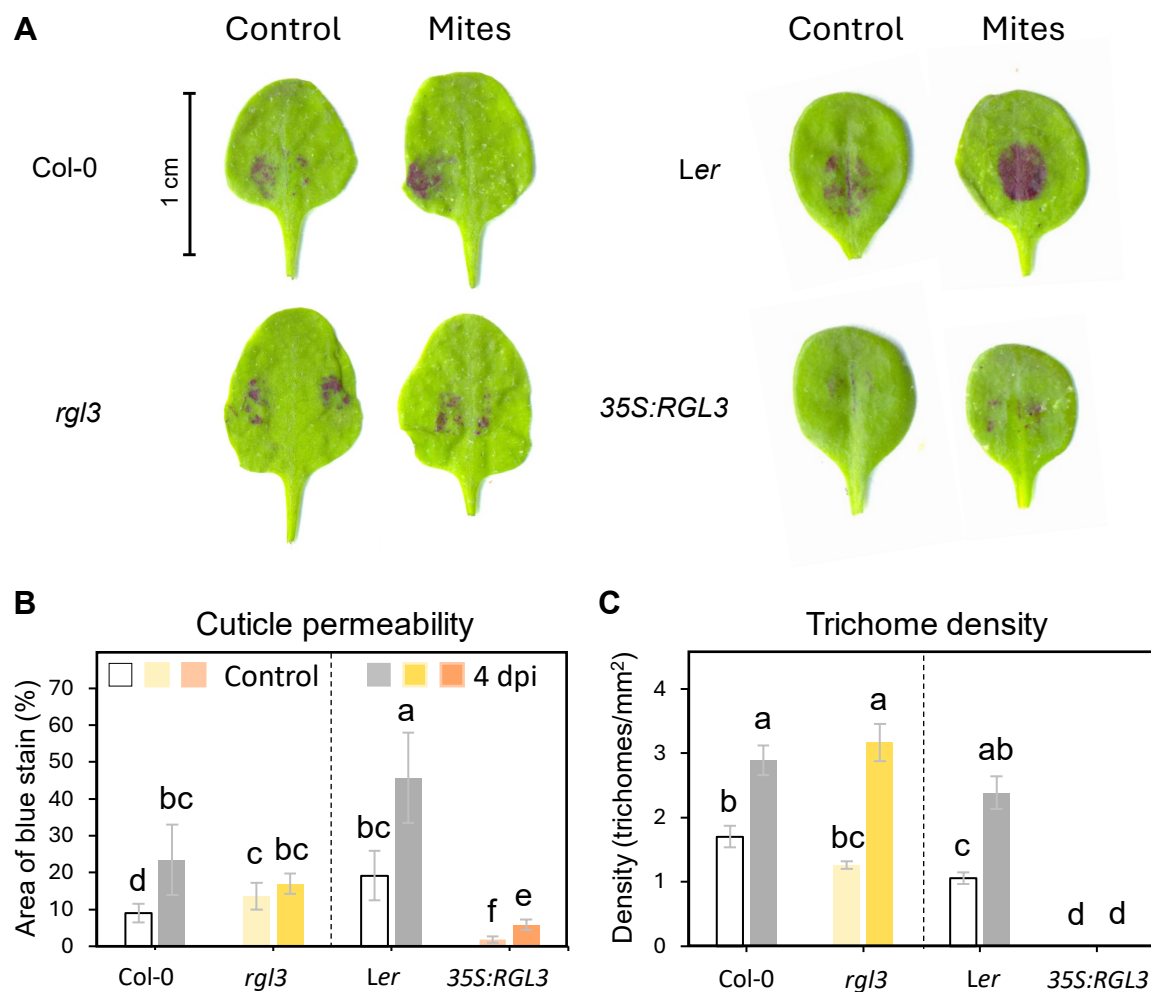
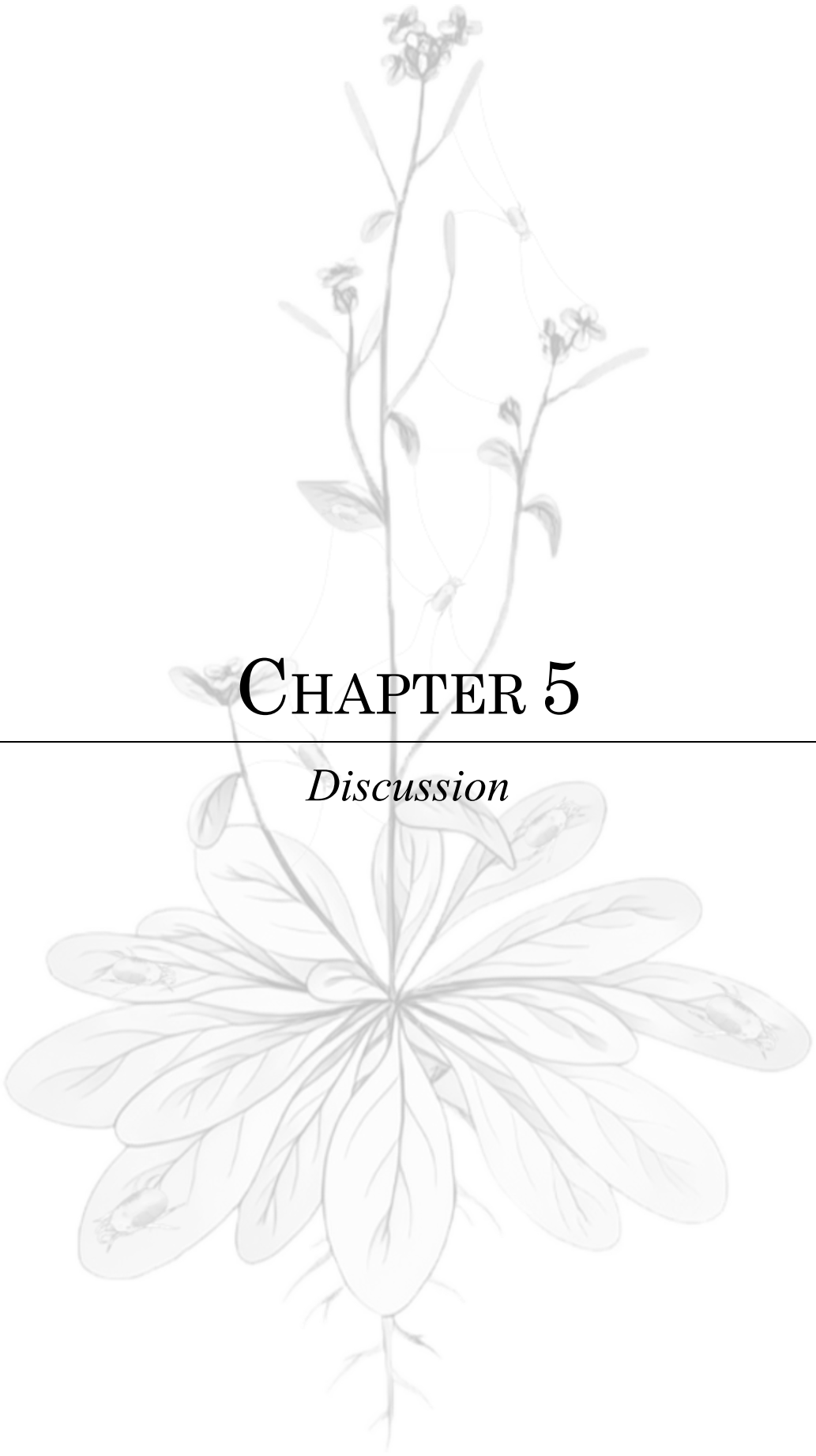


Figure 4.27. Leaf cuticle permeability and trichome density in *RGL3* mutant and overexpressing lines in control and after 4 d of mite infestation. **(A)** Toluidine blue test for cuticle permeability. A droplet was placed on the leaf surface for 2 h. After rinsing the leaf surface with water, the remaining blue stain attached to the cell wall is indicative of a permeable cuticle. **(B)** Percentage of leaf area stained after toluidine blue treatment. Leaf area covered by the toluidine blue droplet was measured before quantification and compared to the remaining blue stained area. Data are means ($\pm$ SE) from 12 biological replicates. **(C)** Trichome densities of the different lines upon control and mite infested conditions. No trichomes were observed in the *35S:RGL3* lines. Data are means ($\pm$ SE) of measurements from eight biological replicates. Yellow colour indicates those mutant and transgenic lines with a Col-0 background. Orange colour indicates those mutant and transgenic lines with a *Ler* background. Cuticle permeability was compared by means of a GLM, using an exponential distribution. Post hoc analysis was performed by applying a pairwise comparison analysis with Bonferroni correction. For trichome density, two-way ANOVA followed by Tukey test was performed after logarithmic transformation of the original data. Different letters indicate significant differences ($p < 0.05$).

A detailed botanical illustration of a plant, likely a species of Ranunculus. The plant features a basal rosette of numerous elongated, lanceolate leaves with prominent venation. A single, slender, upright stem rises from the center of the rosette, bearing several small, five-petaled flowers at the top. The illustration is rendered in a light, sketchy style with fine lines and subtle shading.

CHAPTER 5

Discussion

5. DISCUSSION

5.1. Pest infestation produces an overall impact on plant fitness that varies depending on the different parameters surrounding the plant-pest interactions

Induced plant defences are generally considered energetically costly and can negatively affect plant fitness, a multifaceted concept typically assessed through parameters such as growth, photosynthesis, carbohydrates, and reproductive success (Züst & Agrawal, 2017). Our meta-analysis supports the assumption that pest infestation adversely affects plant growth, photosynthesis, and reproduction. This negative impact was consistent regardless of various factors influencing plant-pest interactions, including the arthropod feeding guild, plant host species, plant developmental stage, and the duration and intensity of the infestation. In contrast, when examining carbohydrate content, no consistent effects were detected, likely due to the limited number of studies available and the variability in outcomes, which ranged from slightly positive to negative impacts on overall plant fitness.

However, although negative impact was observed independently of the factors surrounding the plant-pest interactions, variations in the extent of these negative effects across different subgroups were observed. In the case of the feeding guild, phloem and cell content feeders have been described to cause less mechanical damage to plants compared to chewing, mining or gall-forming phytophagous as only their stylets penetrate the sap or the mesophyll/epidermal cells (Wondrafrash *et al.*, 2013). In our meta-analysis, most of the experiments involved phloem-, cell content- and chewing insects, leading in all cases to a negative impact on plant fitness regardless of the parameter measured. Nonetheless, our data reflect that, infestations with phloem or cell content feeders had a greater impact on plant growth than chewing insects. This result delineates with those from Zvereva *et al.* (2010), who showed that sap-feeder infestations had a stronger negative effect on plant photosynthesis than infestation with chewing insects. While chewing insects remove tissue directly from the plant, feeding, phloem- and cell content feeders do not remove it, resulting in a higher amount of remaining damaged tissue after their attack. Interestingly, the reduction in plant tissue from chewing insect damage did not correlate with lower plant biomass measurements than in phloem- and cell-content infestations, a consequence that could be associated to the different and specific interactions involving different signalling pathways associated with different feeding styles (de Sousa & Peñaflor, 2024). Whereas JA is

involved in regulating defences against chewing insects (Schmiesing *et al.*, 2016), SA-mediated responses are activated by phloem-feeding insects (Kawazu *et al.*, 2012; Thaler *et al.*, 2012). However, both JA and SA responses have been shown in response to mesophyll-sucking mites (Kant *et al.*, 2004; Santamaría *et al.*, 2017; 2019; Garcia *et al.*, 2024). Upon defence activation at the infestation site, systemic defence responses are triggered to protect undamaged tissues, resulting in long-lasting induced resistance established in the form of systemic acquired resistance (SAR) or induced systemic resistance (ISR) (Durrant & Dong, 2004). SAR is associated with elevated SA levels (Mishina & Zeier, 2007; Tsuda *et al.*, 2008), where plants impaired in SA signalling are unable to develop SAR, underscoring the crucial role of SA in this pathway (Durrant & Dong, 2004). Conversely, ISR is a SA-independent pathway dependent on JA and ethylene (ET) signalling (Choudhary *et al.*, 2007). This suggests that infestations by phloem- and cell content feeders may lead to a stronger SAR response, characterized by higher SA expression and altered JA regulation, diverting fitness resources toward defence and significantly reducing plant growth compared to chewing insect infestations. In terms of photosynthesis, the less severe negative effects from chewing insects can be explained by the fact that photosynthesis is measured in the remaining tissue, which is more severely affected in the case of cell content feeders. In the case of the leafminers, only three experiments measuring their impact on photosynthesis were included in the meta-analysis. Leafminer feeding had the most substantial negative effect on photosynthesis among the feeding guilds examined. It has been suggested that plants respond to leafminer damage by balancing the negative impact on photosynthesis with improvements in water use efficiency (Pincebourde *et al.*, 2006). Finally, in the case of gall-forming insects, their impact on plant fitness varied depending on the parameters measured: no effects were detected on growth, while photosynthesis showed either no change or a slight increase. Aldea *et al.* (2006) observed that gall formation led to localized reductions in photosynthesis, with lower quantum yield measurements in damaged patches, potentially due to the formation of pathologically isolated cells upon gall-forming feeding (Huang *et al.*, 2015). However, when photosynthesis was assessed at the whole-plant level, the effects were slightly positive, suggesting a compensatory response to gall damage (Aldea *et al.*, 2006). Therefore, the specificity of induced plant responses is linked to the recognition of specific feeding styles and damage patterns and/or to herbivore-specific elicitors found in salivary secretions and regurgitates introduced during feeding (Santamaría *et al.*, 2018; Ashra & Nair, 2022). These responses lead to distinct resource allocation patterns, which can have positive, negative, or neutral effects on plant fitness. Our

findings strongly suggest that the induction of plant defences by herbivorous arthropods generally results in adverse effects on plant growth and photosynthesis, with the severity of these effects varying according to the feeding guild of the phytophagous species.

The cost of induced defences also varied depending on the specific plant host. Infestations generally had a negative impact on plant fitness across woody, herbaceous, or crop species, with the exception of a neutral or slightly positive effect observed in woody plants. These findings are consistent with a previous meta-analysis on woody plants subjected to natural or simulated herbivory (Nykänen & Koricheva, 2004), where an increase in photosynthesis was attributed to elevated sink demands in plants recovering from damage, an effect that could be attributed to higher tolerance to stress. Nykänen & Koricheva (2004) also highlighted the significance of the specific plant type in determining the cost of induced defences, showing that the growth rate of evergreen species decreased more than that of deciduous species following damage. However, no significant difference was found between deciduous and evergreen woody plants in their ability to tolerate damage from sap-feeders (Zvereva *et al.*, 2010). Additionally, herbivory was reported to have a more pronounced negative effect on monocots compared to dicots or woody plants (Hawkes & Sullivan, 2001).

Variations in the allocation of resources can occur due to differences in the chemical composition of plant structures, the relative mass of organs, or the number of structures produced. For this reason, infestations at different plant stages can differentially affect plant fitness. Our meta-analysis found that infestations during early or late vegetative stages led to a resource reallocation that negatively affected plant growth, photosynthesis, and reproduction, while no negative impact on plant fitness was observed in infestations occurred during the reproductive stage. We hypothesize that, once a plant reaches the reproductive stage, it prioritizes growth over defence to ensure proper seed development. In support of this idea, Rusman *et al.* (2019) observed that the negative effects of herbivory on flowering traits and reproductive output were more pronounced when plants were attacked early in their life cycle compared to when they had already reached the reproductive stage. More recently, Mouden *et al.* (2021) showed that MeJA application on strawberry plants during fruit production did not affect plant growth or fruit yield and quality.

Finally, as expected, our results suggest that prolonged exposure to stress results in greater negative impacts on fitness than shorter infestations, likely because of a resource allocation to defence rather than growth over extended periods of time. Longer infestations may lead to

higher pest population densities, exacerbating the negative effects on plants due to an increased number of feeding individuals. In fact, the meta-analysis underscores the significance of infestation intensity on plant fitness outcomes, with medium or heavy infestation levels consistently showing negative impacts on fitness, while light infestation levels did not produce any significant effects. These results imply that low-density infestations may allow plants to recover from initial stress, potentially enabling both the plant and the arthropod species to achieve reproductive success.

Remarkably, the meta-analysis evinces the variability present in the analysis when evaluating growth, photosynthesis or reproductive parameters. Significant differences among sub-groups were found when evaluating growth and photosynthesis parameters. However, no differences were detected within any variable when reproduction parameters were analysed. This lack of statistical differences could be attributed to the fewer experiments using reproduction parameters to estimate plant fitness, likely due to the complexity and labour-intensive nature of these measurements. It is also possible that data for reproductive parameters are primarily collected from experiments where plants survive until the reproductive phase. Experiments in which plants die prematurely due to biotic stress are unlikely to be included in analyses of reproductive parameters, which could result in an underrepresentation of negative effects in the final analysis. Another explanation could be related to the variability introduced by the parameters used to quantify reproduction. For instance, while the number of flowers can estimate the reproductive potential of a plant, this does not always correlate with seed production, especially if herbivores affect pollination (Herrera *et al.*, 2002) or if flowers are shed prematurely (Niesenbaum, 1996). In such cases, the effect sizes based on the number of seeds may differ from those based on the number of flowers. More research is needed to determine whether the absence of significant differences within variables in reproductive outcomes is due to the limited number of studies, the inherent variability in reproductive measurement methods, or specific physiological responses of plants.

5.2. The automated trichome counting method is robust and reliable for the evaluation of trichomes with basic equipment

Trichomes are unicellular or multicellular hair-like appendages developed on the aerial plant epidermis of most plant species that act as a protective barrier against natural hazards. Evaluating the density of trichomes is a valuable approach for elucidating plant defence

responses to a continuous challenging environment. However, although the interest on trichome analysis and quantification has increased over the last decades (<https://pubmed.ncbi.nlm.nih.gov/?term=trichome&filter=years.1992-2022>) and several protocols for have been developed to quantify trichomes in a rapid and automated manner, not all of them are completely accessible for all laboratories. In general, acquiring high-resolution trichome images demands advanced equipment, complex software interfaces or prior tissue manipulation (Cheng *et al.*, 2013; Pomeranz *et al.*, 2013). To avoid these limitations and make trichome quantification more accessible to researchers, we developed a fast, user-friendly and automated method that enables a direct trichome quantification on the plant epidermis without any pre-treatment step. To this end, we explored the potential of machine learning as a reliable tool for conducting high time-consuming processes in an efficient and accurate manner (Angra & Ahuja, 2017).

By employing a basic scanner and machine learning, our protocol makes trichome analysis more accessible to a wider range of laboratories. The choice to use the adaxial side of the leaves aligns with previous research and ensures consistency in the quantification process (Hauser *et al.*, 2001; Handley *et al.*, 2005). Moreover, previous methods for trichome quantification also focused on this adaxial surface to measure trichome densities and assess differences among different genetic backgrounds and plant species (Handley *et al.*, 2005; Hilscher *et al.*, 2009; Yan *et al.*, 2012; Cheng *et al.*, 2013; Pomeranz *et al.*, 2013; Hung *et al.*, 2020). In addition to this, the removal of the central part of the rosette for the analysis allowed to avoid the inconsistencies present due to dense trichome overlap. In fact, this approach matches with those observed in previously described protocols on *Arabidopsis*, where trichome quantification is performed on detached leaves, excluding the central region of the rosette from the analysis (Handley *et al.*, 2005; Cheng *et al.*, 2013; Pomeranz *et al.*, 2013).

The comparative analysis of manual and automated methods revealed that, while automated methods provide significant time savings and consistency, there were no significant differences between them in terms of accuracy. In addition, the high CCC values suggest that all automated tested methods can reliably replace manual counting, showing a great accuracy and precision with almost perfect agreement with the manual counting. In addition, the absence of significant differences between quantifications with “noisy” and “black” backgrounds confirms the robustness of the Ilastik and Fiji combination in resolving issues related to dust or other interferences previously described to interfere with the proper trichome identification process (Pomeranz *et al.*, 2013). However, among all the methods, the “Ilastik noisy background”

approach was the fastest and simplest among them, being optimal for its ease use and selected for the posterior analyses.

Testing across different *Arabidopsis* accessions with different trichome densities further demonstrated the method's reliability. Differences in leaf trichome distribution and density can lead to varying trichome pattern arrangements, which can potentially cause inaccuracies in trichome identification and final count estimation errors. Based on previous results from Hauser *et al.* (2001) and Hilscher *et al.* (2009), a selection of accessions with a broad range of different trichome densities was performed. The higher the number of trichome on the leaf, the more prone manual quantification is to human error, potentially leading to inaccurate measurements. Similar results for both automated and manual methods were found in the analysis of each accession, even in the “high density” ones, with lower standard errors for the automated method, reflecting a higher data precision through automation. The slight increase in the errors in both manual and “Ilastik noisy background” methods from accessions with lower trichome densities to those with higher densities, put into context the greater variability and difficulty in analyses with increasing trichome densities. These lower standard errors and the high correlation coefficients with the manual counting confirmed the method's precision and robustness of the automated method across varying trichome densities. In addition, the comparison with data from other literature showed that the pattern of trichome density variation was similar to that previously published (Hauser *et al.*, 2001; Hilscher *et al.*, 2009). Concordance analysis by B&A plots also reflected this high accuracy of our method with a slightly negative value that might result from overestimation in the manual counting process or underestimation by the automated approach (**Figure 4.10D**). However, the individual analysis of each of the accessions by B&A plots and their correlations highlighted a high consistency of the results obtained for low- and medium-density accessions, with a probable overestimation of the trichome counting through the manual quantification approach in the analysis of medium-density accessions. For high density accessions, there was a greater variability in the measurements with wider agreement limits between the manual and automated method (**Figure 4.11, C and I**). These larger differences for Can-0 and Rub-1 result from the inherent challenge of accurately quantify trichome when dealing with higher densities and overlapping events. Indeed, manual counts performed by the same researcher on the same leaf from these accessions resulted in different trichome numbers each time. Conversely, the automated method consistently generated the same result, effectively removing the subjectivity that can arise when interpreting regions with overlapping trichomes. However, although the agreement between

manual and automated methods has wider limits with “high” trichome densities, the method has proven to be robust, providing accurate results across all scenarios. In addition, the strong correlation observed across all accessions demonstrates that our automated method provides consistent and robust results that accurately reflect the trichome densities in each accession (**Figure 4.11, D-F and J-L**).

Moreover, the successful adaptation of the method to other plant species like *S. tuberosum* and *B. napus* indicates its flexibility and potential use of the “Ilastik – noisy background” beyond *A. thaliana*. This adaptability could be particularly beneficial in studies involving diverse plant species with varying trichome structures, broadening the method's applicability in plant phenotyping and defence research.

Overall, the “Ilastik noisy background” method presents a valuable tool for trichome quantification, providing a balance of accuracy, speed, and ease of use, avoiding the inconsistencies of variable and inaccurate measurements. Future studies could further refine this approach by integrating more advanced image processing algorithms or by expanding the method's use to other types of trichomes or additional plant species. In fact, new methodologies for trichome quantification have been developed in cotton, maize or Chinese cabbage with the use of deep learning algorithms without pretreatment of the tissues, improving the performance in the detection of overlapping trichomes (Li *et al.*, 2023; Luo *et al.*, 2023; Xu *et al.*, 2023). However, although being more specific in some cases, these analyses suppose higher computational costs with more complex evaluations, implying longer processing times of the analyses. In addition, while for the methods developed on cotton and Chinese cabbage used a stereoscope for the image acquisition, in the case of the method develop on maize they required a more specialized equipment for the image acquisition (Xu *et al.*, 2023). Therefore, the implementation of one method or another would depend on the specific requirements of the analysis and the available equipment, being our method more convenient for a faster analysis with the use of a basic scanner.

5.3. The fine-tune regulation of transcriptomic and hormonal trade-offs allows plant survival with an induced plant developmental delay and a negative effect on seed production and viability

T. urticae perception leads to an initial strong upregulation of genes associated with plant defense and a corresponding downregulation of genes involved in growth processes (**Figure 4.15C, S4.1E**). Accordingly, hormonal evaluation also reveals these defence-growth trade-offs with a temporal separation between these two processes. While the defence response initiates just 30 min after mite perception, the differential growth response begins at 24 hpi (**Figures 4.16A, 4.17A**). Therefore, there is an initial defence induction followed by a repression of the growth responses in which plant morphological and physiological adaptations to the mite occurs (**Figure 4.13**). This resource reallocation allows the plants to defend themselves and survive, entailing a cost in the final plant fitness.

After mite infestation, plants reduce plant growth and suffer a delayed entrance into the reproductive phase, resulting in the production of more rosette leaves before flowering, an effect that allows the plant more time to receive assimilates from the leaves (**Figure 4.13, Supplementary Figure C.7**). In fact, plants may adapt to the stress by slowing the growth of growing tissues while shifting metabolism to produce defence-related secondary metabolites such as indole glucosinolates (IGs), volatiles or anthocyanins (Van den Boom *et al.*, 2004; Widemann *et al.*, 2021) (**Figure 4.14E**). Bekaert *et al.* (2012) demonstrated that glucosinolate production in *A. thaliana* imposes a minimum 15% increase in the plant's photosynthetic requirement. Accumulation of IAN, a precursor of camalexin biosynthesis and a product of IG breakdown, accompanied by the induction of all the genes involved in the IG and camalexin biosynthetic pathway after mite infestation, additionally suggest the accumulation of these defence compounds (**Supplementary Figure C.2**). Moreover, we have observed that upon mite infestation, plants produce higher anthocyanin content up to 4 dpi (**Figure 4.14E**). Anthocyanins are molecules with antioxidant activity that prevent pathogen expansion in host cells (Kaur *et al.*, 2023). However, its accumulation reduces the amount of incident light reaching the photosystem (Landi *et al.*, 2015), correlating with the less efficient photosynthetic performance observed in mite-infested plants (**Figure 4.14, C and D**). Furthermore, after an herbivory attack, levels of internal sugar pools in sink tissues decrease and the photosynthetic metabolic flux suffers a reallocation to the production of defence-related compounds (Gómez

et al., 2012), suggesting that photosynthesis might become a limiting factor affecting defence-growth trade-offs in our experimental system.

Regarding the hormonal defence response, high levels of both JA-Ile and JA, until 30 min and 7 dpi, respectively, are attenuated by the expression of *JAO2* (Rosa-Diaz *et al.* 2023) (**Supplementary Dataset S3.1** Garcia *et al.*, 2024 – DatasetS2) leading to the formation of 12-OH-JA. As hydroxylated JA accumulation correlates with high JA levels, JA hydroxylation by *JAO2* emerges as an essential pathway in the plant adaptation to mite infestation. Conversely, although SA has been described to be induced upon mite infestation, our results suggest SA to be important only at the initial steps of the infestation, until 24 hpi, probably by systemically inducing resistance to *T. urticae* (Santamaría *et al.*, 2019).

In the case of the growth hormone response, auxins were the compounds mainly participating in the defence establishment and growth arrest in mite-infested plants (**Figures 4.16A, 5.1**). Auxins contribute to shoot growth promotion and are also involved in the regulation of defence responses (Ludwig-Müller, 2015). Although mite infestation leads to IAA accumulation, particularly via IAN biosynthesis (**Figure 4.16B, Supplementary Figure C.2**), auxin signalling genes including *SAUR* responsive genes are mainly repressed (**Figure 5.1, Supplementary Dataset S3.1** - Garcia *et al.*, 2024 – DatasetS2). High levels of IAA and IAN may be related to defence activation upon mite perception (Pérez-Alonso *et al.* 2021). However, high auxin levels also inhibit growth (Ludwig-Müller, 2015), requiring a precise control, which explains the accumulation of oxIAA and indole-3-acetyl-aspartate (IAA-Asp) during infestation (**Supplementary Figure C.2**). This tight regulation of auxins levels is also controlled at the transcriptional level through hormonal crosstalks with defence hormones. JA has been shown to promote auxin biosynthesis through the expression of *ANTHRANILATE SYNTHASE α1 (ASAI)* while SA acts antagonistically through stabilization of the Auxin/Indole-3-Acetic Acid (Aux/IAA) repressor proteins, in which SA production is associated with a concomitant reduction in auxin transport and signalling (Zhong *et al.*, 2021). Meanwhile, IAA also enhances JA signalling and suppresses SA biosynthesis and signalling, modulating plant defence response (Yang *et al.*, 2019a; Gilroy & Breen, 2022). Therefore, our results suggest that at the initial stages of infestation, JA starts to accumulate, inducing auxin biosynthesis and repressing SA biosynthesis. Depletion of SA or inactivation through DMR6 and DLO1 hydroxylases or BSMT1 methyltransferase, whose expression is induced at this time point (**Supplementary Dataset S3.1** - Garcia *et al.*, 2024 – DatasetS2), allows auxin accumulation and signalling, disrupting the IAA balance and allowing auxin responses and

plant growth restart (**Figure 5.1**). Moreover, the expression of *TBF1*, a transcriptional factor regulating SA signalling to balance plant growth and immunity (Pajerowska-Mukhtar *et al.*, 2012), was induced until 4 dpi, indicating an initial induction of the defence-related genes by SA, and later, allowing the subsequent resume of plant growth.

In addition, upon mite perception, there is a reduced expression of GA biosynthetic genes (*GA20OX1*) and activation of GA catabolic genes (*GA2OX2*, *GA2OX6* and *GA2OX8*) (**Supplementary Dataset S3.1** - Garcia *et al.*, 2024 – DatasetS2), an effect observed in JA-defence responses (Fukazawa *et al.*, 2023). Furthermore, GA responses are also negatively regulated through the induction of the *RGL3* gene, a DELLA protein involved in JA-responses against biotic stresses inhibiting GA responses and plant growth (Wild *et al.* 2012). Therefore, initial growth inhibition can be also a consequence of *RGL3* transcriptional control after mite perception. Furthermore, at 24 hpi, there is a lower accumulation of active GA₃, a compound described to reduce ABA levels (Ritonga *et al.*, 2023). Since ABA accumulation upon *T. urticae* (**Figure 4.18**) also induces resistance to this pest by stomata aperture regulation (Rosa-Diaz *et al.*, 2024), hormonal crosstalk between ABA and GAs is essential, leading to lower GA levels at this time point. On the other hand, accumulation of GA biosynthetic precursors such as GA₁₉ or GA₂₀ provides insight into the plant developmental status (**Figure 4.17A**, **Supplementary Figure C.4**). Indeed, higher accumulation of GA₁ in mite-infested plants at 7 dpi could result from this developmental delay (**Figure 4.17D**, **Supplementary Figure C.4**), as GAs stimulate the transition from growth to flowering, an effect already induced in control plants. However, ABA was the most relevant indicator of the plant developmental delay (**Figure 4.18**, **Supplementary Figure C.6**). Mite-infested plants accumulated less ABA than control plants at 12 and 15 dpi, resulting in a delayed senescence in these plants (Baron *et al.*, 2012). In support of this idea, ABA accumulation in control plants directly correlates with the decrease in the plant water content at 15 dpi (**Figure 4.14B**). In the case of BRs, phytohormones involved in cell elongation and shoot gravitropism (Fridman & Savaldi-Goldstein, 2013), although BL is the most biologically active compound, precursors of its synthesis such as 28-homoCS, TE or TY, and other BRs like homodolicholide (homoDL) are also biologically active shoot growth-promoting BRs (Pereira-Netto *et al.*, 2006; Xin *et al.*, 2013). During the initial steps of the infestation, control plants accumulate higher levels of TY, 28-homoCS and 28-norcastasterone (28-norCS) compared to mite-infested plants (**Figure 4.17C**, **Supplementary Figure C.3**). Less precursors upon infestation could reflect a resource reallocation process to the production of other compounds. However, BL rapidly accumulates in mite-infested plants via 28-norCS at

4 dpi, also leading to homoDL accumulation and suggesting a growth reactivation by this specific route of synthesis (**Supplementary Figure C.3**). Finally, total CK-B only show a slight reduction in mite infestation conditions (**Figure 4.17E**). Among all CK types, *tZ* and *iP* are considered the most active CKs, acting as signal molecules regulating shoot development (Howell *et al.*, 2003). Additionally, some riboside forms (CK-R), precursors of CK-B, also regulate different traits of shoot growth (Osugi *et al.*, 2017). Both *tZ* and *tZR* were reduced upon mite infestation, while *cZ*, *cZR* and *iPR* were induced (**Supplementary Figure C.5**). *cZ*-types are less active CK forms that function as stress-response markers mediating plant development, pathogen resistance and anti-herbivore responses (Schäfer *et al.*, 2015). Therefore, the reduced shoot growth upon infestation is likely an effect of the reduction of active *tZ* and *tZR* forms. However, the accumulation of *cZ*, *cZR* and *iPR* may result from plant resource reallocation to other CK-types or may be part of a mite manipulation strategy to favour infestation conditions. Therefore, plant adaptation to the mite involves a coordinated balance between transcriptomics and hormonomics, in which hormonal biosynthesis and signalling determine plant physiological readjustments. While transcriptional biosynthesis and signalling correlates during the entire plant life cycle for JA, SA and ABA, determining the initial defence response, growth responses showed an uncoupled relationship between them. Although auxin biosynthesis is induced at the beginning of the infestation, its signalling is repressed, coinciding with the reduced levels of GA, BR and CK that lead to plant growth arrest. Later induction and accumulation of these hormones allow plant growth restart and recovery, showing a delay in development reflected by ABA repression and reduction, marker of plant aging (**Figure 5.1**).

In addition to the previous results, the plant developmental slow-down caused by mite infestation resulted in a delay in plant production (**Figure 4.19, A and B**), an undesirable trait in crops. Interestingly, the number of seeds per silique was lower, with a lower total seed number of larger seeds with similar weight as those from control plants. Nonetheless, despite larger seed size having been correlated to higher tolerance to stressful conditions (Muller-Landau, 2010), contradictory results with seed persistence have been reported (Long *et al.*, 2015). Ellis *et al.* (2021) demonstrated that genetic differences influencing the number of seeds per fruit can make an important contribution to the adaptive differentiation and the genetic basis of fitness variation among natural populations of *A. thaliana*, determining that there was little evidence of trade-off between seed size and number. Ageing processes naturally decrease seed viability (Sano *et al.*, 2016); however, mite infestation exacerbates this negative effect (**Figure 4.19D**).

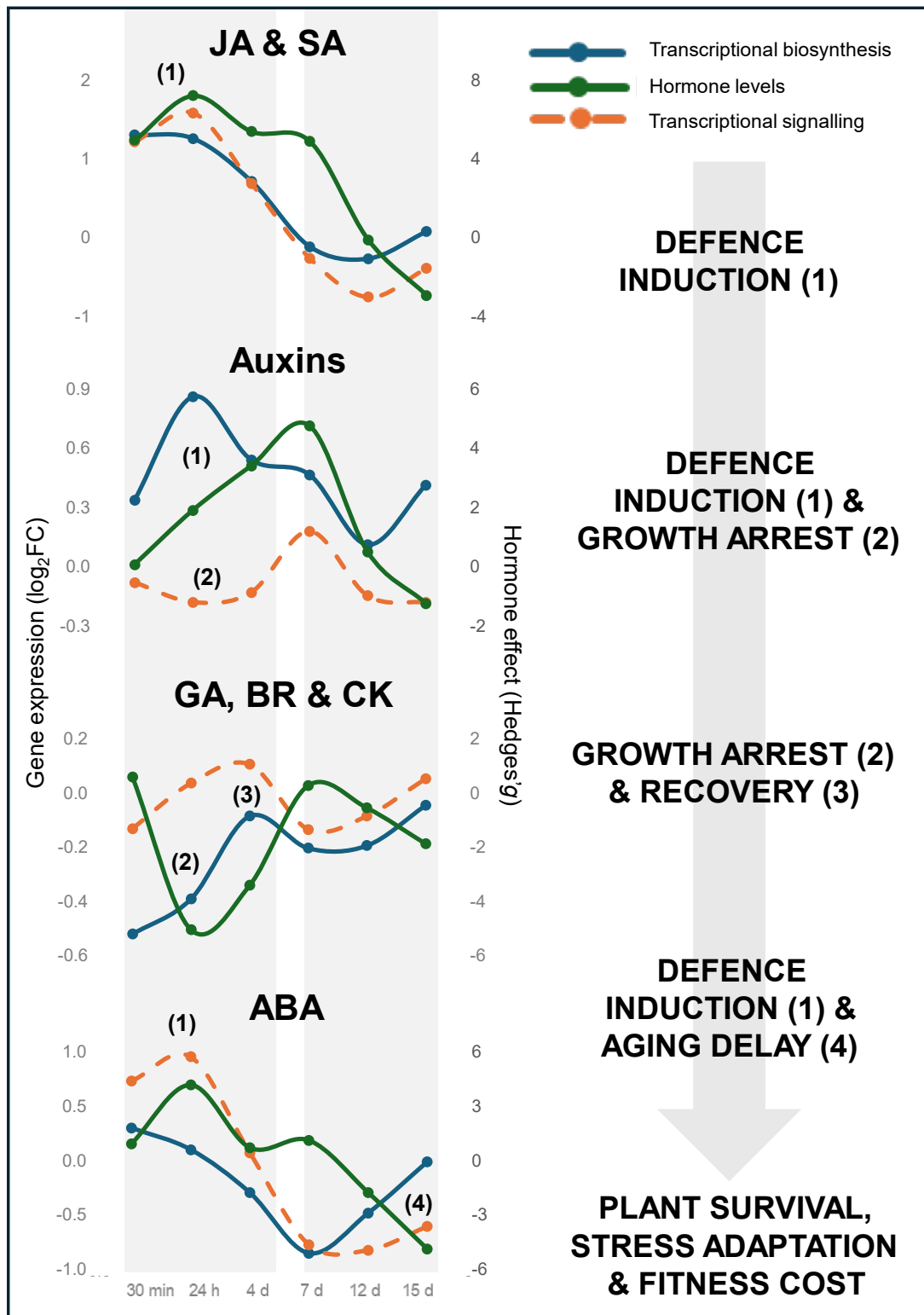


Figure 5.1. Hormone transcriptomic and quantification profiles explain plant phenotypes upon spider mite infestation. The induction of plant defenses (1) entails an initial growth arrest (2) that enable plant adaptation to the stress and subsequent growth resume (3). This growth slow down entails a delay of plant development maintained until the end of the plant life cycle with negative consequences on plant fitness. Transcriptional profiling represents the mean value of the gene expression of the differentially expressed genes involved on each hormonal pathway at each time points. Hormone effect of each hormone at each time point was quantified through Hedges' g coefficients using hormone levels between control and mite infestation conditions.

One possible explanation for this reduced seed longevity is related to resource reallocations during seed development. During seed filling, photosynthesis becomes an essential process for optimal seed formation (Külheim & Jansson, 2005). Since mite-infested plants showed a lower photosynthetic capacity than control plants (**Figure 4.14, C and D**), proper embryo development can be affected through alterations in sugar, lipid and protein seed composition. Additionally, the alterations of the hormone levels observed in the infested plants could unbalance the hormonal levels in the seeds, resulting in negative effects on seed production and viability. In this sense, it has been described that seed dormancy alteration makes seeds less resistant to ageing processes (Long *et al.*, 2015). An imbalance in the ABA-GA seed content could lead to a non-successful seed dormancy. Furthermore, CKs have also been related to seed development and size. Low CK content by overexpression of *CKX1* in Arabidopsis results in larger seeds (Werner *et al.*, 2003), an effect that correlates with the *CKX1* overexpression at 15 dpi (**Supplementary Dataset S3.1** - Garcia *et al.*, 2024 – DatasetS2). Furthermore, higher levels of *tZ*, *cZ* and *iP* in specific stages of different soybean cultivars correlate with higher seed set and improved seed filling, showing that alterations of different CK-types influence the final seed development (Kambhampati *et al.*, 2017). Therefore, determining the specific molecular events underlying this loss of seed production upon mite infestation is essential for improving the final plant fitness.

5.4. GA homeostasis regulates plant defences upon spider mite infestation

As described in the previous chapter, the coordinated transcriptomic and hormonal responses determine the specific host response to *T. urticae* infestation, with both defence and growth hormones tightly regulating each other. To investigate the effects of altered GA levels on these interactions, we analysed the performance of plants with varying GA levels and modified GA regulation. Given its fluctuating levels and the central role of DELLA proteins as a hub for hormonal crosstalk between defence and growth processes, evaluating GA remained a significant focus of this research.

The transcriptomic evaluation of the GA biosynthetic and signalling pathways provided insights into the commonalities and specificities of this route in response to *T. urticae* across different plant species. Remarkably, the higher number of DEGs observed during the early stages of mite infestation suggests that the main role of GA in the response to spider mites occurs during the

initial events of the infestation (**Figure 4.20**). However, despite the variability in the experimental conditions across different studies, our results reveal common response patterns affecting to GA metabolism (**Figure 4.20**).

Regarding GA biosynthesis, shared responses were observed across species, with a general deviation of the GA pathway to the production of volatiles coupled with a reduction in GA biosynthesis through the induction of *GA2OX* catabolic genes and the repression of *GA20OX* and *GA3OX* biosynthetic genes. Interestingly, while induction of DEGs related to the production of the volatile TMTT were not observed in *H. vulgare*, Bui *et al.* (2018) described the induction of *TERPENE SYNTHASE (TPS)* genes *MLOC_56812*, *MLOC_76989* and *MLOC_13618* during the early response to *T. urticae*, potentially linked to volatile production. Therefore, volatile production, particularly TMTT, appears to play a crucial role across species as an indirect defence mechanism, attracting natural enemies of herbivores, such as parasitoid wasps and predatory mites (Kant *et al.*, 2004; Arimura *et al.*, 2009; Tholl *et al.*, 2011; Bui *et al.*, 2018).

On the other hand, the repression of GA biosynthesis through the induction of *GA2OX* and repression of *GA20OX* and *GA3OX* has also been described in response to wounding and simulated herbivory (Pandey *et al.*, 2017; Yang *et al.*, 2020). For instance, *GA2OX6* has been shown to be touch-induced in *Arabidopsis* (Lange & Lange, 2015), while *GA2OX3* and *GA2OX7* are induced in rice in response to brown planthopper (BPH), linking their induction to JA signalling, particularly through the MYC2 transcription factor (Jin *et al.*, 2023). Furthermore, Yang *et al.* (2020) described that the induction of *GA2OX* catabolic genes is activated by JA-Ile, whereas other jasmonates derived from AOC suppress the transcript accumulation of *GA20OX* and *GA3OX*. Therefore, the observed transcriptomic results in response to *T. urticae* can be attributed to the tight regulation between JA and GA, with high JA levels decreasing the *GA20OX* expression and upregulating the GA catabolic *GA2OX* genes (Heinrich *et al.*, 2013b; Fukazawa *et al.*, 2023).

Specificities in the response were also found across the different plant species. The slight variability observed in the transcript levels of *CPS*, *KS*, *KO*, and *KAO*, involved in the early GA biosynthesis, correlated with previous findings in *Nicotiana attenuate* with simulated herbivory, where these genes showed either no change or a slight reduction in expression (Yang *et al.*, 2020). However, the responses related to GA signalling were more specific to each plant host. Particularly, *GID1* and *DELLA* expression appeared to correlate with each other and were

differentially expressed in a wider range of species, suggesting that the GA regulation by *DELLA* is a plant-specific response of high relevance during *T. urticae* infestation.

Interestingly, the observed induction in the *RGL3* expression in Arabidopsis has also been described to occur in JA-dependent responses, involving a tight regulation with the MYC2 factor (Wild *et al.*, 2012, Lange & Lange, 2015). However, DELLA proteins also compete with MYC2 for binding to JAZ factors, activating the JA signalling (Yang *et al.*, 2012). For this reason, the variations in the DELLA regulation determines both the final GA and defence responses, showing a plant species-specific response also shaped by the variable experimental conditions. Concretely, *A. thaliana* and *Z. mays* exhibited a more similar transcriptomic profile to each other compared to other species, showing induction of some DELLA genes and suggesting a shared response strategy. Conversely, the specific response of *C. sativus* involve the repression of *DELLA* genes and the induction of *PIF3* in response to the mite, a pattern not observed in the other plant species analysed.

However, the evaluation of different mutant and transgenic lines provided a broader understanding of the role of GA metabolism and regulation in the defence and growth responses to spider mite. Regarding growth, a high correlation was observed between the GA levels, the *EXPA8* expression and the rosette size. Specifically, the *35S:GA20OX1* and *ga2ox* lines, with higher GA levels, and the *ga20ox1/2* and *ga3ox1/2* lines, with lower GA levels, showing higher and lower expression of *EXPA8*, respectively. EXPA8 is a member of the expansin family of proteins that plays a crucial role in cell wall loosening, regulating cell expansion and processes such as leaf growth (Cosgrove, 2015). Therefore, increased expression of this gene could be related to the enhanced growth observed across different lines.

In the case of the DELLA mutant lines, while *della4x* plants grew similar to *Ler* plants, deregulation of *RGL3* in the *rgl3* mutant line resulted in increased plant growth. In contrast, overexpression of *RGL3* reduced growth, highlighting its involvement in rosette growth regulation. Interestingly, spider mite infestations led to a reduction in growth across all lines, except for the *ga3ox1/2* mutants, suggesting that plants with lower GA levels may exhibit greater tolerance to mite attack, a response similar to the one observed under drought conditions (Colebrook *et al.*, 2014).

The evaluation of the mite performance revealed that lines with low GA levels showed a higher resistance to spider mite while the line *35S:GA20OX1*, with elevated GA levels and increased growth, showed a higher number of oviposited mite eggs on its leaves, reflecting in both cases

an inverse relationship between the defence and growth processes. In addition, *RGL3* expression in *rgl3* and *35S:RGL3* lines also correlated with increased susceptibility and resistance to *T. urticae*, respectively. Interestingly, the similar response of the *della4x*, without any mutation in *RGL3*, compared to *Ler* in response to spider mite, highlighted the pivotal role of *RGL3* in regulating responses to the spider mite. In fact, this gene has also been implicated in growth restriction under biotic stresses (Li *et al.*, 2019b), establishing a link between defence and growth processes.

The transcriptomic evaluation of JA, SA and GA revealed the conserved induction of JA-related genes and *PR1* upon infestation, with the exception of *ga3ox1/2*, which did not show *PR1* induction (**Figure 4.24 and 4.25**). Plant defence responses to spider mite are primarily directed by JA and SA (Santamaría *et al.*, 2017; 2019), leading in the case of JA-related responses to an induction of the *GA2OX* genes, as observed for the case of the *GA2OX6* in all the lines, and of *RGL3*, which competes with JAZ1 and JAZ8 to positively regulate JA responses (Verma *et al.*, 2016; Jin *et al.*, 2023). However, no reduction in *GA20OX1* transcript levels was observed, probably because this reduction occurs before 24 hpi in Arabidopsis, as specified in **Figure 4.20**. Interestingly, due to the lack of variation in the evaluation of the JA and SA pathways across lines, the higher resistance observed in lines with lower GA levels or increased *RGL3* expression may be driven by a specific subset of plant defence responses modulated by GA and DELLA. GA-deficient *gal-3* mutant lines induce some WRKY transcription factors that might represent downstream targets of DELLAs (Navarro *et al.*, 2008). In rice, the OsWRKY71, involved in inducible defence response (Liu *et al.*, 2007), suppress the GA inducible *Amyb32b* α -amylase promoter and is inhibited by GA treatments (Phukan *et al.*, 2016). DELLA stabilization has also been reported to induce resistance to necrotrophs (Navarro *et al.*, 2008), which correlates with the higher resistance observed in *35S:RGL3* plants upon mite infestations. Additionally, foliar low dosage GA treatments in rice enhanced susceptibility to the nematode *Meloidogyne graminicola*, as degradation of the rice DELLA protein SLR1 by GA attenuates the basal JA-defences responses (Yimer *et al.*, 2018).

Interestingly, although low GA levels and *RGL3* regulation alter different steps of the GA pathway, there is an enhanced production of anthocyanins in both lines with lower GA levels and the *RGL3* overexpressing line upon spider mite infestation. As observed in our results, the production of anthocyanins is a specific response known to confer resistance to *T. urticae* (Li *et al.*, 2019c; Hoseinzadeh *et al.*, 2020). In the lines with lower GA levels, uninfested plants showed a higher basal anthocyanin content than uninfested control Col-0 plants, indicating a

higher basal defence response related to the decrease in the active GA content. Upregulation of positive regulatory factor production of anthocyanin pigment1 (PAP1) and other genes involved in the anthocyanin biosynthetic pathway have been described in Arabidopsis plants with low active endogenous GA, showing an opposite correlation between GA levels and anthocyanin content (Loreti *et al.*, 2008). However, in the case of the *RGL3* overexpressing line, enhanced content was only observed after mite infestation, probably due to the induction of enhanced JA-responses that also increase the anthocyanin content (Verma *et al.*, 2016; Xie *et al.*, 2016; Goossens *et al.*, 2017). Therefore, defence regulation by GA levels or DELLA can regulate defence responses by alternative mechanisms of action.

Finally, besides the anthocyanin production, altered expression of *RGL3* specifically affected the cuticle permeability, in which overexpression of *RGL3* reduces the permeability while its absence of expression increases it (**Figure 4.27**). Alterations of the cuticle permeability has been associated with variations in the amount of waxes and composition of the cuticle, an effect described to alter plant-arthropod interactions by reducing phytophagous attachment to the plants (Reina-Pinto & Yephremov, 2009; Gorb & Gorb, 2017; Lewandowska *et al.*, 2020). In fact, thicker leaf cuticles in the barley *HVPap-1* knockdown lines protected them from *T. urticae* feeding, hindering the mite stylet penetration on the leaves and their establishment on plants (Gomez-Sanchez *et al.*, 2019). In addition to these results, the effect of mite feeding on plant increased the cuticle permeability, probably as a consequence of the mite feeding process. In fact, the stylet penetration between the epidermal cells or through the stomata may facilitate the stain penetration. However, *RGL3* overexpressing plants not only showed reduced cuticle permeability but also a glabrous phenotype. The presence of trichomes has been previously described as an important barrier to mites, hampering mite feeding and establishment on plants (Rakha *et al.*, 2017; Resende *et al.*, 2020). Interestingly, although no trichome development was observed, *35S:RGL3* showed higher resistance to *T. urticae*, suggesting an important role of this increased cuticle permeability in the hindering of mite establishment. In fact, plant epicuticular lipids can also influence locomotion, and feeding, but also host acceptance for oviposition (Lewandowska *et al.*, 2020). As lower feeding and mite oviposition was observed in these plants, it is suggested that the altered composition of these lipids is highly affecting mite performance on plants. Furthermore, increasing evidence of a genetic interplay between trichome development and cuticle formation have been described across various species including *A. thaliana*, with common key transcription factors such as AtMYB106 or AtMYB16 participating in the regulation of both processes. However, the specific molecular mechanisms

directing these responses remains largely unexplored (Berhin *et al.*, 2022). Different wax components might trigger signalling cascade impacting trichome development through the repression of CK biosynthesis, a hormone described to induce trichome development, by repressing *IPT3* expression (Berhin *et al.*, 2022). Our results suggest that the *RGL3* overexpression on plants might affect these key factors or alter hormonal balance between defence and growth processes, leading to this glabrous phenotype with reduced cuticle permeability.

In conclusion, the GA homeostasis is essential in the regulation defence-growth processes. Varying levels of GA and altered DELLA expression differently modulate plant growth and defence response by specific mechanisms involving hormonal crosstalk. Although JA responses specifically regulate GA biosynthesis and DELLA expression, higher and lower levels of GA increases susceptibility and resistance to *T. urticae* infestation. In contrast, DELLA regulation, particularly by RGL3, regulates plant resistance through common and specific responses, such as altering anthocyanins and cuticle permeability, but affects the normal plant development as observed in the glabrous plant phenotypes.

5.5. General discussion

The current research delved for the first time into the evaluation of the plant defence-growth trade-off established during Arabidopsis plant development upon spider mite infestation, covering the analysis of the defence and growth processes at the phenotypical, transcriptomic and hormonal level during the entire plant life cycle (**Figure 5.2**). This multifaceted evaluation enables a more detailed analysis of the global plant responses to the spider mite, highlighting the molecular changes and specific adaptations that allow the plant to survive with a cost in the final plant fitness. In fact, this is the first analysis including a hormonal and transcriptional model for the defence-growth trade-off upon infestations with *T. urticae*. So far, previous studies primarily focused on the evaluation of defence and growth processes at a specific developmental stage, not reflecting the dynamic variation in the response and interconnection of these processes over the course of plant development.

Our meta-analysis provided valuable insights into the variation in plant responses across different experimental conditions and the variable estimations of fitness costs when evaluating different parameters (**Figure 5.2**). Generally, the cost of induced defences negatively affects growth, photosynthesis and/or reproduction. However, although some studies reflected a

positive or negative effect on the plant carbohydrate content (Hutchinson *et al.*, 1994; Macedo *et al.*, 2009; Schmidt *et al.*, 2009), these changes appear to be more species-specific. Factors such as feeding guild, host plant, the ontogenetic stage and the duration and intensity of the infestation differently impact final plant fitness, with the arthropod feeding guild and plant host being key determinants of the final effect. In fact, the specific defensive routes elicited by different type of arthropods, involving mainly JA, SA, ET (de Vos *et al.*, 2005; Glazebrook *et al.*, 2005), determine the different responses and hormonal crosstalk established in the plants, potentially modulated by BRs, CKs, auxins or GAs (Erb & Reymond, 2019), as reflected in our dynamic model. In addition, different plant host display different defensive strategies, as in the case of the specific production of glucosinolates in Brassicaceae plants, estimated to be highly costly for them (Bekaert *et al.*, 2012; Wu *et al.*, 2021). Additionally, the absence of negative effects observed in light and short infestations may be linked to the plant's capacity to tolerance the stress, where resource allocation between defence and growth is less impacted than in more intense or prolonged infestations, not resulting in observable defence-growth trade-offs. Furthermore, the analysis of reproductive parameters, which are the closest indicators of fitness costs, is highly relevant for evaluating plant fitness, but fewer studies focused on them. This previous information not only offers a valuable general perspective on plant-pest interactions useful for other researchers (Mouden *et al.*, 2021; Erofeeva, 2023; de Sousa *et al.*, 2024; van Kleeff *et al.*, 2024), but also helped us to determine the specific experimental conditions and parameters to measure in our defence-growth trade-off analyses.

The selection of a time-course experiment with multiple time points of analysis during the vegetative and reproductive stages allowed us to track the dynamic changes in physiological, gene, and hormonal responses, deepening our understanding of their evolution throughout plant development. Usually, evaluation of the plant responses in response to herbivores commonly observes the accumulation of specific defence hormones such as JA and SA at a specific time point, not observing the timing and the duration of its induction during the entire plant life cycle combined with the varying levels of the rest of hormones (de Vos *et al.*, 2005). During this process, the relevant role of trichomes in response to spider mite infestations (Rakha *et al.*, 2017; de Resende *et al.*, 2020), led us to the generation of an easy, rapid and automated trichome quantification protocol to quantify them (**Figure 5.2**). This method could be approachable and valuable for the scientific community, with potential applicability to other plant species, avoiding the limitations of previous methods with complex software interfaces, prior tissue manipulation or advance equipment for obtaining high-resolution images (Cheng *et al.*, 2013;

Pomeranz *et al.*, 2013). In combination with trichome quantification, the analysis of physiological parameters revealed the presence of defence-growth trade-offs at the phenotype level, where the induction of defence mechanism, such as trichome and anthocyanin production, was accompanied by reductions in growth, photosynthetic performance, and reproductive seed set.

Interestingly, in correlation with these phenotypes, the integration of transcriptomic and hormonal data allowed us to generate a model illustrating the defence-growth responses. Particularly, while JA and SA regulate the main defence responses in combination with auxin and ABA, repression of auxin responses together with the reduction of certain GA-, BR- and CK-related compounds lead to the initial growth arrest. From these results, it is remarkable to establish that while previous studies described the role of both JA and SA, and recently, ABA responses in orchestrating the plant defence responses to *T. urticae* at short-term level (Santamaría *et al.*, 2017; 2019; Rosa-Diaz *et al.*, 2024), our long-term evaluation revealed that SA accumulation decreases earlier in the response, while JA levels increase, underscoring the importance of the timing of the responses and the long-lasting effect of JA upon mite infestation. The described SA accumulation in mite-infested plants correlated with the plant growth arrest, in which auxin signalling was repressed along with lower levels of BRs, GAs and CKs, manifesting the antagonistic behaviour of SA with these hormones (Huot *et al.*, 2014; Zhong *et al.*, 2021; Johnston *et al.*, 2024). Later, JA accumulation increase, and likely due to the synergetic effect between JA and auxins (Jiaquiang *et al.*, 2009; Hentrich *et al.*, 2013), as well as their antagonism with SA (Robert-Seilantantz *et al.*, 2011; Zhong *et al.*, 2021), the SA accumulation decrease, allowing growth to resume with increasing growth hormone levels. This timing enables the proper plant adaptation to the mite through establishment of the defence-growth responses with a cost in the final plant fitness, resulting in a lower seed production followed by a reduced seed viability at long-term level. Reduced seed production is usually a common response to herbivores that affects host population densities of plants without site-limited germination (Myers & Sarfraz, 2017). However, infestations can also lead to physiological damage including impairment of DNA, erroneous RNA synthesis, protein synthesis breakdown, lipid peroxidation, among other processes, occasioning detrimental effects on long-term seed viability, that could be relevant during seed storage (Mahapatra *et al.*, 2019). In addition, impairing proper seed filling and development through resource allocation processes or alter seed dormancy by imbalances in the hormone levels derived from the infestation process could also affect to this long-term viability (Werner *et al.*, 2003; Külheim

& Jansson, 2005). Consequently, it is crucial to consider the long-lasting effects of mite infestation on seeds, as seed ageing processes exacerbate the negative impact of the mite on plants, hindering off-spring establishment.

However, as described by Sestari & Campos (2022), the resource allocation described in defence-growth trade-offs is a complex interplay of genetic and metabolic networks that integrates both external and internal signals. In this regard, transcriptional modules involving hormonal signalling pathways could control resource allocation decisions and provide molecular switches between immunity and growth processes, highlighting those affecting the JA and SA regulation, involved in responses to different pest behaviours. Particularly, among these molecular switches, those involving JA-GA and SA-auxin crosstalk have been more extensively studied in biotic interactions (Naseem *et al.*, 2015; Tan *et al.*, 2020; Liu *et al.*, 2021b), although involvement of BRs and CKs have also been described to regulate JA and SA responses (Shigenaga *et al.*, 2017; Jang *et al.*, 2020). In fact, the alteration of the negative SA-CK crosstalk in *ckx3,5* mutant plants with increased endogenous CK levels, is able to release plant growth suppression while providing enhanced pathogen resistance (Johnston *et al.*, 2024), highlighting the involvement of CK in the defence-growth regulations.

Therefore, as suggested by other studies (Huot *et al.*, 2014; Albretch & Argueso, 2017; Erb & Reymond, 2019) and observed in our model, the entire process of plant adaptation involves complex hormonal interactions, in which apart from JA, SA and ET, the rest of hormones including CKs, auxins, BRs and GA shape the final specific response.

Given the complexity of these interactions, even a subtle change in the regulation of a hormone can significantly alter the global plant response. Therefore, the potential existence of molecular switches regulating defence-growth processes, such as the previously described in *ckx3,5* mutants (Johnston *et al.*, 2024), suggest that rewiring of hormone-associated transcriptional pathways may offer a simple and effective strategy to develop crop varieties with enhanced pest resistance without compromising growth, uncoupling defence-growth trade-offs (Sestari & Campos, 2022). For instance, the introduction of a mutation in the photoreceptor PHYTOCHROME B (*phyB*) in stunted Arabidopsis quintuple JAZ mutants (*jazQ*) with increased resistance to insect herbivory through the constitutive activation of the JA pathway, activates the GA transcriptional module producing larger rosettes and faster development than WT plants (Campos *et al.*, 2016).

In this line, based on the relevance of JA in response to mite infestation and the potential of growth hormones in regulating defence responses, the evaluation of plant performance upon spider mite infestation in lines with altered GA regulation provided significance of the GA-mediated defence-growth regulation (**Figure 5.2**). Deviation of the GA pathway towards volatile production followed with a repression of the GA biosynthesis through the main induction of *GA2OX* catabolic genes, was a common response to *T. urticae* infestations across different species, an effect already described to be mainly directed by JA responses (Bui *et al.*, 2018; Yang *et al.*, 2020). However, DELLA regulation was a more species-specific response, with *Arabidopsis* showing the specific induction of *RGL3*, also described to enhance JA responses (Verma *et al.*, 2016).

The JA-GA antagonism was reflected in our results as a module regulating defence-growth trade-offs. As suggested by the resource allocation theory, the reduction in growth and susceptibility to the mite was higher in lines with elevated GA content, potentially related to the mobilization of resources from growth to defence processes. Conversely, opposite results were found in lines with low GA content. These lines showed low or no growth reduction and an enhanced resistance to the mite, that would correlate with a lower resource reallocation from growth to defence. In fact, higher basal contents of anthocyanins were found in these lines, suggesting the higher investment on defences in control conditions at the expense of growth, linking lower GA responses with the modulation of different defensive pathways such as anthocyanin production (Loreti *et al.*, 2008; Jie *et al.*, 2023). Finally, being previously described to be involved in responses to biotic stresses (Wild *et al.*, 2012; Verma *et al.*, 2016), our results revealed the importance of *RGL3* expression, among the rest of DELLA proteins, in plant responses to *T. urticae* infestation. The tight regulation between *RGL3* and *JAZ*, makes *RGL3* an important factor repressing GA and inducing JA responses, and therefore, controlling defence-growth responses. The *RGL3* overexpression resulted in reduced growth but increased resistance to *T. urticae*, while *rgl3* mutants exhibited larger plants with higher susceptibility to mites, implying similar responses to those with low and high levels of GA, respectively, following similar resource allocation processes. Nonetheless, described by Gilroy & Breen, (2022), DELLA proteins are essential hubs regulating hormonal crosstalks. Therefore, altered *RGL3* regulation directly affects the point of convergence between different hormonal pathways and affect to different plant responses. Contrary to low GA levels, overexpression of *RGL3* did not increase basal anthocyanin levels but led to enhanced anthocyanin production in response to mites, probably linked to its interaction with JA (Verma *et al.*, 2016; Xie *et al.*,

2016; Goossens *et al.*, 2017). However, despite being resistant to *T. urticae*, these plants displayed a glabrous phenotype with lower cuticle permeability. RGL3 regulation affects directly to JA (Wild *et al.*, 2012), but also to the regulatory BAP module (BZR1–ARF6–PIF4 complex) involving coordination between BR, auxin and GA signalling that regulates cell proliferation, expansion, and differentiation (Gilroy & Breen, 2022). Therefore, alteration of DELLA levels, may results in changes in both defence, and growth and developmental responses, such as the one observed upon RGL3 overexpression. These results contextualize the relevance of DELLA proteins, particularly RGL3, in the regulating both defence and growth responses upon detection of biotic stresses, determining global plant responses.

In combination, this thesis provides important insights into the evaluation of defence-growth trade-offs and their implications for the final plant fitness. Concretely, the transcriptional and hormonal model offers valuable information on the complex genetic and molecular interplay directing these processes, emphasizing the critical role of the timing of the response in the plant adaptation process to the stress. These regulations imply costs to the final fitness, highlighting the importance of evaluating the long-term effects of the infestation. The analysis of the GA regulation in mediating defence-growth processes in *Arabidopsis* upon mite infestations provides an important starting point in the understanding the role of growth hormones in the regulation of both defence and processes. This opens the door for future research on the role of other growth hormones beyond GAs in regulating defence processes. All this knowledge provides additional tools for the design and development of new pest management strategies that will improve plant resistance without penalties on its fitness.

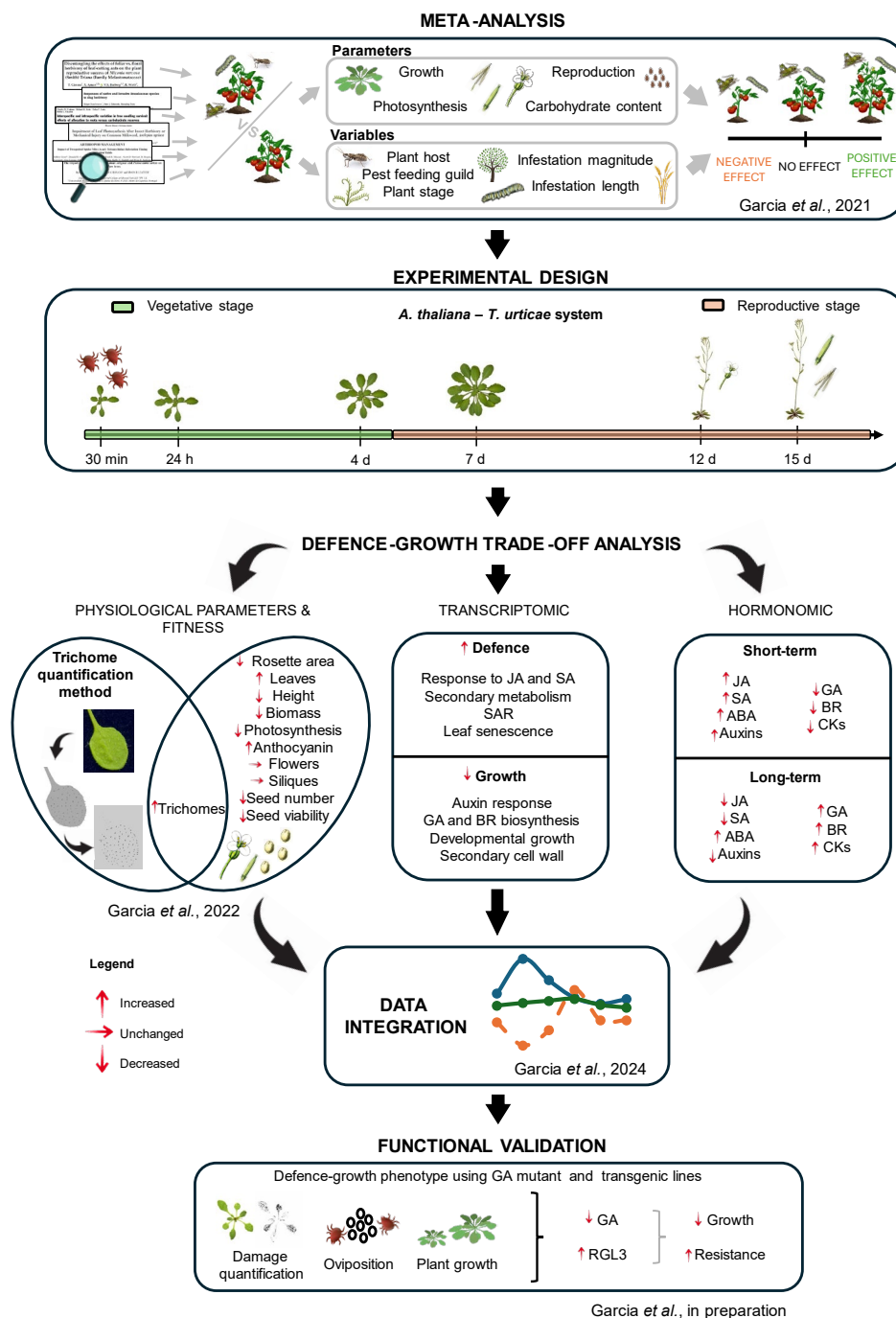
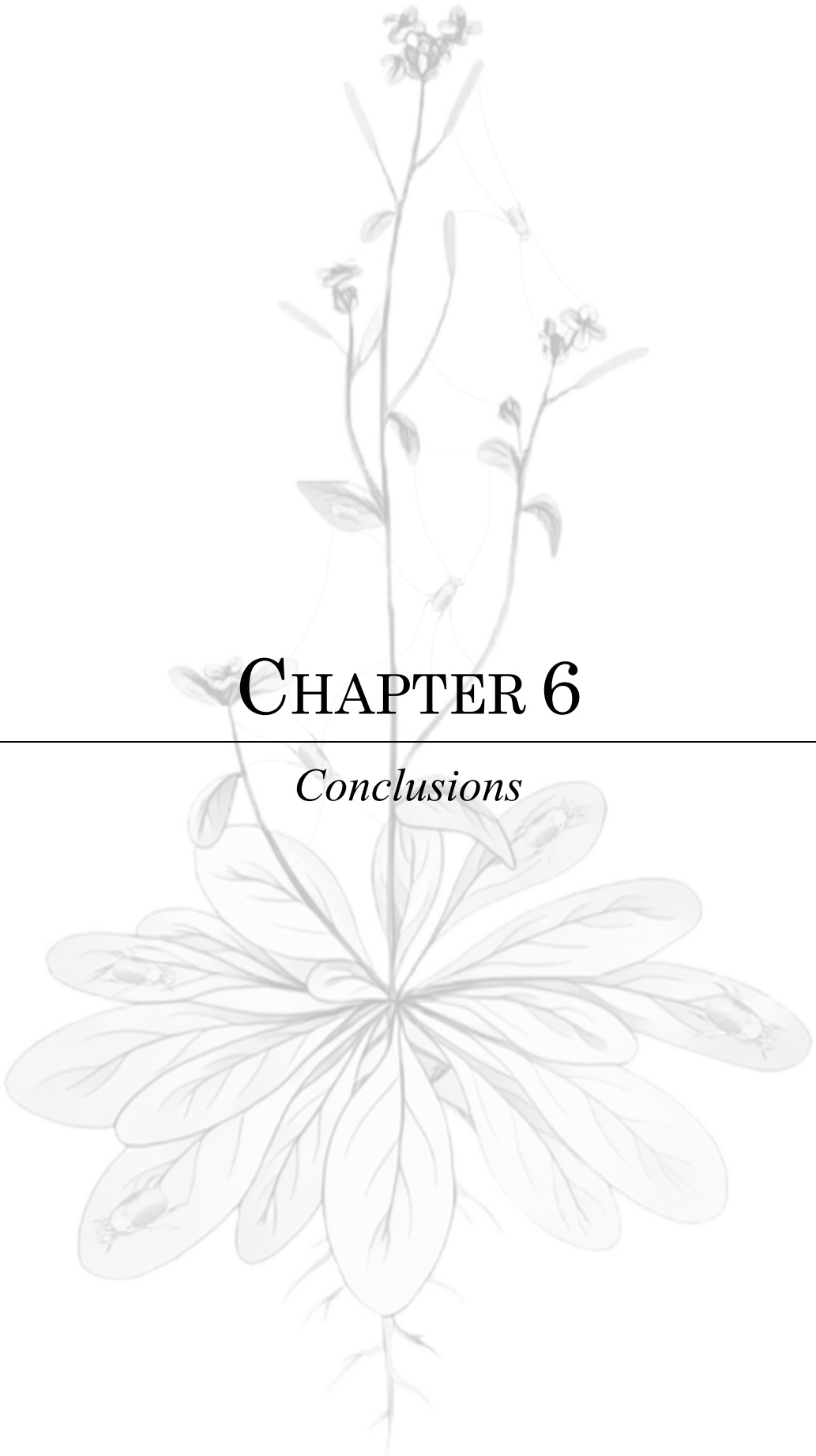


Figure 5.2. Evaluation of the defence-growth trade-off established upon spider mite infestation and the influence of growth hormones in the defence-growth responses. At the top, a summary of the variables and parameters affecting the final evaluation of the plant fitness found in the meta-analysis that enabled the experimental design of the defence-growth trade-off analysis. Physiological, transcriptomic and hormonal responses were measured upon mite infestation. Main results upon mite infestation are depicted. The trichome quantification protocol developed allowed the quantification of trichomes in the different plants. As a result of all these phenotypical, genetic and molecular adaptations, plant fitness is negatively affected. Data integration allowed the generation of a model of response to the mite involving defence-growth responses. In addition, from the data generated, the evaluation of growth hormones suggested the modulation of plant defence-growth responses. Functional validation of GA mutant and transgenic lines revealed that low GA levels or increased *RGL3* expression increase resistance and reduce growth by different regulatory mechanisms, while the role of the rest of hormones in response to spider mite remains unexplored. Publications derived from this thesis are depicted in the step of the analysis they are involved in.



CHAPTER 6

Conclusions

6. CONCLUSIONS

1. The evaluation of plant growth, photosynthetic, and reproductive parameters revealed the negative costs of the plant defences. However, carbohydrate content, due to its variable and species-specific nature, did not show any significant differences in the analysis.
2. The arthropod feeding guild and the plant host used are critical variables in determining the specific defences employed by the plant and their impact on plant fitness. Additionally, the plant's ontogenetic stage and the duration and density of the infestation act as key modulators of the final fitness cost associated with these defences.
3. Differences among subgroups of variables mediating plant-pest interactions are influenced by the parameters used to estimate fitness costs, with growth and photosynthetic parameters better discriminators of their impact.
4. The “Noisy background – Ilastik” method is a fast-forward, accessible and precise tool for trichome quantification that reduces the subjectivity of the analysis, with potential for extrapolation to other species.
5. Spider mite infestation leads to the establishment of defence-growth trade-offs at physiological, transcriptomic, and hormonal level that determines the process of plant adaptation to the stress. These alterations result in a plant developmental delay that impact both seed production at short-term level and longevity at long-term level.
6. Although JA and SA are key regulators of plant responses to *T. urticae*, the timing of these responses, showing an earlier reduction of SA with a high JA accumulation, was found to be crucial in regulating plant growth arrest and resume upon infestation. In this regulation, crosstalk with the rest of hormones determines the specific responses of the plant, particularly, the increase in CK, BR and GA levels also participate in the growth resume.
7. The coordinated transcriptomic and hormonal responses, integrating hormonal biosynthesis, signalling, and hormone levels, provide a comprehensive understanding of the regulation of these processes. Particularly, the increase in auxin levels and biosynthesis, coupled with the repression of auxin signalling, explained its involvement in both induction of defences and repression of growth in response to spider mite.
8. The functional validation using mutant and transgenic lines with altered GA and DELLA content highlights the relevance of GA and its regulation in the modulation of defence-growth responses upon mite infestation. In addition, these alterations determine anthocyanin production under both control and mite infestation conditions, reflecting the importance of the GA homeostasis in controlling plant defence processes.
9. Expression of *RGL3* induces plant resistance to spider mite, with an inverse correlation with plant growth. However, it also alters developmental processes related to trichome formation and leaf cuticle permeability, underscoring the central role of DELLA proteins and particularly, RGL3, as defence-growth modulators.

References

- Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with imageJ. *Biophotonics International*, *11*: 36–42.
- Acevedo, F. E., Rivera-Vega, L. J., Chung, S. H., Ray, S., & Felton, G. W. (2015). Cues from chewing insects - the intersection of DAMPs, HAMPs, MAMPs and effectors. *Current Opinion in Plant Biology*, *26*: 80–86.
- Agut, B., Gamir, J., Jacas, J. A., Hurtado, M., & Flors, V. (2014). Different metabolic and genetic responses in citrus may explain relative susceptibility to *Tetranychus urticae*. *Pest Management Science*, *70*: 1728–1741.
- Agut, B., Gamir, J., Jaques, J. A., & Flors, V. (2015). *Tetranychus urticae*-triggered responses promote genotype-dependent conspecific repellence or attractiveness in citrus. *New Phytologist*, *207*: 790–804.
- Alborn, H. T., & Schmelz, E. A. (2008). Arthropod-associated plant effectors (AAPEs): elicitors and suppressors of crop defense. In Capinera, J. L. (Ed.), *Encyclopedia of Entomology* (2nd ed., pp. 299–303). New York, NY: Springer.
- Albrecht, T., & Argueso, C. T. (2017). Should I fight or should I grow now? The role of cytokinins in plant growth and immunity and in the growth-defence trade-off. *Annals of Botany*, *119*: 725–735.
- Aldea, M., Hamilton, J. G., Resti, J. P., Zangerl, A. R., Berenbaum, M. R., Frank, T. D., & DeLucia, E. H. (2006). Comparison of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood saplings. *Oecologia*, *149*: 221–232.
- Aleman, F., Yazaki, J., Lee, M., Takahashi, Y., Kim, A. Y., Li, Z., Kinoshita, T., Ecker, J. R., & Schroeder, J. I. (2016). An ABA-increased interaction of the PYL6 ABA receptor with MYC2 transcription factor: a putative link of ABA and JA signaling. *Scientific Reports*, *6*: 28941.
- Alhoraibi, H., Bigeard, J., Rayapuram, N., Colcombet, J., & Hirt, H. (2019). Plant immunity: the MTI-ETI model and beyond. *Current Issues in Molecular Biology*, *30*: 39–58.
- Ali, F., Qanmber, G., Li, F., & Wang, Z. (2022). Updated role of ABA in seed maturation, dormancy, and germination. *Journal of Advanced Research*, *35*: 199–214.
- Allison, S. D., & Schultz, J. C. (2005). Biochemical responses of chestnut oak to a galling cynipid. *Journal of Chemical Ecology*, *31*: 151–166.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*: 403–410.
- Ament, K., Kant, M. R., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2004). Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant Physiology*, *135*: 2025–2037.
- Angra, S., & Ahuja, S. (2017). Machine learning and its applications: a review. In *Proceedings of the 2017 International Conference on Big Data Analytics and Computational Intelligence (ICBDAC)* (pp. 57–60). IEEE.
- Appel, H. M., Fescemyer, H., Ehrling, J., Weston, D., Rehrig, E., Joshi, T., Xu, D., Bohlmann, J., & Schultz, J. (2014). Transcriptional responses of *Arabidopsis thaliana* to chewing and sucking insect herbivores. *Frontiers in Plant Science*, *5*: 565.
- Arimura, G. I., Matsui, K., & Takabayashi, J. (2009). Chemical and molecular ecology of herbivore-induced plant volatiles: proximate factors and their ultimate functions. *Plant and Cell Physiology*, *50*: 911–923.
- Arimura, G. I., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kühnemann, F., & Takabayashi, J. (2002). Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *The Plant Journal*, *29*: 87–98.

- Arimura, G. I., Ozawa, R., Shimoda, T., Nishloka, T., Boland, W., & Takabayashi, J. (2000).** Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature*, **406**: 512–515.
- Arnaiz, A. (2019a).** Functional characterization of genes associated with Arabidopsis defence against the two-spotted spider mite feeding. Tesis (Doctoral), E.T.S. de Ingeniería Agronómica, Alimentaria y de Biosistemas (UPM).
- Arnaiz, A., Martinez, M., Gonzalez-Melendi, P., Grbic, V., Diaz, I., & Santamaría, M. E. (2019b).** Plant defenses against pests driven by a bidirectional promoter. *Frontiers in Plant Science*, **10**: 930.
- Arnaiz, A., Romero-Puertas, M. C., Santamaría, M. E., Rosa-Diaz, I., Arbona, V., Muñoz, A., Grbic, V., González-Melendi, P., Mar Castellano, M., Sandalio, L. M., Martinez, M., & Diaz, I. (2023).** The Arabidopsis thioredoxin TRXh5 regulates the S-nitrosylation pattern of the TIRK receptor being both proteins essential in the modulation of defences to *Tetranychus urticae*. *Redox Biology*, **67**: 102902.
- Arnaiz, A., Rosa-Diaz, I., Romero-Puertas, M. C., Sandalio, L. M., & Diaz, I. (2021).** Nitric oxide, an essential intermediate in the plant–herbivore interaction. *Frontiers in Plant Science*, **11**: 620086.
- Arnaiz, A., Santamaría, M. E., Rosa-Diaz, I., Garcia, I., Dixit, S., Vallejos, S., Gotor, C., Martinez, M., Grbic, V., & Diaz, I. (2022).** Hydroxynitrile lyase defends Arabidopsis against *Tetranychus urticae*. *Plant Physiology*, **189**: 2244–2258.
- Arnaiz, A., Talavera-Mateo, L., Gonzalez-Melendi, P., Martinez, M., Diaz, I., & Santamaría, M. E. (2018).** Arabidopsis kunitz trypsin inhibitors in defense against spider mites. *Frontiers in Plant Science*, **9**: 986.
- Ashra, H., & Nair, S. (2022).** Trait plasticity during plant-insect interactions: from molecular mechanisms to impact on community dynamics. *Plant Science*, **317**: 111188.
- Attaran, E., Major, I. T., Cruz, J. A., Rosa, B. A., Koo, A. J. K., Chen, J., Kramer, D. M., He, S. Y., & Howe, G. A. (2014).** Temporal dynamics of growth and photosynthesis suppression in response to jasmonate signaling. *Plant Physiology*, **165**: 1302–1314.
- Balduzzi, S., Rücker, G., & Schwarzer, G. (2019).** How to perform a meta-analysis with R: a practical tutorial. *Evidence-Based Mental Health*, **22**: 153–160.
- Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., Brown, V. K., Butterfield, J., Buse, A., Coulson, J. C., Farrar, J., Good, J. E. G., Harrington, R., Hartley, S., Jones, T. H., Lindroth, R. L., Press, M. C., Symrnioudis, I., Watt, A. D., & Whittaker, J. B. (2002).** Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, **8**: 1–16.
- Balkema-Boomstra, A. G., Zijlstra, S., Verstappen, F. W. A., Inggamer, H., Mercke, P. E., Jongsma, M. A., & Bouwmeester, H. J. (2003).** Role of cucurbitacin C in resistance to spider mite (*Tetranychus urticae*) in cucumber (*Cucumis sativus* L.). *Journal of Chemical Ecology*, **29**: 225–235.
- Barczak-Brzyżek, A., Kielkiewicz, M., Górecka, M., Kot, K., Karpińska, B., & Filipecki, M. (2017).** Absciscic Acid Insensitive 4 transcription factor is an important player in the response of *Arabidopsis thaliana* to two-spotted spider mite (*Tetranychus urticae*) feeding. *Experimental and Applied Acarology*, **73**: 317–326.
- Baron, K. N., Schroeder, D. F., & Stasolla, C. (2012).** Transcriptional response of abscisic acid (ABA) metabolism and transport to cold and heat stress applied at the reproductive stage of development in *Arabidopsis thaliana*. *Plant Science*, **188**: 48–59.
- Bekaert, M., Edger, P. P., Hudson, C. M., Pires, J. C., & Conant, G. C. (2012).** Metabolic and evolutionary costs of herbivory defense: systems biology of glucosinolate synthesis. *New Phytologist*, **196**: 596–605.
- Bell, J. R., Bohan, D. A., Shaw, E. M., & Weyman, G. S. (2005).** Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bulletin of Entomological Research*, **95**: 69–114.
- Bensoussan, N., Estrella Santamaría, M. E., Zhurov, V., Diaz, I., Grbić, M., & Grbić, V. (2016).** Plant-herbivore interaction: dissection of the cellular pattern of *Tetranychus urticae* feeding on the host plant. *Frontiers in Plant Science*, **7**: 1105.

- Bensoussan, N., Zhurov, V., Yamakawa, S., O'Neil, C. H., Suzuki, T., Grbić, M., & Grbić, V. (2018). The digestive system of the two-spotted spider mite, *Tetranychus urticae* Koch, in the context of the mite-plant interaction. *Frontiers in Plant Science*, **9**: 1206.
- Ben-Targem, M., Ripper, D., Bayer, M., & Ragni, L. (2021). Auxin and gibberellin signaling cross-talk promotes hypocotyl xylem expansion and cambium homeostasis. *Journal of Experimental Botany*, **72**: 3647–3660.
- Berhin, A., Nawrath, C., & Hachez, C. (2022). Subtle interplay between trichome development and cuticle formation in plants. *New Phytologist*, **233**: 2036–2046.
- Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. *Frontiers in Plant Science*, **12**: 615114.
- Bi, J., & Wang, Y. F. (2020). The effect of the endosymbiont Wolbachia on the behavior of insect hosts. *Insect Science*, **27**: 846–858.
- Bilgin, D. D., Zavala, J. A., Zhu, J., Clough, S. J., Ort, D. R., & Delucia, E. H. (2010). Biotic stress globally downregulates photosynthesis genes. *Plant, Cell & Environment*, **33**: 1597–1613.
- Boncan, D. A. T., Tsang, S. S. K., Li, C., Lee, I. H. T., Lam, H. M., Chan, T. F., & Hui, J. H. L. (2020). Terpenes and terpenoids in plants: Interactions with environment and insects. *International Journal of Molecular Sciences*, **21**: 7382.
- Borenstein, M., Hedges, L. V., Higgins, J. P. T., & Rothstein, H. R. (2021). *Introduction to meta-analysis*. (2nd ed.). Hoboken, NY: John Wiley and Sons Inc.
- Boter, M., & Diaz, I. (2023). Cyanogenesis, a plant defence strategy against herbivores. *International Journal of Molecular Sciences*, **24**: 6982.
- Boter, M., & Diaz, I. (2024). Contrasting defence mechanisms against spider mite infestation in cyanogenic and non-cyanogenic legumes. *Plant Science*, **345**: 112118.
- Botha, A.-M., Lacock, L., Van Niekerk, C., Matsioloko, M. T., Du Preez, F. B., Loots, S., Venter, E., Kunert, K. J., & Cullis, C. A. (2006). Is photosynthetic transcriptional regulation in *Triticum aestivum* L. cv. 'TugelaDN' a contributing factor for tolerance to *Diuraphis noxia* (Homoptera: Aphididae)? *Plant Cell Reports*, **25**: 41–54.
- Boukouvala, M. C., Kavallieratos, N. G., Skourti, A., Pons, X., Alonso, C. L., Eizaguirre, M., Fernandez, E. B., Solera, E. D., Fita, S., Bohinc, T., Trdan, S., Agrafioti, P., & Athanassiou, C. G. (2022). *Lymantria dispar* (L.) (Lepidoptera: Erebididae): current status of biology, ecology, and management in Europe with notes from North America. *Insects*, **13**: 854.
- Bouré, N., Kumar, S. V., & Arnaud, N. (2019). The BAP module: a multisignal integrator orchestrating growth. *Trends in Plant Science*, **24**: 602–610.
- Bowman, J. (Ed.). (2012). *Arabidopsis: an atlas of morphology and development* (1st ed.). Berlin: Springer Science & Business Media.
- Brown, J. K. M., & Hovmöll, M. S. (2002). Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science*, **297**: 537–541.
- Bruce, T. J., & Pickett, J. A. (2007). Plant defence signalling induced by biotic attacks. *Current Opinion in Plant Biology*, **10**: 387–392.
- Bruin, J., Dicke, M., & Sabelis, M. W. (1992). Plants are better protected against spider-mites after exposure to volatiles from infested conspecifics. *Experientia*, **48**: 525–529.
- Buda, G. J., Isaacson, T., Matas, A. J., Paolillo, D. J., & Rose, J. K. C. (2009). Three-dimensional imaging of plant cuticle architecture using confocal scanning laser microscopy. *The Plant Journal*, **60**: 378–385.
- Buffon, G., Blasi, É. A. dos R., Lamb, T. I., Adamski, J. M., Schwambach, J., Ricachenevsky, F. K., Bertolazi, A., Silveira, V., Lopes, M. C. B., & Sperotto, R. A. (2021). *Oryza sativa* cv. Nipponbare and *Oryza*

barthii as unexpected tolerance and susceptibility sources against *Schizotetranychus oryzae* (Acari: Tetranychidae) mite infestation. *Frontiers in Plant Science*, **12**: 613568.

Bui, H., Greenhalgh, R., Ruckert, A., Gill, G. S., Lee, S., Ramirez, R. A., & Clark, R. M. (2018). Generalist and specialist mite herbivores induce similar defense responses in maize and barley but differ in susceptibility to benzoxazinoids. *Frontiers in Plant Science*, **9**: 1222.

Cagle, L. R. (1949). Life history of the two-spotted spider mite. *Virginia Agriculture Experimental Station Technical Bulletin*, **113**: 1-31.

Campos, M. L., Yoshida, Y., Major, I. T., De Oliveira Ferreira, D., Weraduwege, S. M., Froehlich, J. E., Johnson, B. F., Kramer, D. M., Jander, G., Sharkey, T. D., & Howe, G. A. (2016). Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nature Communications*, **7**: 12570.

Campos, M. L., De Almeida, M., Rossi, M. L., Martinelli, A. P., Litholdo Junior, C. G., Figueira, A., Rampelotti-Ferreira, F. T., Vendramim, J. D., Benedito, V. A., & Pereira Peres, L. E. (2009). Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. *Journal of Experimental Botany*, **60**: 4347–4361.

Canet, J. V., Dobón, A., Ibáñez, F., Perales, L., & Tornero, P. (2010). Resistance and biomass in Arabidopsis: a new model for salicylic acid perception. *Plant Biotechnology Journal*, **8**: 126–141.

Carella, P., Wilson, D. C., & Cameron, R. K. (2015). Some things get better with age: differences in salicylic acid accumulation and defense signaling in young and mature Arabidopsis. *Frontiers in Plant Science*, **5**: 775.

Carrillo, L., Martinez, M., Ramessar, K., Cambra, I., Castañera, P., Ortego, F., & Díaz, I. (2011). Expression of a barley cystatin gene in maize enhances resistance against phytophagous mites by altering their cysteine-proteases. *Plant Cell Reports*, **30**: 101–112.

Casanova-Sáez, R., & Voß, U. (2019). Auxin metabolism controls developmental decisions in land plants. *Trends in Plant Science*, **24**: 741–754.

Castrillón-Arbeláez, P. A., Martínez-Gallardo, N., Arnaut, H. A., Tiessen, A., & Délano-Frier, J. P. (2012). Metabolic and enzymatic changes associated with carbon mobilization, utilization and replenishment triggered in grain amaranth (*Amaranthus cruentus*) in response to partial defoliation by mechanical injury or insect herbivory. *BMC Plant Biology*, **12**: 1–22.

Cazaux, M., Navarro, M., Bruinsma, K. A., Zhurov, V., Negrave, T., Van Leeuwen, T., Grbic, V., & Grbic, M. (2014). Application of two-spotted spider mite *Tetranychus urticae* for plant-pest interaction studies. *Journal of Visualized Experiments*, **89**: 51738.

Chandler, D., Davidson, G., & Jacobson, R. J. (2005). Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. *Biocontrol Science and Technology*, **15**: 37–54.

Charaya, M. U., Upadhyay, A., Bhati, H. P., & Kumar, A. (2021). Plant disease forecasting: Past practices to emerging technologies. In: Nehra, S., (Ed.), *Plant Disease: Management Strategies* (pp. 1-30). Rajasthan, India: Agrobios Research.

Chen, L., & Yu, D. (2014). ABA regulation of plant response to biotic stresses. In L. T. Popova & M. T. Wang (Eds.), *Abscisic acid: Metabolism, transport and signaling* (pp. 409–429). Berlin, Germany: Springer.

Chen, Y., & Williams, K. A. (2006). Quantifying western flower thrips (*Frankliniella occidentalis* Pergande) (Thysanoptera: Thripidae) damage on ivy geranium (*Pelargonium peltatum* (L.) L'Her ex Ait.) (Geraniaceae Juss.) with adobe® Photoshop™ and Scion® image software. *Journal of the Kansas Entomological Society*, **79**: 83–87.

Chen, Y., Pang, Q., Dai, S., Wang, Y., Chen, S., & Yan, X. (2011). Proteomic identification of differentially expressed proteins in Arabidopsis in response to methyl jasmonate. *Journal of Plant Physiology*, **168**: 995–1008.

- Cheng, Y., Cao, L., Wang, S., Li, Y., Shi, X., Liu, H., Li, L., Zhang, Z., Fowke, L. C., Wang, H., & Zhou, Y.** (2013). Downregulation of multiple CDK inhibitor ICK/KRP genes upregulates the E2F pathway and increases cell proliferation, and organ and seed sizes in Arabidopsis. *The Plant Journal*, **75**: 642–655.
- Choi, J., Huh, S. U., Kojima, M., Sakakibara, H., Paek, K. H., & Hwang, I.** (2010). The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in Arabidopsis. *Developmental Cell*, **19**: 284–295.
- Choudhary, D. K., Prakash, A., & Johri, B. N.** (2007). Induced systemic resistance (ISR) in plants: mechanism of action. *Indian Journal of Microbiology*, **47**: 289–297.
- Chung, Y., & Choe, S.** (2013). The regulation of brassinosteroid biosynthesis in Arabidopsis. *Critical Reviews in Plant Sciences*, **32**: 396–410.
- Cipollini, D., & Heil, M.** (2010). Costs and benefits of induced resistance to herbivores and pathogens in plants. *CABI Reviews*, **5**: 1–25.
- Cipollini, D., Walters, D., & Voelckel, C.** (2018). Costs of resistance in plants: from theory to evidence. In S. Roberts (Ed.), *Annual plant reviews online* (pp. 263–307). Hoboken, NJ: Wiley.
- Clarke, J. D., Volko, S. M., Ledford, H., Ausubel, F. M., & Dong, X.** (2000). Roles of salicylic acid, jasmonic acid, and ethylene in cpr-induced resistance in Arabidopsis. *The Plant Cell*, **12**: 2175–2190.
- Clotuche, G., Mailleux, A. C., Fernández Astudillo, A., Deneubourg, J. L., Detrain, C., & Hance, T.** (2011). The formation of collective silk balls in the spider mite *Tetranychus urticae* Koch. *PLoS ONE*, **6**: e18854.
- Coakley, S. M., Scherm, H., & Chakraborty, S.** (1999). Climate change and plant disease management. *Annual Review of Phytopathology*, **37**: 399–426.
- Colebrook, E. H., Thomas, S. G., Phillips, A. L., & Hedden, P.** (2014). The role of gibberellin signalling in plant responses to abiotic stress. *Journal of Experimental Biology*, **217**: 67–75.
- Coles, J. P., Phillips, A. L., Croker, S. J., García-Lepe, R., Lewis, M. J., & Hedden, P.** (1999). Modification of gibberellin production and plant development in Arabidopsis by sense and antisense expression of gibberellin 20-oxidase genes. *The Plant Journal*, **17**: 547–556.
- Cortleven, A., Leuendorf, J. E., Frank, M., Pezzetta, D., Bolt, S., & Schmölling, T.** (2019). Cytokinin action in response to abiotic and biotic stresses in plants. *Plant, Cell & Environment*, **42**: 998–1018.
- Coruzzi, G. M., & Last, R. L.** (2000) Amino Acids. In B. Buchanan, W. Gruissem & R. Jones (Eds.), *Biochemistry and Molecular Biology of Plants* (pp. 358–410). Rockville, MD: American Society of Plant Physiology; Wiley Press.
- Cosgrove, D. J.** (2015). Plant expansins: diversity and interactions with plant cell walls. *Current Opinion in Plant Biology*, **25**: 1662–172.
- Cui, J. R., Bing, X. L., Tang, Y. J., Liu, F., Ren, L., Zhou, J. Y., Liu, H. H., Wang, M. K., Hoffmann, A. A., & Hong, X. Y.** (2023). A conserved protein disulfide isomerase enhances plant resistance against herbivores. *Plant Physiology*, **191**: 660–678.
- Dai, X., Mashiguchi, K., Chen, Q., Kasahara, H., Kamiya, Y., Ojha, S., DuBois, J., Ballou, D., & Zhao, Y.** (2013). The biochemical mechanism of auxin biosynthesis by an Arabidopsis YUCCA flavin-containing monooxygenase. *Journal of Biological Chemistry*, **288**: 1448–1457.
- Davière, J. M., & Achard, P.** (2016). A pivotal role of DELLAs in regulating multiple hormone signals. *Molecular Plant*, **9**: 10–20.
- De Lorenzo, G., & Cervone, F.** (2022). Plant immunity by damage-associated molecular patterns (DAMPs). *Essays in Biochemistry*, **66**: 459–469.
- de Souza, L. A., & Peñaflor, M. F. G. V.** (2024). Small but strong: herbivory by sap-feeding insect reduces plant progeny growth but enhances direct and indirect anti-herbivore defenses. *Oecologia*, **205**: 191–201.

- de Vleeschauwer, D., van Buyten, E., Satoh, K., Balidion, J., Mauleon, R., Choi, I. R., Vera-Cruz, C., Kikuchi, S., & Höfte, M. (2012). Brassinosteroids antagonize gibberellin- and salicylate-mediated root immunity in rice. *Plant Physiology*, **158**: 1833–1846.
- De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., Buchala, A. J., Métraux, J.-P., Van Loon, L. C., Dicke, M., & Pieterse, M. J. (2005). Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Molecular Plant-Microbe Interactions*, **18**: 923–937.
- Del Re, A. C. (2015). A practical tutorial on conducting meta-analysis in R. *The Quantitative Methods for Psychology*, **11**: 37–50.
- DeLucia, E. H., Nabity, P. D., Zavala, J. A., & Berenbaum, M. R. (2012). Climate change: resetting plant-insect interactions. *Plant Physiology*, **160**: 1677–1685.
- Díaz-Mendoza, M., Velasco-Arroyo, B., Santamaría, M. E., Díaz, I., & Martínez, M. (2017). HvPap-1 C1A protease participates differentially in the barley response to a pathogen and an herbivore. *Frontiers in Plant Science*, **8**: 1585.
- Dosse, G. (1952). *The greenhouse spider mite Tetranychus urticae Koch forma dianthica and its control*. Wallingford, UK: CABI.
- Durrant, W. E., & Dong, X. (2004). Systemic acquired resistance. *Annual Review of Phytopathology*, **42**: 185–209.
- Duval, S., & Tweedie, R. (2000). Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics*, **56**: 455–463.
- Egbuna, C., & Sawicka, B. (Eds.) (2019). *Natural Remedies for Pest, Disease and Weed Control*. Cambridge, MA: Academic Press.
- Egger, M., Smith, G. D., Schneider, M., & Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. *British Medical Journal*, **315**: 629–634.
- Eichenseer, H., Mathews, M. C., Bi, J. L., Murphy, J. B., & Felton, G. W. (1999). Salivary glucose oxidase: multifunctional roles for *Helicoverpa zea*? *Archives of Insect Biochemistry and Physiology*, **42**: 99–109.
- Ellis, T. J., Postma, F. M., Oakley, C. G., & Ågren, J. (2021). Life-history trade-offs and the genetic basis of fitness in *Arabidopsis thaliana*. *Molecular Ecology*, **30**: 2846–2858.
- Emam, H. (2021). Efficiency of three bacterial strains against *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions. *Arab Universities Journal of Agricultural Sciences*, **29**: 447–457.
- Emamverdian, A., Ding, Y., & Mokhberdoran, F. (2020). The role of salicylic acid and gibberellin signaling in plant responses to abiotic stress with an emphasis on heavy metals. *Plant Signaling & Behavior*, **15**: 1777372.
- Engelberth, J., Seidl-Adams, I., Schultz, J. C., & Tumlinson, J. H. (2007). Insect elicitors and exposure to green leafy volatiles differentially upregulate major octadecanoids and transcripts of 12-oxo phytodienoic acid reductases in *Zea mays*. *Molecular Plant-Microbe Interactions*, **20**: 707–716.
- Erb, M. (2018). Plant defenses against herbivory: closing the fitness gap. *Trends in Plant Science*, **23**: 187–194.
- Erb, M., Meldau, S., & Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science*, **17**: 250–259.
- Erb, M., & Reymond, P. (2019). Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology*, **70**: 527–557.
- Erofeeva, E. A. (2023). Environmental hormesis in living systems: the role of hormetic trade-offs. *Science of the Total Environment*, **901**: 166022.
- Ferrieri, A. P., Appel, H., Ferrieri, R. A., & Schultz, J. C. (2012). Novel application of 2-[18F]fluoro-2-deoxy-d-glucose to study plant defenses. *Nuclear Medicine and Biology*, **39**: 1152–1160.

- Fridman, Y., & Savaldi-Goldstein, S.** (2013). Brassinosteroids in growth control: how, when and where. *Plant Science*, **209**: 24–31.
- Frost, C. J., Mescher, M. C., Carlson, J. E., & De Moraes, C. M.** (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, **146**: 818–824.
- Fukazawa, J., Mori, K., Ando, H., Mori, R., Kanno, Y., Seo, M., & Takahashi, Y.** (2023). Jasmonate inhibits plant growth and reduces gibberellin levels via microRNA5998 and transcription factor MYC2. *Plant Physiology*, **193**: 2197–2214.
- Gallego-Bartolomé, J., Minguet, E. G., Grau-Enguix, F., Abbas, M., Locascio, A., Thomas, S. G., Alabadí, D., & Blázquez, M. A.** (2012). Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **109**: 13446–13451.
- Gallego-Giraldo, L., Jikumaru, Y., Kamiya, Y., Tang, Y., & Dixon, R. A.** (2011). Selective lignin downregulation leads to constitutive defense response expression in alfalfa (*Medicago sativa* L.). *New Phytologist*, **190**: 627–639.
- Garcia, A., Martinez, M., Diaz, I., & Santamaría, M. E.** (2021b). The price of the induced defense against pests: a meta-analysis. *Frontiers in Plant Science*, **11**: 61512.
- Garcia, A., Santamaría, M. E., Diaz, I., & Martinez, M.** (2021a). Disentangling transcriptional responses in plant defense against arthropod herbivores. *Scientific Reports*, **11**: 12996.
- Garcia, A., Talavera-Mateo, L., Petrik, I., Oklestkova, J., Novak, O., & Santamaría, M. E.** (2024). Spider mite infestation triggers coordinated hormonal trade-offs enabling plant survival with a fitness cost. *Physiologia Plantarum*, **176**: e14479.
- Garcia, A., Talavera-Mateo, L., & Santamaría, M. E.** (2022). An automatic method to quantify trichomes in *Arabidopsis thaliana*. *Plant Science*, **323**: 111391.
- Gilroy, E., & Breen, S.** (2022). Interplay between phytohormone signalling pathways in plant defence - other than salicylic acid and jasmonic acid. *Essays in Biochemistry*, **66**: 657–671.
- Giri, A. P., Wünsche, H., Mitra, S., Zavala, J. A., Muck, A., Svatoš, A., & Baldwin, I. T.** (2006). Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. *Plant Physiology*, **142**: 1621–1641.
- Glazebrook, J.** (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, **43**: 205–227.
- Gómez, S., Steinbrenner, A. D., Osorio, S., Schueller, M., Ferrieri, R. A., Fernie, A. R., & Orians, C. M.** (2012). From shoots to roots: transport and metabolic changes in tomato after simulated feeding by a specialist lepidopteran. *Entomologia Experimentalis et Applicata*, **144**: 101–111.
- Gomez-Sanchez, A., Gonzalez-Melendi, P., Santamaría, M. E., Arbona, V., Lopez-Gonzalez, A., Garcia, A., Hensel, G., Kumlehn, J., Martinez, M., & Diaz, I.** (2019). Repression of drought-induced cysteine-protease genes alters barley leaf structure and responses to abiotic and biotic stresses. *Journal of Experimental Botany*, **70**: 2143–2155.
- Gonzalez, A., Zhao, M., Leavitt, J. M., & Lloyd, A. M.** (2008). Regulation of the anthocyanin biosynthetic pathway by the TTG1/bHLH/Myb transcriptional complex in Arabidopsis seedlings. *The Plant Journal*, **53**: 814–827.
- Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S.** (2012). Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, **40**: D1178–D1186.
- Goossens, J., Mertens, J., & Goossens, A.** (2017). Role and functioning of bHLH transcription factors in jasmonate signalling. *Journal of Experimental Botany*, **68**: 1333–1347.

- Gorb, E. V., & Gorb, S. N.** (2017). Anti-adhesive effects of plant wax coverage on insect attachment. *Journal of Experimental Botany*, **68**: 5323–5337.
- Grbić, M., Van Leeuwen, T., Clark, R. M., Rombauts, S., Rouzé, P., Grbić, V., Osborne, E. J., Dermauw, W., Ngoc, P. C. T., Ortego, F., Hernández-Crespo, P., Diaz, I., Martinez, M., Navajas, M., Sucena, É., Magalhães, S., Nagy, L., Pace, R. M., Djuranović, S., Smagghe, G., Iga, M., Christiaens, O., Veenstra, J. A., Ewer, J., Villalobos, R. M., Hutter, J. L., Hudson, S. D., Velez, M., Yi, S. V., Zeng, J., Pires-daSilva, A., Roch, F., Cazaux, M., Navarro, M., Zhurov, V., Acevedo, G., Gjelic, A., Fawcett, J. A., Bonnet, E., Martens, C., Baele, G., Wissler, L., Sanchez-Rodrigues, A., Tirry, L., Blais, C., Demeestere, K., Henz, S. R., Gregory, T. R., Mathie, J., Verdon, L., Farinelli, L., Schmutz, J., Lindquist, E., Feyereisen, R., & Van De Peer, Y.** (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature*, **479**: 487–492.
- Grunewald, W., Vanholme, B., Pauwels, L., Plovie, E., Inzé, D., Gheysen, G., & Goossens, A.** (2009). Expression of the Arabidopsis jasmonate signalling repressor JAZ1/TIFY10A is stimulated by auxin. *EMBO Reports*, **10**: 923–928.
- Guan, C., Wang, X., Feng, J., Hong, S., Liang, Y., Ren, B., & Zuo, J.** (2014). Cytokinin antagonizes abscisic acid-mediated inhibition of cotyledon greening by promoting the degradation of ABSCISIC ACID INSENSITIVE5 protein in Arabidopsis. *Plant Physiology*, **164**: 1515–1526.
- Gudesblat, G. E., & Russinova, E.** (2011). Plants grow on brassinosteroids. *Current Opinion in Plant Biology*, **14**: 530–537.
- Guo, Q., Yoshida, Y., Major, I. T., Wang, K., Sugimoto, K., Kapali, G., Havko, N. E., Benning, C., & Howe, G. A.** (2018). JAZ repressors of metabolic defense promote growth and reproductive fitness in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **115**: E10768–E10777.
- Gupta, S., & Roy, A.** (2021). Deciphering the role of phytoanticipins, phytoalexins, and polyphenols in plant-insect defense. In I. K. Singh & A. Singh, (Eds.), *Plant-Pest Interactions: From Molecular Mechanisms to Chemical Ecology* (pp 305–335). Berlin: Springer Science & Business Media.
- Gurevitch, J., & Hedges, L.V.** (1993). Meta-analysis: combining the results of independent experiments. In S. Scheiner & J. Gurevitch (Eds.), *Design and Analysis of Ecological Experiments* (pp. 378–398). New York, NY: Chapman & Hall.
- Gurevitch, J., & Hedges, L.V.** (2001). Meta-analysis: combining the results of independent experiments. In S. Scheiner & J. Gurevitch (Eds.), *Design and Analysis of Ecological Experiments* (pp. 347–369). New York, NY: Oxford Press University.
- Gutsche, A., Heng-Moss, T., Sarath, G., Twigg, P., Xia, Y., Lu, G., & Mornhinweg, D.** (2009). Gene expression profiling of tolerant barley in response to *Diuraphis noxia* (Hemiptera: Aphididae) feeding. *Bulletin of Entomological Research*, **99**: 163–173.
- Halitschke, R., Hamilton, J. G., & Kessler, A.** (2011). Herbivore-specific elicitation of photosynthesis by mirid bug salivary secretions in the wild tobacco *Nicotiana attenuata*. *New Phytologist*, **191**: 528–535.
- Handley, R., Ekbom, B., & Ågren, J.** (2005). Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. *Ecological Entomology*, **30**: 284–292.
- Hanik, N., Gómez, S., Best, M., Schueller, M., Orians, C. M., & Ferrieri, R. A.** (2010). Partitioning of new carbon as ^{11}C in *Nicotiana tabacum* reveals insight into methyl jasmonate induced changes in metabolism. *Journal of Chemical Ecology*, **36**: 1058–1067.
- Hare, J. D.** (1990). Ecology and management of the Colorado potato beetle. *Annual Review of Entomology*, **35**: 81–100.
- Hare, J. D., Elle, E., & Van Dam, N. M.** (2003). Costs of glandular trichomes in *Datura wrightii*: a three-year study. *Evolution*, **57**: 793–805.

- Harrer, M., Cuijpers, P., Furukawa, T., & Ebert, D.D. (2019). *dmeter: companion R package for the guide doing meta-analysis in R* (Version 0.0.9000) [R package]. <http://dmeter.protectlab.org>
- Hatsugai, N., Igarashi, D., Mase, K., Lu, Y., Tsuda, Y., Chakravarthy, S., Wei, H., Foley, J. W., Collmer, A., Glazebrook, J., & Katagiri, F. (2017). A plant effector-triggered immunity signaling sector is inhibited by pattern-triggered immunity. *The EMBO Journal*, **36**: 2758–2769.
- Hauser, M. T., Harr, B., & Schlötterer, C. (2001). Trichome distribution in *Arabidopsis thaliana* and its close relative *Arabidopsis lyrata*: molecular analysis of the candidate gene GLABROUS1. *Molecular Biology and Evolution*, **18**: 1754–1763.
- Hawkes, C. V., & Sullivan, J. J. (2001). The impact of herbivory on plants in different resource conditions: a meta-analysis. *Ecology*, **82**: 2045–2058.
- He, H. M., Liu, L. N., Munir, S., Bashir, N. H., Wang, Y., Yang, J., & LI, C. Y. (2019). Crop diversity and pest management in sustainable agriculture. *Journal of Integrative Agriculture*, **18**: 1945–1952.
- He, Y., Zhang, H., Sun, Z., Li, J., Hong, G., Zhu, Q., Zhou, X., MacFarlane, S., Yan, F., & Chen, J. (2017). Jasmonic acid-mediated defense suppresses brassinosteroid-mediated susceptibility to Rice black streaked dwarf virus infection in rice. *New Phytologist*, **214**: 388–399.
- He, Z., Webster, S., & He, S. Y. (2022). Growth–defense trade-offs in plants. *Current Biology*, **32**: R634–R639.
- Hedden, P. (2020). The current status of research on gibberellin biosynthesis. *Plant and Cell Physiology*, **61**: 1832–1849.
- Hedden, P., & Thomas, S. G. (2012). Gibberellin biosynthesis and its regulation. *Biochemical Journal*, **444**: 11–25.
- Hedges, L. V. (1981). Distribution theory for glass’s estimator of effect size and related estimators. *Journal of Educational Statistics*, **6**: 107–128.
- Heil, M., & Baldwin, I. T. (2002). Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends in Plant Science*, **7**: 61–67.
- Heinrich, M., Hettenhausen, C., Lange, T., Wünsche, H., Fang, J., Baldwin, I. T., & Wu, J. (2013). High levels of jasmonic acid antagonize the biosynthesis of gibberellins and inhibit the growth of *Nicotiana attenuata* stems. *The Plant Journal*, **73**: 591–606.
- Hentrich, M., Böttcher, C., Dücking, P., Cheng, Y., Zhao, Y., Berkowitz, O., Masle, J., Medina, J., & Pollmann, S. (2013). The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of *YUCCA8* and *YUCCA9* gene expression. *The Plant Journal*, **74**: 626–637.
- Herms, D. A., & Mattson, W. J. (1992). The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**: 283–335.
- Herrera, C. M., Medrano, M., Rey, P. J., Sánchez-Lafuente, A. M., Garcia, M. B., Guitián, J., & Manzaneda, A. J. (2002). Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. *Proceedings of the National Academy of Sciences of the United States of America*, **99**: 16823–16828.
- Hessman, F.V. (2009). *Figure Calibration*. [Software] Astrophysics Department, University of Göttingen. http://www.astro.physik.uni-goettingen.de/~hessman/ImageJ/Figure_Calibration/ [Accessed on July 23, 2020]
- Hilscher, J., Schlötterer, C., & Hauser, M. T. (2009). A single amino acid replacement in ETC2 shapes trichome patterning in natural *Arabidopsis* populations. *Current Biology*, **19**: 1747–1751.
- Hogenhout, S. A., & Bos, J. I. B. (2011). Effector proteins that modulate plant-insect interactions. *Current Opinion in Plant Biology*, **14**: 422–428.

- Holtorf, M., Lenaerts, C., Cullen, D., & Vanden Broeck, J. (2019). Extracellular nutrient digestion and absorption in the insect gut. *Cell and Tissue Research*, **377**: 397–414.
- Hoseinzadeh, A. H., Soorni, A., Shoorooei, M., Mahani, M. T., Amiri, R. M., Allahyari, H., & Mohammadi, R. (2020). Comparative transcriptome provides molecular insight into defense-associated mechanisms against spider mite in resistant and susceptible common bean cultivars. *PLoS ONE*, **15**: e0228680.
- Hou, X., Lee, L. Y. C., Xia, K., Yan, Y., & Yu, H. (2010). DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Developmental Cell*, **19**: 884–894.
- Howe, G. A., Major, I. T., & Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annual Review of Plant Biology*, **69**: 387–415.
- Howell, S. H., Lall, S., & Che, P. (2003). Cytokinins and shoot development. *Trends in Plant Science*, **8**: 453–459.
- Hu, Y., Han, X., Yang, M., Zhang, M., Pan, J., & Yu, D. (2019). The transcription factor INDUCER OF CBF EXPRESSION1 interacts with ABSCISIC ACID INSENSITIVE5 and DELLA proteins to fine-tune abscisic acid signaling during seed germination in Arabidopsis. *The Plant Cell*, **31**: 1520–1538.
- Huang, H., Gao, H., Liu, B., Qi, T., Tong, J., Xiao, L., Xie, D., & Song, S. (2017). Arabidopsis MYB24 regulates jasmonate-mediated stamen development. *Frontiers in Plant Science*, **8**: 1525.
- Huang, M. Y., Huang, W. D., Chou, H. M., Chen, C. C., Chen, P. J., Chang, Y. T., & Yang, C. M. (2015). Structural, biochemical, and physiological characterization of photosynthesis in leaf-derived cup-shaped galls on *Litsea acuminata*. *BMC Plant Biology*, **15**: 1–12.
- Huang, Y., Han, C., Peng, W., Peng, Z., Xiong, X., Zhu, Q., Gao, B., Xie, D., & Ren, C. (2010). Brassinosteroid negatively regulates jasmonate inhibition of root growth in Arabidopsis. *Plant Signaling & Behavior*, **5**: 140–142.
- Hung, F. Y., Chen, J. H., Feng, Y. R., Lai, Y. C., Yang, S., & Wu, K. (2020). Arabidopsis JMJ29 is involved in trichome development by regulating the core trichome initiation gene GLABRA3. *The Plant Journal*, **103**: 1735–1743.
- Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant*, **7**: 1267–1287.
- Hutchison, W. D., & Campbell, C. D. (1994). Economic impact of sugarbeet root aphid (Homoptera: Aphididae) on sugarbeet yield and quality in southern Minnesota. *Journal of Economic Entomology*, **87**: 465–475.
- Hyodo, H. (2018). Stress/wound ethylene. In A. K. Mattoo & J. C. Suttle (Eds.), *The Plant Hormone Ethylene* (1st ed., pp. 43–63). Boca Raton, FL: CRC Press.
- Iida, J., Desaki, Y., Hata, K., Uemura, T., Yasuno, A., Islam, M., Maffei, M. E., Ozawa, R., Nakajima, T., Galis, I., & Arimura, G. ichiro. (2019). Tetranins: new putative spider mite elicitors of host plant defense. *New Phytologist*, **224**: 875–885.
- Ishiwari, H., Suzuki, T., & Maeda, T. (2007). Essential compounds in herbivore-induced plant volatiles that attract the predatory mite *Neoseiulus womersleyi*. *Journal of Chemical Ecology*, **33**: 1670–1681.
- Jakubowska, M., Dobosz, R., Zawada, D., & Kowalska, J. (2022). A review of crop protection methods against the twospotted spider mite—*Tetranychus urticae* Koch (Acari: Tetranychidae)—with special reference to alternative methods. *Agriculture*, **12**: 898.
- Jang, G., Chang, S. H., Um, T. Y., Lee, S., Kim, J. K., & Choi, Y. Do. (2017). Antagonistic interaction between jasmonic acid and cytokinin in xylem development. *Scientific Reports*, **7**: 10212.
- Jang, G., Yoon, Y., & Choi, Y. Do. (2020). Crosstalk with jasmonic acid integrates multiple responses in plant development. *International Journal of Molecular Sciences*, **21**: 305.
- Jiaqiang, S., Yingxiu, X., Songqing, Y., Hongling, J., Qian, C., Fang, L., Wenkun, Z., Rong, C., Xugang, L., Tietz, O., Xiaoyan, W., Cohen, J. D., Palme, K., & Chuanyou, L. (2009). Arabidopsis ASA1 is important for

jasmonate-mediated regulation of auxin biosynthesis and transport during lateral root formation. *The Plant Cell*, **21**: 1495–1511.

Jie, H., Zhao, L., Ma, Y., Rasheed, A., & Jie, Y. (2023). Integrated transcriptome and metabolome analysis reveal that exogenous gibberellin application regulates lignin synthesis in ramie. *Agronomy*, **13**: 1450.

Jin, G., Qi, J., Zu, H., Liu, S., Gershenzon, J., Lou, Y., Baldwin, I. T., & Li, R. (2023). Jasmonate-mediated gibberellin catabolism constrains growth during herbivore attack in rice. *The Plant Cell*, **35**: 3828–3844.

Johnston, G. A., Berry, H. M., Kojima, M., Sakakibara, H., & Argueso, C. T. (2024). Concerted plant growth and defense through targeted phytohormone crosstalk modification. *BioRxiv*, 2024–04.

Jonckheere, W., Dermauw, W., Zhurov, V., Wybouw, N., Van Den Bulcke, J., Villarroel, C. A., Greenhalgh, R., Grbić, M., Schuurink, R. C., Tirry, L., Baggerman, G., Clark, R. M., Kant, M. R., Vanholme, B., Menschaert, G., & Van Leeuwen, T. (2016). The salivary protein repertoire of the polyphagous spider mite *Tetranychus urticae*: a quest for effectors. *Molecular & Cellular Proteomics*, **15**: 3594–3613.

Jung, C., Lyou, S. H., Yeu, S., Kim, M. A., Rhee, S., Kim, M., Lee, J. S., Choi, Y. Do, & Cheong, J. J. (2007). Microarray-based screening of jasmonate-responsive genes in *Arabidopsis thaliana*. *Plant Cell Reports*, **26**: 1053–1063.

Kambhampati, S., Kurepin, L. V., Kisiala, A. B., Bruce, K. E., Cober, E. R., Morrison, M. J., & Emery, R. J. N. (2017). Yield associated traits correlate with cytokinin profiles in developing pods and seeds of field-grown soybean cultivars. *Field Crops Research*, **214**: 175–184.

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, **28**: 27–30.

Kant, M. R., Jonckheere, W., Knecht, B., Lemos, F., Liu, J., Schimmel, B. C. J., Villarroel, C. A., Ataíde, L. M. S., Dermauw, W., Glas, J. J., Egas, M., Janssen, A., Van Leeuwen, T., Schuurink, R. C., Sabelis, M. W., & Alba, J. M. (2015). Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. *Annals of Botany*, **115**: 1015–1051.

Kant, M. R., Ament, K., Sabelis, M. W., Haring, M. A., & Schuurink, R. C. (2004). Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology*, **135**: 483–495.

Kaplan, I., Dively, G. P., & Denno, R. F. (2009). The costs of anti-herbivore defense traits in agricultural crop plants: A case study involving leafhoppers and trichomes. *Ecological Applications*, **19**: 864–872.

Karban, R. (2011). The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, **25**: 339–347.

Kasahara, H. (2016). Current aspects of auxin biosynthesis in plants. *Bioscience, Biotechnology and Biochemistry*, **80**: 34–42.

Kassambara, A., & Mundt, F. (2017). *factoextra: Extract and visualize the results of multivariate data analyses* (Version 1.0.3) [R package] <https://CRAN.R-project.org/package=factoextra>

Kattupalli, D., Barbadikar, K. M., Baliya, V., Balichatla, S., Athulya, R., Padmakumari, A. P., Saxena, S., Gaikwad, K., Yerram, S., Kokku, P., & Madhav, M. S. (2021). The draft genome of yellow stem borer, an agriculturally important pest, provides molecular insights into its biology, development and specificity towards rice for infestation. *Insects*, **12**: 563.

Katyayini, N. U., Rinne, P. L. H., Tarkowská, D., Strnad, M., & van der Schoot, C. (2020). Dual role of gibberellin in perennial shoot branching: inhibition and activation. *Frontiers in Plant Science*, **11**: 736.

Kaur, S., Tiwari, V., Kumari, A., Chaudhary, E., Sharma, A., Ali, U., & Garg, M. (2023). Protective and defensive role of anthocyanins under plant abiotic and biotic stresses: an emerging application in sustainable agriculture. *Journal of Biotechnology*, **361**: 12–29.

- Kawazu, K., Mochizuki, A., Sato, Y., Sugeno, W., Murata, M., Seo, S., & Mitsuhashi, I.** (2012). Different expression profiles of jasmonic acid and salicylic acid inducible genes in the tomato plant against herbivores with various feeding modes. *Arthropod-Plant Interactions*, **6**: 221–230.
- Kazan, K.** (2013). Auxin and the integration of environmental signals into plant root development. *Annals of Botany*, **112**: 1655–1665.
- Kazan, K., & Manners, J. M.** (2009). Linking development to defense: auxin in plant-pathogen interactions. *Trends in Plant Science*, **14**: 373–382.
- Khan, B. A., Nadeem, M. A., Nawaz, H., Amin, M. M., Abbasi, G. H., Nadeem, M., Ali, M., Ameen, M., Javaid, M. M., Maqbool, R., Ikram, M., & Ayub, M. A.** (2023). Pesticides: impacts on agriculture productivity, environment, and management strategies. In T. Aftab (Ed.), *Emerging contaminants and plants: Interactions, adaptations and remediation technologies* (pp. 109–134). Cham: Springer International Publishing.
- Kieber, J. J., & Schaller, G. E.** (2018). Cytokinin signaling in plant development. *Development*, **145**: dev149344.
- Kim, D., Langmead, B., & Salzberg, S. L.** (2015). HISAT: a fast spliced aligner with low memory requirements. *Nature Methods*, **12**: 357–360.
- Kim, J. Y., Park, Y. J., Lee, J. H., Kim, Z. H., & Park, C. M.** (2021). EIN3-mediated ethylene signaling attenuates auxin response during hypocotyl thermomorphogenesis. *Plant and Cell Physiology*, **62**: 708–720.
- Kindt, F., Joosten, N. N., Peters, D., & Tjallingii, W. F.** (2003). Characterisation of the feeding behaviour of western flower thrips in terms of electrical penetration graph (EPG) waveforms. *Journal of Insect Physiology*, **49**: 183–191.
- Kirichenko, N., Augustin, S., & Kenis, M.** (2019). Invasive leafminers on woody plants: a global review of pathways, impact, and management. *Journal of Pest Science*, **92**: 93–106.
- Kolde, R.** (2022). *pheatmap: Pretty heatmaps* (Version 1.0.12). [R package] <https://CRAN.R-project.org/package=pheatmap>
- Külheim, C., & Jansson, S.** (2005). What leads to reduced fitness in non-photochemical quenching mutants? *Physiologia Plantarum*, **125**: 202–211.
- Laby, R. J., Kincaid, M. S., Kim, D., & Gibson, S. I.** (2000). The Arabidopsis sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *The Plant Journal*, **23**: 587–596.
- Lackman, P., González-Guzmán, M., Tilleman, S., Carqueijeiro, I., Pérez, A. C., Moses, T., Seo, M., Kanno, Y., Häkkinen, S. T., Van Montagu, M. C. E., Thevelein, J. M., Maaheimo, H., Oksman-Caldentey, K. M., Rodríguez, P. L., Rischer, H., & Goossens, A.** (2011). Jasmonate signaling involves the abscisic acid receptor PYL4 to regulate metabolic reprogramming in Arabidopsis and tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, **108**: 5891–5896.
- Landi, M., Tattini, M., & Gould, K. S.** (2015). Multiple functional roles of anthocyanins in plant-environment interactions. *Environmental and Experimental Botany*, **119**: 4–17.
- Lange, M. J. P., & Lange, T.** (2015). Touch-induced changes in Arabidopsis morphology dependent on gibberellin breakdown. *Nature Plants*, **1**: 1–5.
- Lata, C., & Prasad, M.** (2011). Role of DREBs in regulation of abiotic stress responses in plants. *Journal of Experimental Botany*, **62**: 4731–4748.
- Lewandowska, M., Keyl, A., & Feussner, I.** (2020). Wax biosynthesis in response to danger: its regulation upon abiotic and biotic stress. *New Phytologist*, **227**: 698–713.
- Li, N., Han, X., Feng, D., Yuan, D., & Huang, L. J.** (2019a). Signaling crosstalk between salicylic acid and ethylene/Jasmonate in plant defense: do we understand what they are whispering? *International Journal of Molecular Sciences*, **20**: 671.

- Li, X., Ouyang, X., Zhang, Z., He, L., Wang, Y., Li, Y., Zhao, J., Chen, Z., Wang, C., Ding, L., Pei, Y., & Xiao, Y. (2019c). Over-expression of the red plant gene R1 enhances anthocyanin production and resistance to bollworm and spider mite in cotton. *Molecular Genetics and Genomics*, **294**: 469–478.
- Li, X., Chen, J., He, Y., Yang, G., Li, Z., Tao, Y., Li, Y., Li, Y., Huang, L., & Feng, X. (2023). High-through counting of Chinese cabbage trichomes based on deep learning and trinocular stereo microscope. *Computers and Electronics in Agriculture*, **212**: 108134.
- Li, Y., Yang, Y., Hu, Y., Liu, H., He, M., Yang, Z., Kong, F., Liu, X., & Hou, X. (2019b). DELLA and EDS1 form a feedback regulatory module to fine-tune plant growth–defense tradeoff in Arabidopsis. *Molecular Plant*, **12**: 1485–1498.
- Liao, K., Peng, Y. J., Yuan, L. B., Dai, Y. S., Chen, Q. F., Yu, L. J., Bai, M. Y., Zhang, W. Q., Xie, L. J., & Xiao, S. (2020). Brassinosteroids antagonize jasmonate-activated plant defense responses through BRI1-EMS-Suppressor1 (BES1)1. *Plant Physiology*, **182**: 1066–1082.
- Liao, Y., Smyth, G. K., & Shi, W. (2014). FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*, **30**: 923–930.
- Little, D., Gouhier-Darimont, C., Bruessow, F., & Reymond, P. (2007). Oviposition by pierid butterflies triggers defense responses in Arabidopsis. *Plant Physiology*, **143**: 784–800.
- Liu, B., Seong, K., Pang, S., Song, J., Gao, H., Wang, C., Zhai, J., Zhang, Y., Gao, S., Li, X., Qi, T., & Song, S. (2021). Functional specificity, diversity, and redundancy of Arabidopsis JAZ family repressors in jasmonate and COI1-regulated growth, development, and defense. *New Phytologist*, **231**: 1525–1545.
- Liu, F., Jiang, H., Ye, S., Chen, W. P., Liang, W., Xu, Y., Sun, B., Sun, J., Wang, Q., Cohen, J. D., & Li, C. (2010). The Arabidopsis P450 protein CYP82C2 modulates jasmonate-induced root growth inhibition, defense gene expression and indole glucosinolate biosynthesis. *Cell Research*, **20**: 539–552.
- Liu, H., & Timko, M. P. (2021). Jasmonic acid signaling and molecular crosstalk with other phytohormones. *International Journal of Molecular Sciences*, **22**: 2914.
- Liu, L., Li, H., Zeng, H., Cai, Q., Zhou, X., & Yin, C. (2016). Exogenous jasmonic acid and cytokinin antagonistically regulate rice flag leaf senescence by mediating chlorophyll degradation, membrane deterioration, and senescence-associated genes expression. *Journal of Plant Growth Regulation*, **35**: 366–376.
- Liu, X., Bai, X., Wang, X., & Chu, C. (2007). OsWRKY71, a rice transcription factor, is involved in rice defense response. *Journal of Plant Physiology*, **164**: 969–979.
- Liu, X., & Hou, X. (2018). Antagonistic regulation of ABA and GA in metabolism and signaling pathways. *Frontiers in Plant Science*, **9**: 251.
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) method. *Methods*, **25**: 402–408.
- Lombardero, M. J., Ayres, M. P., Lorio, P. L., & Ruel, J. J. (2000). Environmental effects on constitutive and inducible resin defences of *Pinus taeda*. *Ecology Letters*, **3**: 329–339.
- Long, R. L., Gorecki, M. J., Renton, M., Scott, J. K., Colville, L., Goggin, D. E., Commander, L. E., Westcott, D. A., Cherry, H., & Finch-Savage, W. E. (2015). The ecophysiology of seed persistence: a mechanistic view of the journey to germination or demise. *Biological Reviews*, **90**: 31–59.
- Loreti, E., Povero, G., Novi, G., Solfanelli, C., Alpi, A., & Perata, P. (2008). Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. *New Phytologist*, **179**: 1004–1016.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, **15**: 1–21.

- Luczynski, A., Isman, M. B., & Raworth, D. A.** (1990). Strawberry foliar phenolics and their relationship to development of the twospotted spider mite. *Journal of Economic Entomology*, **83**: 557–563.
- Lüdecke, D.** (2017). *esc: Effect size computation for meta analysis* (Version 0.3.1) [R Package] <https://CRAN.R-project.org/package=esc>
- Ludwig-Müller, J.** (2015). Bacteria and fungi controlling plant growth by manipulating auxin: balance between development and defense. *Journal of Plant Physiology*, **172**: 4–12.
- Luo, K., Jin, Y., Wen, S., Li, Y., Rong, J., & Ding, M.** (2023). Detection and quantification of cotton trichomes by deep learning algorithm. *Computers and Electronics in Agriculture*, **210**: 107936.
- Macedo, T. B., Peterson, R. K. D., Weaver, D. K., & Ni, X.** (2009). Impact of *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae) on primary physiology of four near-isogenic wheat lines. *Journal of Economic Entomology*, **102**: 412–421.
- Machado, R. A. R., Baldwin, I. T., & Erb, M.** (2017). Herbivory-induced jasmonates constrain plant sugar accumulation and growth by antagonizing gibberellin signaling and not by promoting secondary metabolite production. *New Phytologist*, **215**: 803–812.
- Macke, E., Magalhães, S., Khan, H. D. T., Luciano, A., Frantz, A., Facon, B., & Olivieri, I.** (2011). Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society B: Biological Sciences*, **278**: 1054–1063.
- 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**: e1005760.
- Martel, C., Zhurov, V., Navarro, M., Martinez, M., Cazaux, M., Auger, P., Migeon, A., Santamaría, M. E., Wybouw, N., Diaz, I., Van Leeuwen, T., Navajas, M., Grbic, M., & Grbic, V.** (2015). Tomato whole genome transcriptional response to *Tetranychus urticae* identifies divergence of spider mite-induced responses between tomato and Arabidopsis. *Molecular Plant-Microbe Interactions*, **28**: 343–361.
- Martinez, M., & Diaz, I.** (2024). Plant cyanogenic-derived metabolites and herbivore counter-defences. *Plants*, **13**: 1239.
- Martinez, M., Santamaría, M. E., Diaz-Mendoza, M., Arnaiz, A., Carrillo, L., & Ortego, F.** (2016). Phytocystatins: defense proteins against phytophagous insects and acari. *International Journal of Molecular Sciences*, **17**: 1747.
- Matson, P. A., Parton, W. J., Power, A. G., & Swift, M. J.** (1997). Agricultural intensification and ecosystem properties. *Science*, **277**: 504–509.
- Mazzi, D., & Dorn, S.** (2012). Movement of insect pests in agricultural landscapes. *Annals of Applied Biology*, **160**: 97–113.
- Mehra, P., Fairburn, R., Leftley, N., Banda, J., & Bennett, M. J.** (2023). Turning up the volume: how root branching adaptive responses aid water foraging. *Current Opinion in Plant Biology*, **75**: 102405.
- Miao, Z. Q., Zhao, P. X., Mao, J. L., Yu, L. H., Yuan, Y., Tang, H., Liu, Z. B., & Xiang, C. Bin.** (2018). HOMEBOX PROTEIN52 mediates the crosstalk between ethylene and auxin signaling during primary root elongation by modulating auxin transport-related gene expression. *The Plant Cell*, **30**: 276.
- Migeon, A. & Dorkeld, F.** (2024) *Spider Mites Web: a comprehensive database for the Tetranychidae*. [Website] <https://www1.montpellier.inra.fr/CBGP/spmweb/> [Accessed on 18.03.2024].
- Mikkelsen, M. D., Hansen, C. H., Wittstock, U., & Halkier, B. A.** (2000). Cytochrome P450 CYP79B2 from Arabidopsis catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. *Journal of Biological Chemistry*, **275**: 33712–33717.

- Millard, P., & Grelet, G. A.** (2010). Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. *Tree Physiology*, **30**: 1083–1095.
- Mishina, T. E., & Zeier, J.** (2007). Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in *Arabidopsis*. *The Plant Journal*, **50**: 500–513.
- Mitchum, M. G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, S., Kamiya, Y., & Sun, T. P.** (2006). Distinct and overlapping roles of two gibberellin 3-oxidases in *Arabidopsis* development. *The Plant Journal*, **45**: 804–818.
- Mittler, R.** (2017). ROS Are Good. *Trends in Plant Science*, **22**: 11–19.
- Moghe, G., Irfan, M., & Sarmah, B.** (2023). Dangerous sugars: structural diversity and functional significance of acylsugar-like defense compounds in flowering plants. *Current Opinion in Plant Biology*, **73**: 102348.
- Mohamed, N., Mohamed, M., El-Ebiarie, A., & Gamal, N.** (2019). Biochemical characterization of some digestive enzymes in the midgut of *Eristalis megacephala* (Diptera: Syrphidae). *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, **11**: 77–95.
- Mohammadi, K., Fathi, S. A. A., Razmjou, J., & Naseri, B.** (2021). Evaluation of the effect of strip intercropping green bean/garlic on the control of *Tetranychus urticae* in the field. *Experimental and Applied Acarology*, **83**: 183–195.
- Moher, D., Liberati, A., Tetzlaff, J., & Altman, D.G.** (2009). The PRISMA group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *PLoS Medicine*, **6**: e1000097.
- Mok, M. C.** (2020). Cytokinins and plant development — an overview. In D. W. S. Mock & M. C. Mock (Eds.), *Cytokinins* (1st ed., pp. 55–166). Boca Raton, FL: CRC Press.
- Moreira, X., Mooney, K. A., Rasmann, S., Petry, W. K., Carrillo-Gavilán, A., Zas, R., & Sampedro, L.** (2014). Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters*, **17**: 537–546.
- Morris, K., Mackerness, S. A. H., Page, T., Fred John, C., Murphy, A. M., Carr, J. P., & Buchanan-Wollaston, V.** (2000). Salicylic acid has a role in regulating gene expression during leaf senescence. *The Plant Journal*, **23**: 677–685.
- Mouden, S., Bac-Molenaar, J. A., Kappers, I. F., Beerling, E. A. M., & Leiss, K. A.** (2021). Elicitor application in strawberry results in long-term increase of plant resilience without yield loss. *Frontiers in Plant Science*, **12**: 695908.
- Muday, G. K., Rahman, A., & Binder, B. M.** (2012). Auxin and ethylene: collaborators or competitors? *Trends in Plant Science*, **17**: 181–195.
- Müller, M.** (2021). Foes or friends: ABA and ethylene interaction under abiotic stress. *Plants*, **10**: 448.
- Müller, T. M., Böttcher, C., & Glawischnig, E.** (2019). Dissection of the network of indolic defence compounds in *Arabidopsis thaliana* by multiple mutant analysis. *Phytochemistry*, **161**: 11–20.
- Muller-Landau, H. C.** (2010). The tolerance-fecundity trade-off and the maintenance of diversity in seed size. *Proceedings of the National Academy of Sciences of the United States of America*, **107**: 4242–4247.
- Murashige, T., & Skoog, F.** (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, **15**.
- Murphy, A.** (2015). Hormone crosstalk in plants. *Journal of Experimental Botany*, **66**: 4853–4854.
- Musser, R. O., Hum-Musser, S. M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J. B., & Felton, G. W.** (2002). Caterpillar saliva beats plant defences. *Nature*, **416**: 599–600.

- Myers, J. H., & Sarfraz, R. M.** (2017). Impacts of insect herbivores on plant populations. *Annual Review of Entomology*, **62**: 207–230.
- Mymko, D., & Avila-Sakar, G.** (2019). The influence of leaf ontogenetic stage and plant reproductive phenology on trichome density and constitutive resistance in six tomato varieties. *Arthropod-Plant Interactions*, **13**: 797–803.
- Nachappa, P., Margolies, D. C., Nechols, J. R., Whitfield, A. E., & Rotenberg, D.** (2013). Tomato spotted wilt virus benefits a non-vector arthropod, *Tetranychus urticae*, by modulating different plant responses in tomato. *PLoS ONE*, **8**: e75909.
- Naher, N., Islam, M. W., Khalequzzaman, M., & Haque, M. M.** (1970). Study on the developmental stages of spider mite (*Tetranychus urticae* Koch) infesting country bean. *Journal of Bio-Science*, **16**: 109–114.
- Nakata, M., Mitsuda, N., Herde, M., Koo, A. J. K., Moreno, J. E., Suzuki, K., Howe, G. A., & Ohme-Takagi, M.** (2013). A bHLH-type transcription factor, ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR/JA-ASSOCIATED MYC2-LIKE1, acts as a repressor to negatively regulate jasmonate signaling in Arabidopsis. *The Plant Cell*, **25**: 1641–1656.
- Naseem, M., & Dandekar, T.** (2012). The role of auxin-cytokinin antagonism in plant-pathogen interactions. *PLoS Pathogens*, **8**: e1003026.
- Naseem, M., Srivastava, M., Tehseen, M., & Ahmed, N.** (2015). Auxin crosstalk to plant immune networks: a plant-pathogen interaction perspective. *Current Protein & Peptide Science*, **16**: 389–394.
- Nault, L. R.** (1997). Arthropod transmission of plant viruses: a new synthesis. *Annals of the Entomological Society of America*, **90**: 521–541.
- Navarro, L., Bari, R., Achard, P., Lisón, P., Nemri, A., Harberd, N. P., & Jones, J. D. G.** (2008). DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. *Current Biology*, **18**: 650–655.
- Nemhauser, J. L., Hong, F., & Chory, J.** (2006). Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell*, **126**: 467–475.
- Ngou, B. P. M., Ahn, H. K., Ding, P., & Jones, J. D. G.** (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature*, **592**: 110–115.
- Ngou, B. P. M., Heal, R., Wyler, M., Schmid, M. W., & Jones, J. D. G.** (2022). Concerted expansion and contraction of immune receptor gene repertoires in plant genomes. *Nature Plants*, **8**: 1146–1152.
- Nguyen, C. H., Yan, D., & Nambara, E.** (2023). Persistence of abscisic acid analogs in plants: chemical control of plant growth and physiology. *Genes*, **14**: 1078.
- Niesenbaum, R. A.** (1996). Linking herbivory and pollination: defoliation and selective fruit abortion in linden benzoin. *Ecology*, **77**: 2324–2331.
- Noir, S., Bömer, M., Takahashi, N., Ishida, T., Tsui, T. L., Balbi, V., Shanahan, H., Sugimoto, K., & Devoto, A.** (2013). Jasmonate controls leaf growth by repressing cell proliferation and the onset of endoreduplication while maintaining a potential stand-by mode. *Plant Physiology*, **161**: 1930–1951.
- Nolan, T., Chen, J., & Yin, Y.** (2017). Cross-talk of brassinosteroid signaling in controlling growth and stress responses. *Biochemical Journal*, **474**: 2641–2661.
- Núñez-Farfán, J., Fornoni, J., & Valverde, P. L.** (2007). The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics*, **38**: 541–566.
- Nykänen, H., & Koricheva, J.** (2004). Damage-induced changes in woody plants and their effects on insect herbivore performance: a meta-analysis. *Oikos*, **104**: 247–268.
- Ojeda-Martínez, D., Díaz, I., & Santamaría, M. E.** (2022). Transcriptomic landscape of herbivore oviposition in Arabidopsis: a systematic review. *Frontiers in Plant Science*, **12**: 772492.

- Ojeda-Martinez, D., Martinez, M., Diaz, I., & Estrella Santamaría, M. E. (2021). Spider mite egg extract modifies Arabidopsis response to future infestations. *Scientific Reports*, *11*: 17692.
- Ojeda-Martinez, D., Martinez, M., Diaz, I., & Santamaría, M. E. (2020). Saving time maintaining reliability: a new method for quantification of *Tetranychus urticae* damage in Arabidopsis whole rosettes. *BMC Plant Biology*, *20*: 1–19.
- Oklestkova, J., Tarkowská, D., Eyer, L., Elbert, T., Marek, A., Smržová, Z., Novák, O., Fránek, M., Zhabinskii, V. N., & Strnad, M. (2017). Immunoaffinity chromatography combined with tandem mass spectrometry: a new tool for the selective capture and analysis of brassinosteroid plant hormones. *Talanta*, *170*: 432–440.
- Oku, K., Magalhães, S., & Dicke, M. (2009). The presence of webbing affects the oviposition rate of two-spotted spider mites, *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, *49*: 167–172.
- Olawale Adisa, Bamidele Segun Ilugbusi, Odunayo Adewunmi Adelekan, Onyeka Franca Asuzu, & Ndubuisi Leonard Ndubuisi. (2024). A comprehensive review of redefining agricultural economics for sustainable development: overcoming challenges and seizing opportunities in a changing world. *World Journal of Advanced Research and Reviews*, *21*: 2329–2341.
- Oñate-Sánchez, L., & Vicente-Carbajosa, J. (2008). DNA-free RNA isolation protocols for *Arabidopsis thaliana*, including seeds and siliques. *BMC Research Notes*, *1*: 1–7.
- Ortiz-García, P., González Ortega-Villaizán, A., Onejeme, F. C., Müller, M., & Pollmann, S. (2023). Do opposites attract? Auxin-abscisic acid crosstalk: new perspectives. *International Journal of Molecular Sciences*, *24*: 3090.
- Osakabe, M., Uesugi, R., & Goka, K. (2009). Evolutionary aspects of acaricide-resistance development in spider mites. *Psyche*, *2009*: 947439.
- Osugi, A., Kojima, M., Takebayashi, Y., Ueda, N., Kiba, T., & Sakakibara, H. (2017). Systemic transport of *trans*-zeatin and its precursor have differing roles in Arabidopsis shoots. *Nature Plants*, *3*: 1–6.
- Pagán, I., Alonso-Blanco, C., & García-Arenal, F. (2007). The relationship of within-host multiplication and virulence in a plant-virus system. *PLoS ONE*, *2*: e786.
- Pagán, I., & García-Arenal, F. (2018). Tolerance to plant pathogens: theory and experimental evidence. *International Journal of Molecular Sciences*, *19*: 810.
- Pajerowska-Mukhtar, K. M., Wang, W., Tada, Y., Oka, N., Tucker, C. L., Fonseca, J. P., & Dong, X. (2012). The HSF-like transcription factor TBF1 is a major molecular switch for plant growth-to-defense transition. *Current Biology*, *22*: 103–112.
- Pakyari, H., Fathipour, Y., & Enkegaard, A. (2011). Effect of temperature on life table parameters of predatory thrips *Scolothrips longicornis* (Thysanoptera: Thripidae) fed on twospotted spider mites (Acari: Tetranychidae). *Journal of Economic Entomology*, *104*: 799–805.
- Pan, J., Hu, Y., Wang, H., Guo, Q., Chen, Y., Howe, G. A., & Yu, D. (2020). Molecular mechanism underlying the synergetic effect of jasmonate on abscisic acid signaling during seed germination in Arabidopsis. *The Plant Cell*, *32*: 3846–3865.
- Pandey, S. P., Srivastava, S., Goel, R., Lakhwani, D., Singh, P., Asif, M. H., & Sane, A. P. (2017). Simulated herbivory in chickpea causes rapid changes in defense pathways and hormonal transcription networks of JA/ethylene/GA/auxin within minutes of wounding. *Scientific Reports*, *7*: 44729.
- Paponov, M., Antonyan, M., Slimestad, R., & Paponov, I. A. (2021). Decoupling of plant growth and accumulation of biologically active compounds in leaves, roots, and root exudates of *Hypericum perforatum* L. by the combination of jasmonate and far-red lighting. *Biomolecules*, *11*: 1283.
- Park, C. J., & Shin, R. (2022). Calcium channels and transporters: roles in response to biotic and abiotic stresses. *Frontiers in Plant Science*, *13*: 964059.

- Park, S. J., Kwak, K. J., Oh, T. R., Kim, Y. O., & Kang, H.** (2009). Cold shock domain proteins affect seed germination and growth of *Arabidopsis thaliana* under abiotic stress conditions. *Plant and Cell Physiology*, **50**: 869–878.
- Peiffer, M., & Felton, G. W.** (2009). Do caterpillars secrete “oral secretions”? *Journal of Chemical Ecology*, **35**: 326–335.
- Pereira-Netto, A. B., Carvalho-Oliveira, M. M. C., Ramírez, J. A., & Galagovsky, L. R.** (2006). Shooting control in *Eucalyptus Grandis* x *E. urophylla* hybrid: comparative effects of 28-homocastasterone and a 5 α -monofluoro derivative. *Plant Cell, Tissue and Organ Culture*, **86**: 329–335.
- Pérez-Alonso, M. M., Sánchez-Parra, B., Ortiz-García, P., Santamaría, M. E., Díaz, I., & Pollmann, S.** (2021). Jasmonic acid-dependent myc transcription factors bind to a tandem g-box motif in the *YUCCA8* and *YUCCA9* promoters to regulate biotic stress responses. *International Journal of Molecular Sciences*, **22**: 9768.
- Phukan, U. J., Jeena, G. S., & Shukla, R. K.** (2016). WRKY transcription factors: molecular regulation and stress responses in plants. *Frontiers in Plant Science*, **7**: 760.
- Pieterse, C. M. J., & Van Loon, L. C.** (2004). NPR1: the spider in the web of induced resistance signaling pathways. *Current Opinion in Plant Biology*, **7**: 456–464.
- Pincebourde, S., Frak, E., Sinoquet, H., Regnard, J. L., & Casas, J.** (2006). Herbivory mitigation through increased water-use efficiency in a leaf-mining moth-apple tree relationship. *Plant, Cell & Environment*, **29**: 2238–2247.
- Plackett, A. R. G., Powers, S. J., Fernandez-Garcia, N., Urbanova, T., Takebayashi, Y., Seo, M., Jikumaru, Y., Benlloch, R., Nilsson, O., Ruiz-Rivero, O., Phillips, A. L., Wilson, Z. A., Thomas, S. G., & Hedden, P.** (2012). Analysis of the developmental roles of the *Arabidopsis* gibberellin 20-oxidases demonstrates that *GA20ox1*, -2, and -3 are the dominant paralogs. *The Plant Cell*, **24**: 941–960.
- Pomeranz, M., Campbell, J., Siegal-Gaskins, D., Engelmeier, J., Wilson, T., Fernandez, V., Brkljacic, J., & Grotewold, E.** (2013). High-resolution computational imaging of leaf hair patterning using polarized light microscopy. *The Plant Journal*, **73**: 701–708.
- Posit team** (2023) *RStudio: Integrated development environment for R*. (Version 2023.9.0.463). [Software]. Boston, MA: Posit Software, PBC. <https://www.posit.co/>
- Praslička, J., & Huszár, J.** (2004). Influence of temperature and host plants on the development and fecundity of the spider mite *Tetranychus urticae* (Acarina: Tetranychidae). *Plant Protection Science*, **40**: 141.
- Puentes, A., & Ågren, J.** (2014). No trade-off between trichome production and tolerance to leaf and inflorescence damage in a natural population of *Arabidopsis lyrata*. *Journal of Plant Ecology*, **7**: 373–383.
- Qi, L., Yan, J., Li, Y., Jiang, H., Sun, J., Chen, Q., Li, H., Chu, J., Yan, C., Sun, X., Yu, Y., Li, C., & Li, C.** (2012). *Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. *New Phytologist*, **195**: 872–882.
- Qi, T., Huang, H., Song, S., & Xie, D.** (2015). Regulation of jasmonate-mediated stamen development and seed production by a bHLH-MYB complex in *Arabidopsis*. *The Plant Cell*, **27**: 1620–1633.
- Qin, H., Wang, J., Chen, X., Wang, F., Peng, P., Zhou, Y., Miao, Y., Zhang, Y., Gao, Y., Qi, Y., & others.** (2019). Rice OsDOF 15 contributes to ethylene-inhibited primary root elongation under salt stress. *New Phytologist*, **223**: 798–813.
- R Core Team** (2020). *R: A language and environment for statistical computing* (Version 4.0.0) [Software]. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>
- R Core Team** (2021). *R: A language and environment for statistical computing* (Version 4.1.1) [Software]. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>

- Rakha, M., Bouba, N., Ramasamy, S., Regnard, J. L., & Hanson, P.** (2017). Evaluation of wild tomato accessions (*Solanum* spp.) for resistance to two-spotted spider mite (*Tetranychus urticae* Koch) based on trichome type and acylsugar content. *Genetic Resources and Crop Evolution*, **64**: 1011–1022.
- R Raman, A., Schaefer, C. W., & Withers, T. M.** (2005). Galls and gall-inducing arthropods: an overview of their biology, ecology, and evolution. In A. Raman, C. W. Schaefer, & T. M. Withers (Eds.), *Biology, ecology and evolution of gall-inducing arthropods* (pp. 1–33). Enfield, NH: Science Publishers.
- Ray, S., Helms, A. M., Matulis, N. L., Davidson-Lowe, E., Grisales, W., & Ali, J. G.** (2020). Asymmetry in herbivore effector responses: caterpillar frass effectors reduce performance of a subsequent herbivore. *Journal of Chemical Ecology*, **46**: 76–83.
- Rehman, F., Khan, F. A., & Badruddin, S. M. A.** (2012). Role of phenolics in plant defense against insect herbivory. In A. K. Verma, A. K. Prasad, & S. M. A. Badruddin (Eds.), *Chemistry of phytopotentials: Health, energy and environmental perspectives* (pp. 309–313). Berlin, Germany: Springer.
- Reina-Pinto, J. J., & Yephremov, A.** (2009). Surface lipids and plant defenses. *Plant Physiology and Biochemistry*, **47**: 540–549.
- Resende, J. T. V., Filho, R. B. de L., Ribeiro, L. K., Corrêa, J. V. W., Maciel, C. D. de G., & Youssef, K.** (2020). Strawberry genotypes with resistance to *Tetranychus urticae* mediated by leaf trichomes. *Ciencia e Agrotecnologia*, **44**: e006920.
- Resh, V. H., & Cardé, R. T.** (2009). *Encyclopedia of insects* (2nd ed.). London, United Kingdom: Academic press.
- Reymond, P.** (2013). Perception, signaling and molecular basis of oviposition-mediated plant responses. *Planta*, **238**: 247–258.
- Riahi, E., Shishehbor, P., Nemati, A. R., & Saeidi, Z.** (2013). Temperature effects on development and life table parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Journal of Agricultural Science and Technology*, **15**: 661–672.
- Rieu, I., Eriksson, S., Powers, S. J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S. G., Hedden, P., & Phillips, A. L.** (2008a). Genetic analysis reveals that C₁₉-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. *The Plant Cell*, **20**: 2420–2436.
- Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S. J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S. G., Phillips, A. L., & Hedden, P.** (2008b). The gibberellin biosynthetic genes *AtGA20ox1* and *AtGA20ox2* act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *The Plant Journal*, **53**: 488–504.
- Rioja, C., Zhurov, V., Bruinsma, K., Grbic, M., & Grbic, V.** (2017). Plant-herbivore interactions: a case of an extreme generalist, the two-spotted spider mite *Tetranychus urticae*. *Molecular Plant-Microbe Interactions*, **30**: 935–945.
- Ritonga, F. N., Zhou, D., Zhang, Y., Song, R., Li, C., Li, J., & Gao, J.** (2023). The roles of gibberellins in regulating leaf development. *Plants*, **12**: 1243.
- Robert, C. A. M., Ferrieri, R. A., Schirmer, S., Babst, B. A., Schueller, M. J., Machado, R. A. R., Arce, C. C. M., Hibbard, B. E., Gershenson, J., Turlings, T. C. J., & Erb, M.** (2014). Induced carbon reallocation and compensatory growth as root herbivore tolerance mechanisms. *Plant, Cell & Environment*, **37**: 2613–2622.
- Robert-Seilanianantz, A., MacLean, D., Jikumar, Y., Hill, L., Yamaguchi, S., Kamiya, Y., & Jones, J. D. G.** (2011). The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. *The Plant Journal*, **67**: 218–231.
- Romero-Hernandez, G., & Martinez, M.** (2022b). Plant kinases in the perception and signaling networks associated with arthropod herbivory. *Frontiers in Plant Science*, **13**: 824422.

- Romero-Hernandez, G., & Martinez, M.** (2022a). Opposite roles of MAPKKK17 and MAPKKK21 against *Tetranychus urticae* in Arabidopsis. *Frontiers in Plant Science*, **13**: 1038866.
- Rosa-Diaz, I.** (2024). Fine-tune regulatory mechanisms required for efficient defenses to herbivores. Tesis (Doctoral), E.T.S. de Ingeniería Agronómica, Alimentaria y de Biosistemas (UPM).
- Rosa-Diaz, I., Rowe, J., Cayuela-Lopez, A., Arbona, V., Diaz, I., & Jones, A. M.** (2024). Spider mite herbivory induces an ABA-driven stomatal defense. *Plant Physiology*, **195**: 2970-2984.
- Rosa-Diaz, I., Santamaría, M. E., Acien, J. M., & Diaz, I.** (2023). Jasmonic acid catabolism in Arabidopsis defence against mites. *Plant Science*, **334**: 111784.
- Rosenberg, M. S.** (2005). The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution*, **59**: 464–468.
- Rosenthal, R.** (1991). *Meta-analytic procedures for social research* (Rev. ed). Newbury Park. CA: Sage.
- RStudio Team** (2016). *RStudio: Integrated Development for R*. (Version 1.1.463) [Software] Boston, MA: RStudio, PBC. <http://www.rstudio.com/>
- RStudio Team** (2022). *RStudio: Integrated Development Environment for R*. (Version 2022.02.0+433pro2) [Software] Boston, MA: RStudio, PBC. <http://www.rstudio.com/>
- Rusman, Q., Poelman, E. H., Nowrin, F., Polder, G., & Lucas-Barbosa, D.** (2019). Floral plasticity: herbivore-species-specific-induced changes in flower traits with contrasting effects on pollinator visitation. *Plant, Cell & Environment*, **42**: 1882–1896.
- Saito, Y.** (1977). Study on the spinning behavior of the spider mite (Acarina: Tetranychidae). I. method for the quantitative evaluation of the mite webbing, and the relationship between webbing and walking. *Japanese Journal of Applied Entomology and Zoology*, **21**: 27–34.
- Sano, N., Rajjou, L., North, H. M., Debeaujon, I., Marion-Poll, A., & Seo, M.** (2016). Staying alive: molecular aspects of seed longevity. *Plant and Cell Physiology*, **57**: 660–674.
- Santamaría, M. E., Arnaiz, A., Gonzalez-Melendi, P., Martinez, M., & Diaz, I.** (2018a). Plant perception and short-term responses to phytophagous insects and mites. *International Journal of Molecular Sciences*, **19**: 1356.
- Santamaría, M. E., Arnaiz, A., Rosa-Diaz, I., González-Melendi, P., Romero-Hernandez, G., Ojeda-Martinez, D. A., Garcia, A., Contreras, E., Martinez, M., & Diaz, I.** (2020). Plant defenses against *Tetranychus urticae*: mind the gaps. *Plants*, **9**: 464.
- Santamaría, M. E., Arnaiz, A., Velasco-Arroyo, B., Grbic, V., Diaz, I., & Martinez, M.** (2018b). Arabidopsis response to the spider mite *Tetranychus urticae* depends on the regulation of reactive oxygen species homeostasis. *Scientific Reports*, **8**: 9432.
- Santamaría, M. E., Garcia, A., Arnaiz, A., Rosa-Diaz, I., Romero-Hernandez, G., Diaz, I., & Martinez, M.** (2021). Comparative transcriptomics reveals hidden issues in the plant response to arthropod herbivores. *Journal of Integrative Plant Biology*, **63**: 312–326.
- Santamaría, M. E., Martinez, M., Arnaiz, A., Ortego, F., Grbic, V., & Diaz, I.** (2017). MATI, a novel protein involved in the regulation of herbivore-associated signaling pathways. *Frontiers in Plant Science*, **8**: 975.
- Santamaría, M. E., Martínez, M., Arnaiz, A., Rioja, C., Burow, M., Grbic, V., & Díaz, I.** (2019). An arabidopsis TIR-lectin two-domain protein confers defense properties against *Tetranychus urticae*. *Plant Physiology*, **179**: 1298–1314.
- Santamaría, M. E., Martínez, M., Cambra, I., Grbic, V., & Diaz, I.** (2013). Understanding plant defence responses against herbivore attacks: an essential first step towards the development of sustainable resistance against pests. *Transgenic Research*, **22**: 697–708.

- Santamaría, M. E., González-Cabrera, J., Martínez, M., Grbic, V., Castañera, P., Díaz, L., & Ortego, F. (2015). Digestive proteases in bodies and faeces of the two-spotted spider mite, *Tetranychus urticae*. *Journal of Insect Physiology*, **78**: 69–77.
- Santamaría, M. E., Cambra, I., Martínez, M., Pozancos, C., González-Melendi, P., Grbic, V., Castañera, P., Ortego, F., & Díaz, I. (2012). Gene pyramiding of peptidase inhibitors enhances plant resistance to the spider mite *Tetranychus urticae*. *PLoS ONE*, **7**: e43011.
- Sato, M. E., Silva, M. Z. da, Raga, A., & Souza Filho, M. F. de. (2005). Abamectin resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): selection, cross-resistance and stability of resistance. *Neotropical Entomology*, **34**: 991–998.
- Schäfer, M., Brütting, C., Meza-Canales, I. D., Großkinsky, D. K., Vankova, R., Baldwin, I. T., & Meldau, S. (2015). The role of *cis*-zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. *Journal of Experimental Botany*, **66**: 4873–4884.
- Schaller, G. E., & Kieber, J. J. (2002). Ethylene. *The Arabidopsis Book*, **1**: e0071.
- Schaller, G. E., Bishopp, A., & Kieber, J. J. (2015). The yin-yang of hormones: cytokinin and auxin interactions in plant development. *The Plant Cell*, **27**: 44–63.
- Schimmel, B. C. J., Alba, J. M., Wybouw, N., Glas, J. J., Meijer, T. T., Schuurink, R. C., & Kant, M. R. (2018). Distinct signatures of host defense suppression by plant-feeding mites. *International Journal of Molecular Sciences*, **19**: 3265.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, **9**: 676–682.
- Schmidt, L., Schurr, U., & Röse, U. S. R. (2009). Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. *Plant, Cell & Environment*, **32**: 893–903.
- Schmiesing, A., Emonet, A., Gouhier-Darimont, C., & Reymond, P. (2016). Arabidopsis MYC transcription factors are the target of hormonal salicylic acid/jasmonic acid cross talk in response to *Pieris brassicae* egg extract. *Plant Physiology*, **170**: 2432–2443.
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**: 671–675.
- Schultz, J. C., Appel, H. M., Ferrieri, A. P., & Arnold, T. M. (2013). Flexible resource allocation during plant defense responses. *Frontiers in Plant Science*, **4**: 324.
- Schwachtje, J., Minchin, P. E. H., Jahnke, S., Van Dongen, J. T., Schittko, U., & Baldwin, I. T. (2006). SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proceedings of the National Academy of Sciences of the United States of America*, **103**: 12935–12940.
- Schweighofer, A., Kazanaviciute, V., Scheikl, E., Teige, M., Doczi, R., Hirt, H., Schwanninger, M., Kant, M., Schuurink, R., Mauch, F., Buchala, A., Cardinale, F., & Meskiene, I. (2007). The PP2C-type phosphatase AP2C1, which negatively regulates MPK4 and MPK6, modulates innate immunity, jasmonic acid, and ethylene levels in Arabidopsis. *The Plant Cell*, **19**: 2213–2224.
- Sestari, I., & Campos, M. L. (2022). Into a dilemma of plants: the antagonism between chemical defenses and growth. *Plant Molecular Biology*, **109**: 469–482.
- Sharma, A., Khan, A. N., Subrahmanyam, S., Raman, A., Taylor, G. S., & Fletcher, M. J. (2014). Salivary proteins of plant-feeding hemipteroids-implication in phytophagy. *Bulletin of Entomological Research*, **104**: 117–136.
- Shigenaga, A. M., Berens, M. L., Tsuda, K., & Argueso, C. T. (2017). Towards engineering of hormonal crosstalk in plant immunity. *Current Opinion in Plant Biology*, **38**: 164–172.

- Shih, C. T., Poe, S. L., & Cromroy, H. L. (1976). Biology, life table, and intrinsic rate of increase of *Tetranychus urticae*. *Annals of the Entomological Society of America*, **69**: 362–364.
- Šimura, J., Antoniadi, I., Šíroká, J., Tarkowská, D., Strnad, M., Ljung, K., & Novák, O. (2018). Plant hormonomics: multiple phytohormone profiling by targeted metabolomics. *Plant Physiology*, **177**: 476–489.
- Skubacz, A., Daszkowska-Golec, A., & Szarejko, I. (2016). The role and regulation of ABI5 (ABA-insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. *Frontiers in Plant Science*, **7**: 1884.
- Sommer, C., Straehle, C., Koethe, U., & Hamprecht, F. A. (2011). Ilastik: interactive learning and segmentation toolkit. In *2011 IEEE International Symposium on Biomedical Imaging: From Nano to Macro* (pp. 230–233). IEEE.
- Spíchal, L., Rakova, N. Y., Riefler, M., Mizuno, T., Romanov, G. A., Strnad, M., & Schmülling, T. (2004). Two cytokinin receptors of *Arabidopsis thaliana*, CRE1/AHK4 and AHK3, differ in their ligand specificity in a bacterial assay. *Plant and Cell Physiology*, **45**: 1299–1305.
- Stahl, E., Hilfiker, O., & Reymond, P. (2018). Plant–arthropod interactions: who is the winner? *The Plant Journal*, **93**: 703–728.
- Steinbrenner, A. D., Gómez, S., Osorio, S., Fernie, A. R., & Orrians, C. M. (2011). Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: a whole plant perspective. *Journal of Chemical Ecology*, **37**: 1294–1303.
- Strauss, S. Y. (1997). Floral characters link herbivores, pollinators, and plant fitness. *Ecology*, **78**: 1640–1645.
- Strauss, S. Y., Rudgers, J. A., Lau, J. A., & Irwin, R. E. (2002). Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution*, **17**: 278–285.
- Street, I. H., Aman, S., Zubo, Y., Ramzan, A., Wang, X., Shakeel, S. N., Kieber, J. J., & Eric Schaller, G. (2015). Ethylene inhibits cell proliferation of the Arabidopsis root meristem. *Plant Physiology*, **169**: 338–350.
- Suzuki, T., España, M. U., Nunes, M. A., Zhurov, V., Dermauw, W., Osakabe, M., Van Leeuwen, T. V., Grbic, M., & Grbic, V. (2017). Protocols for the delivery of small molecules to the two-spotted spider mite, *Tetranychus urticae*. *PLoS ONE*, **12**: e0180658.
- Tan, S., Abas, M., Verstraeten, I., Glanc, M., Molnár, G., Hajný, J., Lasák, P., Petřík, I., Russinova, E., Petrášek, J., Novák, O., Pospíšil, J., & Friml, J. (2020). Salicylic acid targets protein phosphatase 2A to attenuate growth in plants. *Current Biology*, **30**: 381–395.
- Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., Zhu, T., Zou, G., & Katagiri, F. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *The Plant Cell*, **15**: 317–330.
- Tarkowská, D., Novák, O., Oklestkova, J., & Strnad, M. (2016). The determination of 22 natural brassinosteroids in a minute sample of plant tissue by UHPLC–ESI–MS/MS. *Analytical and Bioanalytical Chemistry*, **408**: 6799–6812.
- Terra, W. R., & Ferreira, C. (2012). Biochemistry and molecular biology of digestion. In L. I. Gilbert (Ed.), *Insect molecular biology and biochemistry* (pp. 365–418). London, United Kingdom: Academic Press.
- Thaler, J. S., Humphrey, P. T., & Whiteman, N. K. (2012). Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, **17**: 260–270.
- Tholl, D., Sohrabi, R., Huh, J. H., & Lee, S. (2011). The biochemistry of homoterpenes - common constituents of floral and herbivore-induced plant volatile bouquets. *Phytochemistry*, **72**: 1635–1646.
- Tian, H., Qi, T., Li, Y., Wang, C., Ren, C., Song, S., & Huang, H. (2016). Regulation of the WD-repeat/bHLH/MYB complex by gibberellin and jasmonate. *Plant Signaling & Behavior*, **11**: 1118–113.
- Tsuda, K., Sato, M., Glazebrook, J., Cohen, J. D., & Katagiri, F. (2008). Interplay between MAMP-triggered and SA-mediated defense responses. *The Plant Journal*, **53**: 763–775.

- Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T.** (2021). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, **18**: 1112.
- Tunaz, H., & Stanley, D. W.** (2004). Phospholipase A2 in salivary glands isolated from tobacco hornworms, *Manduca sexta*. *Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology*, **139**: 27–33.
- Ullmann-Zeunert, L., Stanton, M. A., Wielsch, N., Bartram, S., Hummert, C., Svatoš, A., Baldwin, I. T., & Groten, K.** (2013). Quantification of growth–defense trade-offs in a common currency: nitrogen required for phenolamide biosynthesis is not derived from ribulose-1, 5-bisphosphate carboxylase/oxygenase turnover. *The Plant Journal*, **75**: 417–429.
- Um, T. Y., Lee, H. Y., Lee, S., Chang, S. H., Chung, P. J., Oh, K. B., Kim, J. K., Jang, G., & Choi, Y. Do.** (2018). Jasmonate zim-domain protein 9 interacts with slender rice 1 to mediate the antagonistic interaction between jasmonic and gibberellic acid signals in rice. *Frontiers in Plant Science*, **9**: 1866.
- Urbanová, T., Tarkowská, D., Novák, O., Hedden, P., & Strnad, M.** (2013). Analysis of gibberellins as free acids by ultra performance liquid chromatography-tandem mass spectrometry. *Talanta*, **112**: 85–94.
- Ursache, R., Andersen, T. G., Marhavý, P., & Geldner, N.** (2018). A protocol for combining fluorescent proteins with histological stains for diverse cell wall components. *The Plant Journal*, **93**: 399–412.
- Vacante, V.** (2015). *The handbook of mites of economic plants: Identification, bio-ecology and control*. Wallingford, United Kingdom: CABI.
- Van De Velde, V., Duarte, M. V. A., Benavente, A., Vangansbeke, D., Wäckers, F., & De Clercq, P.** (2021). Quest for the allmitey: potential of *Pronematus ubiquitus* (Acari: Iolinidae) as a biocontrol agent against *Tetranychus urticae* and *Tetranychus evansi* (Acari: Tetranychidae) on tomato (*Solanum lycopersicum* L.). *BioRxiv*, 2021–04.
- Van Den Boom, C. E. M., Van Beek, T. A., Posthumus, M. A., De Groot, A., & Dicke, M.** (2004). Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *Journal of Chemical Ecology*, **30**: 69–89.
- van Kleeff, P. J. M., Mastop, M., Sun, P., Dangol, S., van Doore, E., Dekker, H. L., Kramer, G., Lee, S., Ryu, C. M., de Vos, M., & Schuurink, R. C.** (2024). Discovery of three *Bemisia tabaci* effectors and their effect on gene expression in planta. *Molecular Plant-Microbe Interactions*, **37**: 380–395.
- Van Leeuwen, T., & Dermauw, W.** (2016). The molecular evolution of xenobiotic metabolism and resistance in chelicerate mites. *Annual Review of Entomology*, **61**: 475–498.
- Van Leeuwen, T., Tirry, L., Yamamoto, A., Nauen, R., & Dermauw, W.** (2015). The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. *Pesticide Biochemistry and Physiology*, **121**: 12–21.
- Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., & Tirry, L.** (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. *Insect Biochemistry and Molecular Biology*, **40**: 563–572.
- Verma, V., Ravindran, P., & Kumar, P. P.** (2016). Plant hormone-mediated regulation of stress responses. *BMC Plant Biology*, **16**: 1–10.
- Vieira, L. M., Marinho, L. M. G., Rocha, J. de C. G., Barros, F. A. R., & Stringheta, P. C.** (2019). Chromatic analysis for predicting anthocyanin content in fruits and vegetables. *Food Science and Technology (Brazil)*, **39**: 415–422.
- Villarroel, C. A., Jonckheere, W., Alba, J. M., Glas, J. J., Dermauw, W., Haring, M. A., Van Leeuwen, T., Schuurink, R. C., & Kant, M. R.** (2016). Salivary proteins of spider mites suppress defenses in *Nicotiana benthamiana* and promote mite reproduction. *The Plant Journal*, **86**: 119–131.

- Vincent, T. R., Avramova, M., Canham, J., Higgins, P., Bilkey, N., Mugford, S. T., Pitino, M., Toyota, M., Gilroy, S., Miller, A. J., Hogenhout, S. A., & Sanders, D. (2017). Interplay of plasma membrane and vacuolar ion channels, together with BAK1, elicits rapid cytosolic calcium elevations in Arabidopsis during aphid feeding. *The Plant Cell*, **29**: 1460–1479.
- Wang, D., Pajerowska-Mukhtar, K., Culler, A. H., & Dong, X. (2007). Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology*, **17**: 1784–1790.
- Wang, X., Yang, X., Chen, S., Li, Q., Wang, W., Hou, C., Gao, X., Wang, L., & Wang, S. (2016). Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in Arabidopsis. *Frontiers in Plant Science*, **6**: 1243.
- Wang, Z. Y., Bai, M. Y., Oh, E., & Zhu, J. Y. (2012). Brassinosteroid signaling network and regulation of photomorphogenesis. *Annual Review of Genetics*, **46**: 701–724.
- Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W. H. A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., Schwartz, M., & Venables, B. (2016). *gplots: Various R programming tools for plotting data*. (Version 2.17.0) [R package] <https://CRAN.R-project.org/package=gplots>
- Wasternack, C. (2014). Action of jasmonates in plant stress responses and development - applied aspects. *Biotechnology Advances*, **32**: 31–39.
- Wei, Z., Hu, W., Lin, Q., Cheng, X., Tong, M., Zhu, L., Chen, R., & He, G. (2009). Understanding rice plant resistance to the brown planthopper (*Nilaparvata lugens*): a proteomic approach. *Proteomics*, **9**: 2798–2808.
- Weihmann, T., & Wipfler, B. (2019). The generalized feeding apparatus of cockroaches: a model for biting and chewing insects. In G. W. Chapman & R. A. Borrell (Eds.), *Insect mouthparts: Form, function, development and performance* (pp. 203–262). London, United Kingdom: Academic Press.
- Weiss, D., & Ori, N. (2007). Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiology*, **144**: 1240–1246.
- Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., & Schmölling, T. (2003). Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell*, **15**: 2532–2550.
- Widemann, E., Bruinsma, K., Walshe-Roussel, B., Rioja, C., Arbona, V., Saha, R. K., Letwin, D., Zhurov, V., Gómez-Cadenas, A., Bernards, M. A., Grbic, M., & Grbic, V. (2021). Multiple indole glucosinolates and myrosinases defend Arabidopsis against *Tetranychus urticae* herbivory. *Plant Physiology*, **187**: 116–132.
- Wild, M., Davière, J. M., Cheminant, S., Regnault, T., Baumberger, N., Heintz, D., Baltz, R., Genschik, P., & Achard, P. (2012). The Arabidopsis DELLA RGA-like3 is a direct target of MYC2 and modulates jasmonate signaling responses. *The Plant Cell*, **24**: 3307–3319.
- Wondafrash, M., Van Dam, N. M., & Tytgat, T. O. G. (2013). Plant systemic induced responses mediate interactions between root parasitic nematodes and aboveground herbivorous insects. *Frontiers in Plant Science*, **4**: 87.
- Wu, J., & Baldwin, I. T. (2009). Herbivory-induced signalling in plants: perception and action. *Plant, Cell & Environment*, **32**: 1161–1174.
- Wu, W., Cao, S. F., Shi, L. Y., Chen, W., Yin, X. R., & Yang, Z. F. (2023). Absciscic acid biosynthesis, metabolism and signaling in ripening fruit. *Frontiers in Plant Science*, **14**: 1279031.
- Wu, X., Huang, H., Childs, H., Wu, Y., Yu, L., & Pehrsson, P. R. (2021). Glucosinolates in Brassica vegetables: characterization and factors that influence distribution, content, and intake. *Annual Review of Food Science and Technology*, **12**: 485–511.
- 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**: 639–647.

- Xie, Y., Tan, H., Ma, Z., & Huang, J. (2016). DELLA Proteins promote anthocyanin biosynthesis via sequestering MYBL2 and JAZ suppressors of the MYB/bHLH/WD40 complex in *Arabidopsis thaliana*. *Molecular Plant*, **9**: 711–721.
- Ximénez-Embún, M. G., Castañera, P., & Ortego, F. (2017). Drought stress in tomato increases the performance of adapted and non-adapted strains of *Tetranychus urticae*. *Journal of Insect Physiology*, **96**: 73–81.
- Ximénez-Embún, M. G., González-Guzmán, M., Arbona, V., Gómez-Cadenas, A., Ortego, F., & Castañera, P. (2018). Plant-mediated effects of water deficit on the performance of *Tetranychus evansi* on tomato drought-adapted accessions. *Frontiers in Plant Science*, **9**: 1490.
- Xin, P., Yan, J., Fan, J., Chu, J., & Yan, C. (2013). An improved simplified high-sensitivity quantification method for determining brassinosteroids in different tissues of rice and *Arabidopsis*. *Plant Physiology*, **162**: 2056–2066.
- Xu, D., Wang, K., Zhang, Y., Wang, H., Wu, Q., & Wang, S. (2019). The performance of *Tetranychus urticae* on five melon cultivars is correlated with leaf thickness. *Systematic and Applied Acarology*, **24**: 645–658.
- Xu, J., Yao, J., Zhai, H., Li, Q., Xu, Q., Xiang, Y., Liu, Y., Liu, T., Ma, H., Mao, Y., Wu, F., Wang, Q., Feng, X., Mu, J., & Lu, Y. (2023). TrichomeYOLO: a neural network for automatic maize trichome counting. *Plant Phenomics*, **5**: 0024.
- Yan, A., Pan, J., An, L., Gan, Y., & Feng, H. (2012). The responses of trichome mutants to enhanced ultraviolet-B radiation in *Arabidopsis thaliana*. *Journal of Photochemistry and Photobiology B: Biology*, **113**: 29–35.
- Yan, Z., Liu, X., Ljung, K., Li, S., Zhao, W., Yang, F., Wang, M., & Tao, Y. (2017). Type B response regulators act as central integrators in transcriptional control of the auxin biosynthesis enzyme TAA1. *Plant Physiology*, **175**: 1438–1454.
- Yang, D. L., Yao, J., Mei, C. S., Tong, X. H., Zeng, L. J., Li, Q., Xiao, L. T., Sun, T. P., Li, J., Deng, X. W., Lee, C. M., Thomashow, M. F., Yang, Y., He, Z., & He, S. Y. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America*, **109**: E1192–E1200.
- Yang, F., Tang, J., Yang, D., Yang, T., Liu, H., Luo, W., Wu, J., Jianqiang, W., & Wang, L. (2020). Jasmonoyl-L-isoleucine and allene oxide cyclase-derived jasmonates differently regulate gibberellin metabolism in herbivory-induced inhibition of plant growth. *Plant Science*, **300**: 110627.
- Yang, J., Duan, G., Li, C., Liu, L., Han, G., Zhang, Y., & Wang, C. (2019a). The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. *Frontiers in Plant Science*, **10**: 1349.
- Yang, J., Wang, G. Q., Zhou, Q., Lu, W., Ma, J. Q., & Huang, J. H. (2019b). Transcriptomic and proteomic response of *Manihot esculenta* to *Tetranychus urticae* infestation at different densities. *Experimental and Applied Acarology*, **78**: 273–293.
- Yimer, H. Z., Nahar, K., Kyndt, T., Haeck, A., Van Meulebroek, L., Vanhaecke, L., Demeestere, K., Höfte, M., & Gheysen, G. (2018). Gibberellin antagonizes jasmonate-induced defense against *Meloidogyne graminicola* in rice. *New Phytologist*, **218**: 646–660.
- Yoshinaga, N., Alborn, H. T., Nakanishi, T., Suckling, D. M., Nishida, R., Tumlinson, J. H., & Mori, N. (2010). Fatty acid-amino acid conjugates diversification in lepidopteran caterpillars. *Journal of Chemical Ecology*, **36**: 319–325.
- Younginger, B. S., Sirová, D., Cruzan, M. B., & Ballhorn, D. J. (2017). Is biomass a reliable estimate of plant fitness? *Applications in Plant Sciences*, **5**: 1600094.
- Zangerl, A. R. (2003). Evolution of induced plant responses to herbivores. *Basic and Applied Ecology*, **4**: 91–103.

- Zebelo, S. A., & Maffei, M. E.** (2015). Role of early signalling events in plant-insect interactions. *Journal of Experimental Botany*, **66**: 435–448.
- Zhang, Y., Liu, Y., Liang, X., Wu, C., Liu, X., Wu, M., Yao, X., Qiao, Y., Zhan, X., & Chen, Q.** (2023). Exogenous methyl jasmonate induced cassava defense response and enhanced resistance to *Tetranychus urticae*. *Experimental and Applied Acarology*, **89**: 45–60.
- Zhang, Y., Bouwmeester, H. J., & Kappers, I. F.** (2020). Combined transcriptome and metabolome analysis identifies defence responses in spider mite-infested pepper (*Capsicum annuum*). *Journal of Experimental Botany*, **71**: 330–343.
- Zhang, Y., Goritschnig, S., Dong, X., & Li, X.** (2003). A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of *npr1-1*, *constitutive 1*. *The Plant Cell*, **15**: 2636–2646.
- Zhang, Y., & Li, X.** (2019). Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Current Opinion in Plant Biology*, **50**: 29–36.
- Zhang, Z., Wang, M., Li, Z., Li, Q., & He, Z.** (2008). Arabidopsis GH3.5 regulates salicylic acid-dependent and both NPR1-dependent and independent defense responses. *Plant Signaling & Behavior*, **3**: 537–542.
- Zhao, M., Morohashi, K., Hatlestad, G., Grotewold, E., & Lloyd, A.** (2008). The TTG1-bHLH-MYB complex controls trichome cell fate and patterning through direct targeting of regulatory loci. *Development*, **135**: 1991–1999.
- Zhong, Q., Hu, H., Fan, B., Zhu, C., & Chen, Z.** (2021). Biosynthesis and roles of salicylic acid in balancing stress response and growth in plants. *International Journal of Molecular Sciences*, **22**: 11672.
- Zhou, S., Lou, Y. R., Tzin, V., & Jander, G.** (2015). Alteration of plant primary metabolism in response to insect herbivory. *Plant Physiology*, **169**: 1488–1498.
- Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., & Chanda, S. K.** (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, **10**: 1523.
- Zhurov, V., Navarro, M., Bruinsma, K. A., Arbona, V., Estrella Santamaría, M. E., Cazaux, M., Wybouw, N., Osborne, E. J., Ens, C., Rioja, C., Vermeirssen, V., Rubio-Somoza, I., Krishna, P., Diaz, I., Schmid, M., Gómez-Cadenas, A., Van de Peer, Y., Grbić, M., Clark, R. M., Van Leeuwen, T., & Grbić, V.** (2014). Reciprocal responses in the interaction between Arabidopsis and the cell-content-feeding chelicerate herbivore spider mite. *Plant Physiology*, **164**: 384–399.
- Zhu-Salzman, K., Luthe, D. S., & Felton, G. W.** (2008). Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. *Plant Physiology*, **146**: 852–858.
- Zubo, Y. O., & Schaller, G. E.** (2020). Role of the cytokinin-activated type-B response regulators in hormone crosstalk. *Plants*, **9**: 166.
- Züst, T., & Agrawal, A. A.** (2017). Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annual Review of Plant Biology*, **68**: 513–534.
- Züst, T., Joseph, B., Shimizu, K. K., Kliebenstein, D. J., & Turnbull, L. A.** (2011). Using knockout mutants to reveal the growth costs of defensive traits. *Proceedings of the Royal Society B: Biological Sciences*, **278**: 2598–2603.
- Züst, T., Rasmann, S., & Agrawal, A. A.** (2015). Growth-defense tradeoffs for two major anti-herbivore traits of the common milkweed *Asclepias syriaca*. *Oikos*, **124**: 1404–1415.
- Zvereva, E. L., Lanta, V., & Kozlov, M. V.** (2010). Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. *Oecologia*, **163**: 949–960.

Zwack, P. J., & Rashotte, A. M. (2015). Interactions between cytokinin signalling and abiotic stress responses. *Journal of Experimental Botany*, **66**: 4863–4871.

Annexes

Annex A. Steps and parameters used for the automated approaches

1) Ilastik noisy background – Steps

a. Ilastik – Pixel classification

- Prediction export > source: simple segmentation
- Prediction export > image settings > renormalize from [0,3] to [0,255]
- Prediction export > image settings > format: jpeg
- Batch processing > select all images as input files
- Process all files

b. Fiji

- Fiji > plugins > new > macro
- File > open > open the three macros for Fiji analysis (damage, noise and area)
- Open the images obtained from the initial Ilastik step
- Macro “damage” > run --> Images will be stored in the Ilastik folder
- Open the images obtained in the previous step.
- Macro “noise” > run --> Images will be stored in the Ilastik folder
- In the window of results > results > distribution > parameter: area, uncheck “automatic binning” and specify 100 bins with a range of 0-100
- In the histogram with the data distribution it is observed a long tail represented as the noise present in the analysis. Check this box in order to select the size of the clusters of pixels to remove from the analysis. In our case, for Arabidopsis, clusters of noise pixels were found with a size ≤ 7 .
- Macro “area” > “size= *selected size of noise in the previous step* (7 in our case) – infinity...” > run
- Finally, a window with the results is generated. We use the “Count” column in order to obtain the number of final trichomes detected by this analysis.

2) Ilastik black background – Steps

a. Ilastik – Pixel classification

- Prediction export > source: simple segmentation
- Prediction export > image settings > renormalize from [0,3] to [0,255]
- Prediction export > image settings > format: jpeg
- Batch processing > select all images as input files
- Process all files

b. Fiji

- Fiji > plugins > new > macro
- File > open > open the three macros for Fiji analysis (damage, noise and area)
- Open the images obtained from the initial Ilastik step
- Macro “damage” > run --> Images will be stored in the Ilastik folder
- Open the images obtained in the previous step.
- Macro “noise” > run --> Images will be stored in the Ilastik folder
- In the window of results > results > distribution > parameter: area, uncheck “automatic binning” and specify 100 bins with a range of 0-100
- In the histogram with the data distribution it is observed a long tail represented as the noise present in the analysis. Check this box in order to select the size of the clusters of pixels to remove from the analysis. In our case, for Arabidopsis, clusters of noise pixels were found with a size ≤ 7 .
- Macro “area” > “size= *selected size of noise in the previous step* (7 in our case) – infinity...” > run
- Finally, a window with the results is generated. We use the “Count” column in order to obtain the number of final trichomes detected by this analysis.

3) **Ilastik (Pixel + Object Classification) – Noisy background**

- **Pixel classification:**
 - Prediction export > source: Probabilities
 - Prediction export > image settings > renormalize from [0,3] to [0,255]
 - Prediction export > image settings > format: jpeg
 - Specify the destiny folder in order to save all files in a unique folder
 - Batch processing > select all images as input files
 - Process all files
- **Object classification:**
 - Introduce as “Input data” both the original images in the “Raw data” tab and the probability maps images extracted in the previous step in the “Prediction Maps” tab.
 - Right click on the name of your input data > Edit properties > Storage > Copy into project file
 - “Treshold and size filter” > Double click on label 1 to rename the label to “Trichome” > Double click on label 1 to rename the label to “Trichome”
 - Object Information Export > Configure Feature Table Export > General > Format > csv
 - Object Information Export > Configure Feature Table Export > General > Filename > Specify the destiny folder and filename
 - Batch Processing > Raw data > Select all original images
 - Batch Processing > Prediction Maps > Select all probability maps generated in the Pixel classification process for the images selected in the Raw data tab. It is important to introduce the probability maps of the images in the same order they are input in the Raw data tab.
 - Process all files
- **Script for trichome quantification:**
 - **Windows:**
 - Search for "Command Prompt" app of Windows and open it. This can also be done by using the command (Windows + R) and typing "cmd" in the emergent window and clicking the "OK" button.
 - Specify the next command in the prompt window:

cd Desktop

for /f "delims=" %i in ('dir /s /b "C:\Users\PATH\TO\DIRECTORY\FOLDER*.csv"')

do @(

```
echo "%~n" >>results.txt
```

```
type "%~i" | findstr ",Trichome," | find /c "Trichome" >>results.txt)
```

- For the code above, you have to specify the path for the folder where you have saved your csv files. Once done, the code read all the csv files generated and create a text file with the results of the number of trichomes counted for every file included in the analysis. This txt file will be located at the desktop of your computer with the name “results”.

Annex B. Macros used for the trichome quantification analysis

Macro B.1. Commands used to transform grey scales images to black/white using Fiji.

```
imageCount = nImages  
  
n=nImages;  
  
path=getDirectory("Trichomes/");  
  
for(i=0,1; i<n; i++){  
    setAutoThreshold("Default");  
  
    run("Threshold...");  
  
    setThreshold(0, 110);  
  
    setOption("BlackBackground", false);  
  
    run("Convert to Mask");  
  
    title=getTitle;  
  
  
    //Give the correct directory where you want to save the output images (marked red)  
  
    saveAs("TIFF", path+title);  
  
    close();  
  
}
```

Macro B.2. Commands used for the identification of the pixel cluster size from which trichomes are detected using Fiji.

```
imageCount = nImages  
n=nImages;  
for(i=0,1; i<n; i++){  
    setAutoThreshold("Default");  
    run("Threshold...");  
    setThreshold(129, 255);  
    setOption("BlackBackground", false);  
    run("Convert to Mask");  
    run("Analyze Particles...", "size=0-Infinity show=Nothing display");  
    title=getTitle;  
  
    //Give the correct directory where you want to save the output images (marked red)  
    saveAs("TIFF", title);  
    close();  
}
```

Macro B.3. Commands used to calculate and extract the number of trichomes on white/black images using Fiji.

```
imageCount = nImages

n=nImages;

for(i=0,1; i<n; i++){

    setAutoThreshold("Default");

    run("Threshold...");

    setThreshold(129, 255);

    setOption("BlackBackground", false);

    run("Convert to Mask");

    run("Analyze Particles...", "size=7-Infinity show=Nothing clear include summarize");

    title=getTitle;

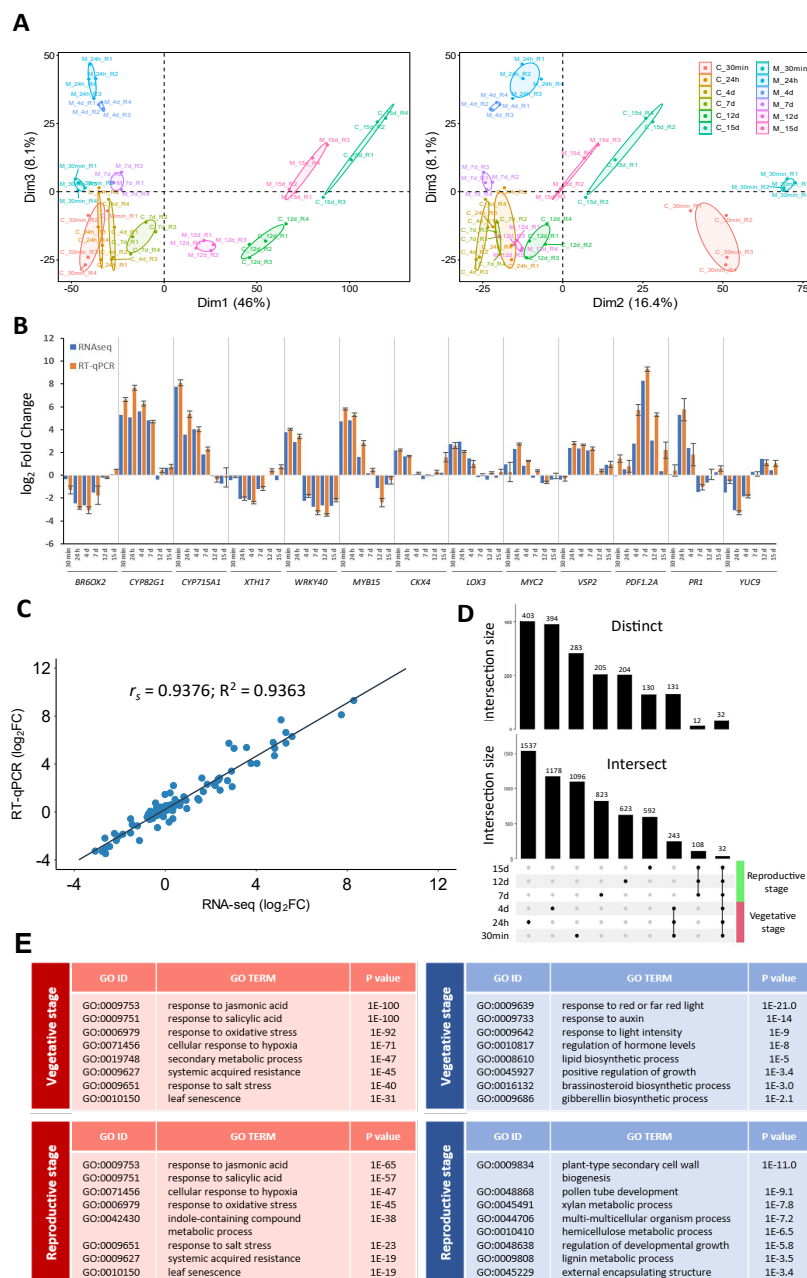
    //Give the correct directory where you want to save the output images (marked red)

    saveAs("TIFF", title);

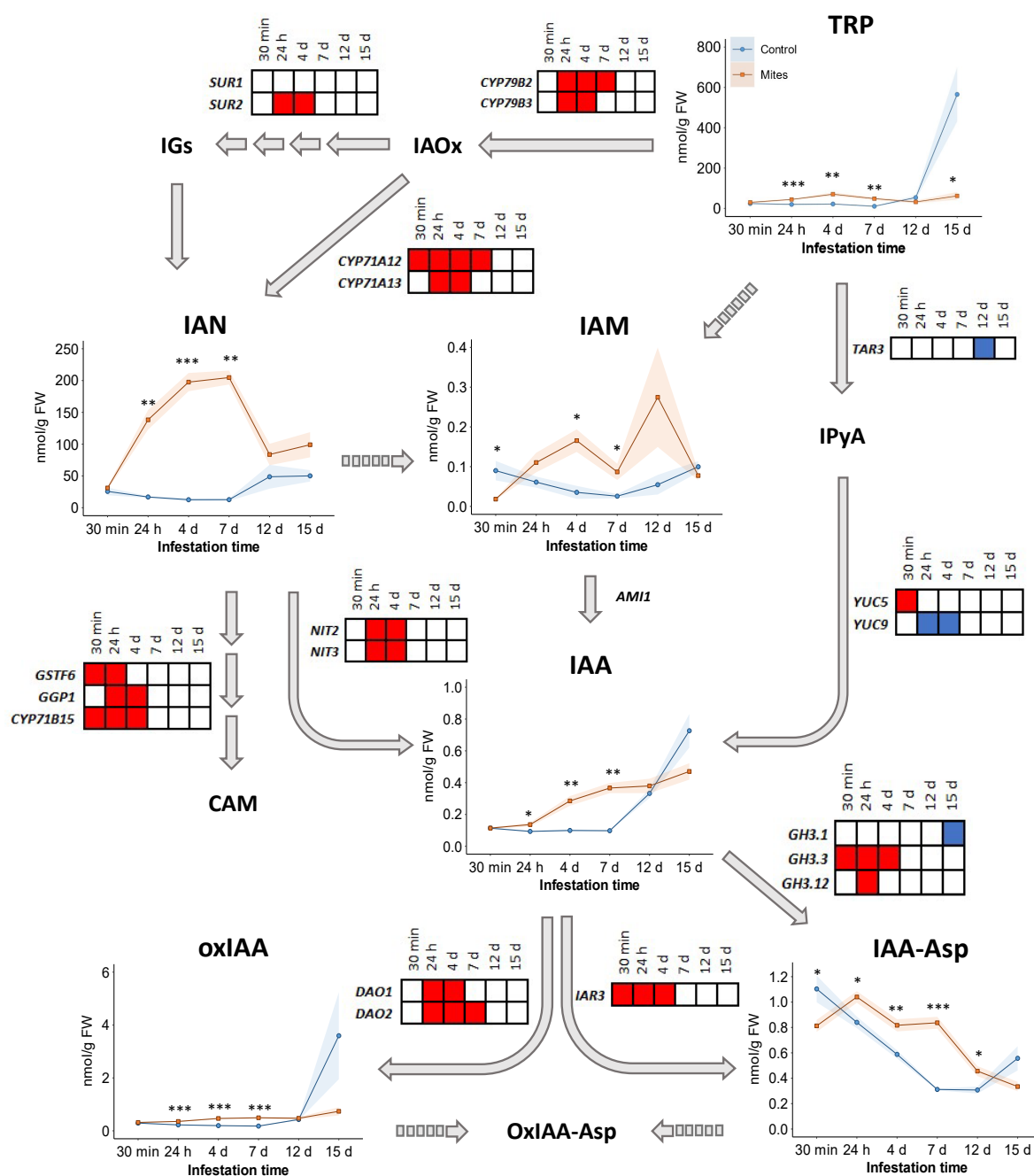
    close();

}
```

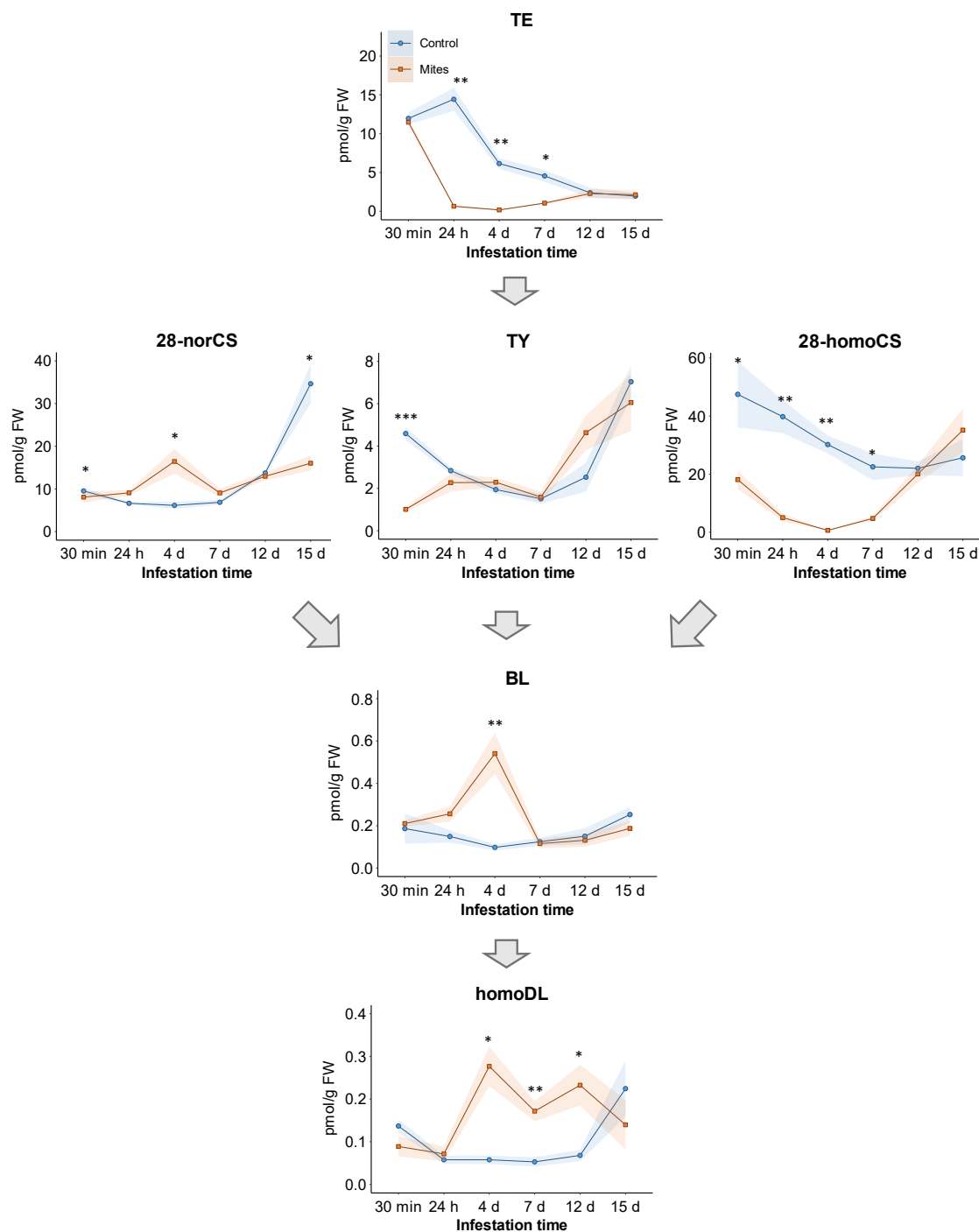
Annex C. Supplementary Figures



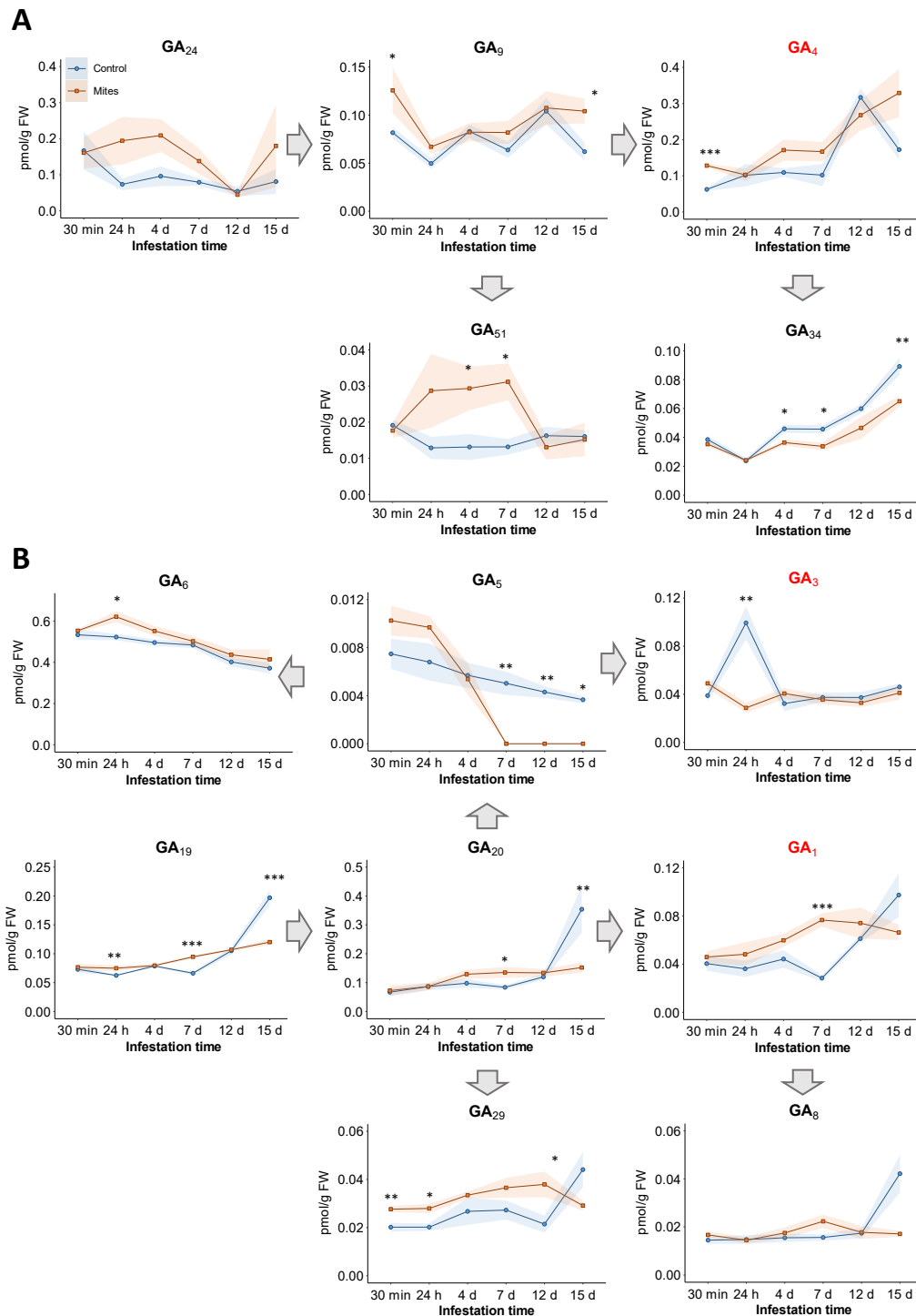
Supplementary Figure C.1. RNA-seq analysis and validation show patterns of defense and growth processes. (A) Principal Component Analysis (PCA) of control and mite-infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation in the first 3 PCA dimensions. Ellipses define confidence areas (95%) for each specific treatment and time. Each circle represents a biological replicate. Samples are identified using a specific color code for different time and treatment. (B) Bar plot representing the expression values in log₂FC from RNA-seq and quantitative real-time polymerase chain reaction assays for thirteen selected genes. (C) Dispersion graphic representing the correlation between the log₂FC values from RNA-seq and quantitative real-time polymerase chain reaction assays for the selected genes. Spearman rank correlation coefficient (r_s) is showed in combination with the regression coefficient (R^2). (D) UpSet plots showing the number of specific (distinct) and shared (intersect) DEGs among infestation time points. Vegetative (30 min, 24 h and 4 d) and reproductive stages (7 d, 12 d and 15 d) were grouped together as independent groups of interest. (E) Enriched biological processes of the clusters with up- (red) or down-regulated (blue) detected DEGs upon mite infestation during the vegetative and reproductive plant stages.



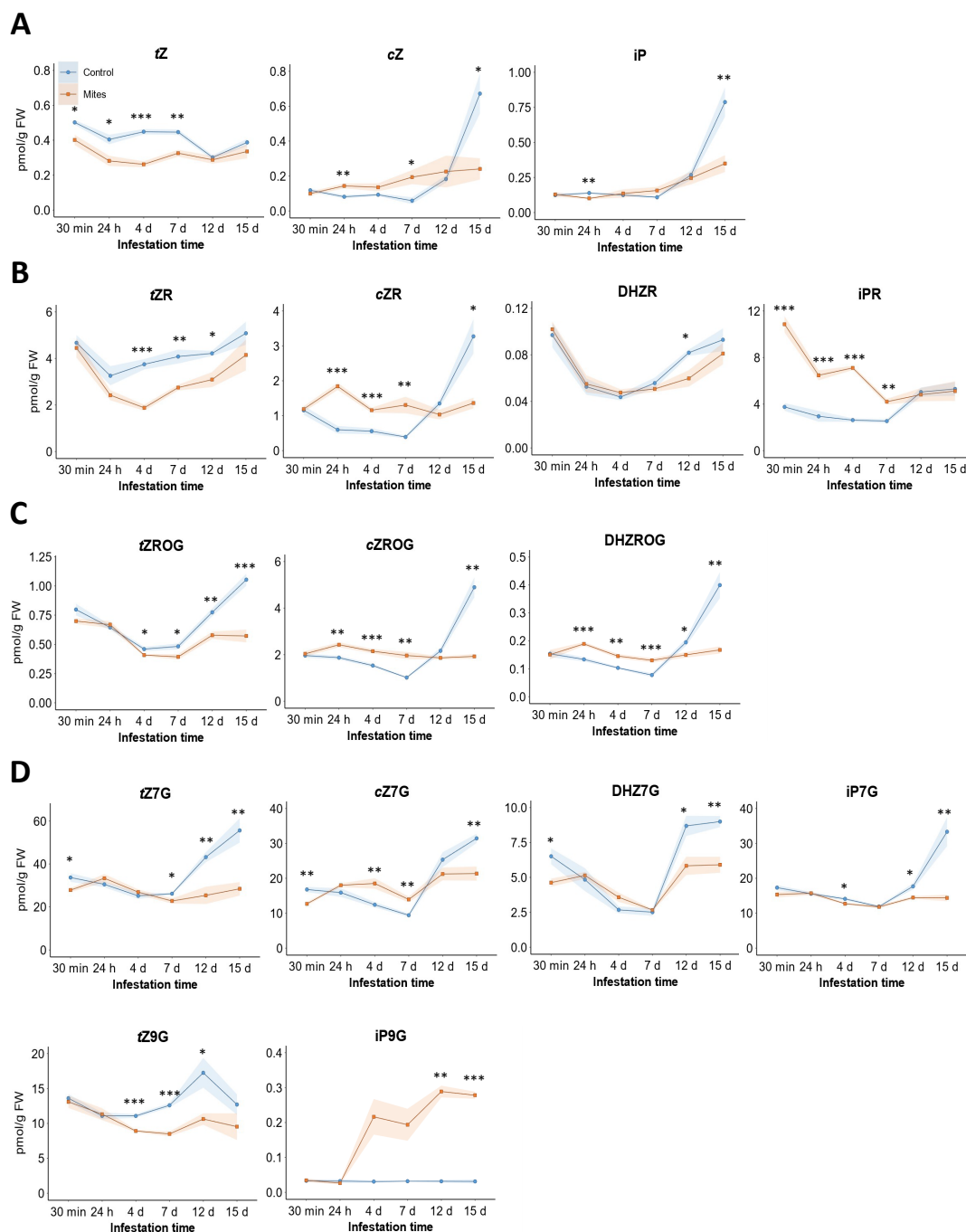
Supplementary Figure C.2. Mite infestation induces IAN and auxin accumulation. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Levels of different auxin-related compounds of the auxin biosynthesis were quantified and differentially expressed genes involved participating in each step of the pathway were plotted. Red colour indicates differential upregulation of genes at a specific time point. Blue colour indicates differential downregulation of genes for a specific time point. For metabolomic analyses, results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. TRP: tryptophan; IAOx: indole-3-acetaldoxime; IGs: indole glucosinolates; IAN: indole-3-acetonitrile; IAM: indole-3-acetamide; IPyA: indole-3-pyruvic acid; IAA: indole-3-acetic acid; oxIAA: 2-oxindole-3-acetic acid; IAA-Asp: indole-3-acetylaspatic acid; oxIAA-Asp: 2-oxindole-3-acetylaspatic acid.



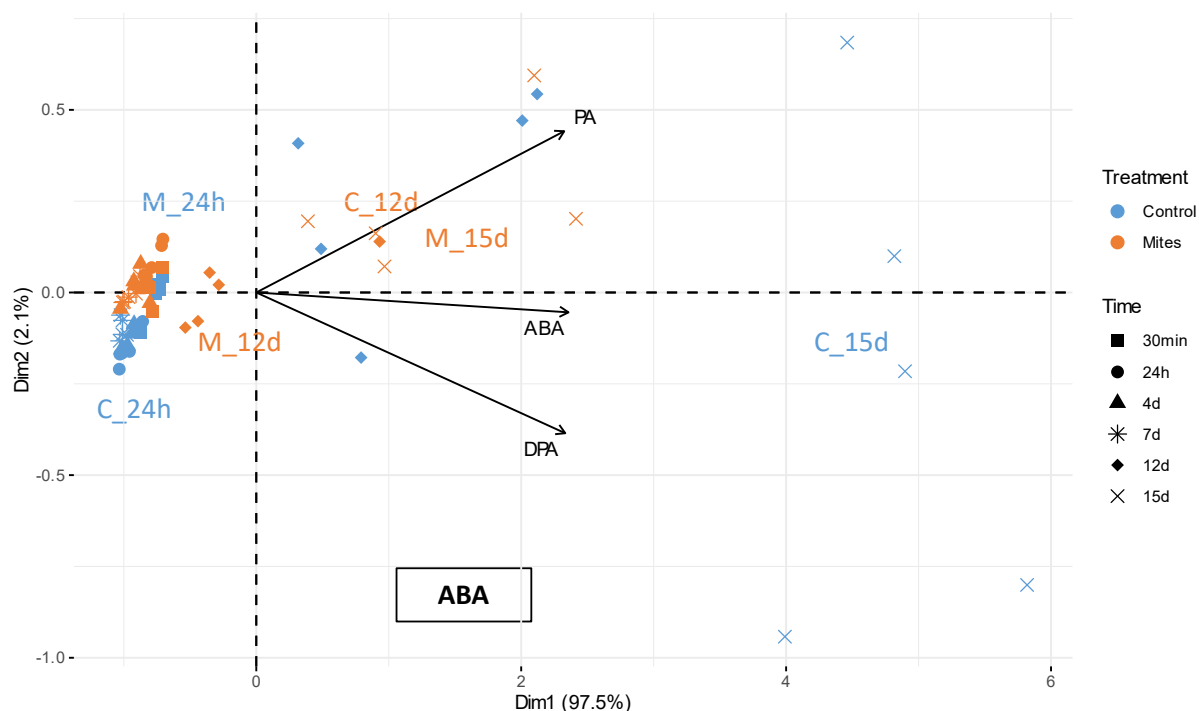
Supplementary Figure C.3. Mite infestation induces changes in the BRs levels. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Levels of different BR-related compounds of the BR biosynthesis were quantified. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. TE: teasterone; TY: typhasterol; 28-norCS: 28-norcastasterone; 28-homoCS: 28-homocastasterone; BL: brassinolide; homoDL: homodolicholide.



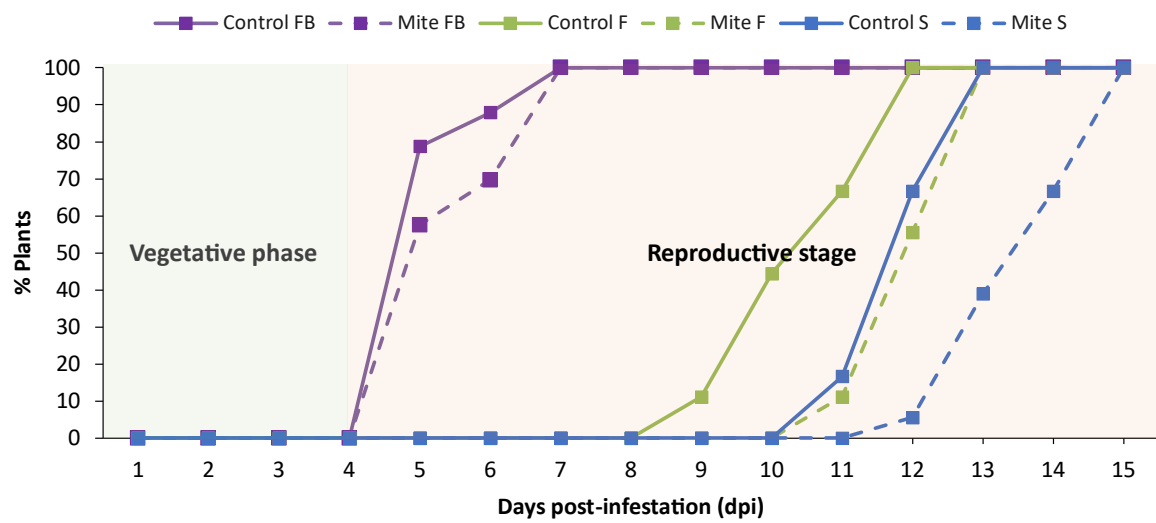
Supplementary Figure C.4. Levels of different GA compounds in control and mite infestation conditions. GA compounds were classified according to its involvement in the (A) 13-non-hydroxylated biosynthetic pathway for GA₄ biosynthesis or in the (B) 13-hydroxylated biosynthetic pathway for GA₁ and GA₃ biosynthesis. Red colour is used to highlight the active gibberellins. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. GA: gibberellin.



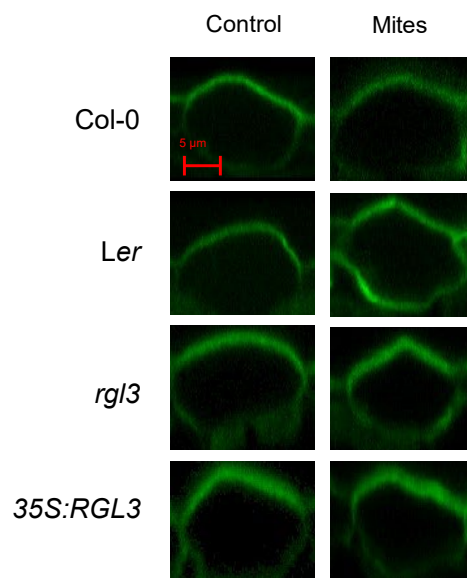
Supplementary Figure C.5. Mite infestation induces changes in the CK levels. Levels of the different CK-related compounds were classified into four groups based on its biological activity: **(A)** active CK bases (CK-B), **(B)** CK ribosides (CK-R), **(C)** CK O-glucosides (CK-OG) and **(D)** CK N-glucoside metabolites (CK-NG). Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants. For comparisons between control and infested plants, statistical analyses were performed at each time point using the Mann-Whitney U-test for non-parametric data and the Student's *t*-test for parametric data, with or without Welch's correction for unequal or equal variances, respectively. Coloured shadows represent the standard error of the samples. A single asterisk indicates that $p < 0.05$; a double asterisk indicates that $p < 0.01$; and a triple asterisk indicates that $p < 0.001$. *tZ*: trans-zeatin; *cZ*: cis-zeatin; *iP*: isopentenyl adenine; *tZR*: trans-zeatin riboside; *cZR*: cis-zeatin riboside; *DHZR*: dihydrozeatin riboside; *iPR*: isopentenyl adenosine; *tZROG*: trans-zeatin riboside *O*-glucoside; *cZROG*: cis-zeatin riboside *O*-glucoside; *DHZROG*: dihydrozeatin riboside *O*-glucoside; *tZ7G*: trans-zeatin-7-glucoside; *cZ7G*: cis-zeatin-7-glucoside; *DHZ7G*: dihydrozeatin-7-glucoside; *iP7G*: isopentenyladenin-7-glucoside; *tZ9G*: trans-zeatin-9-glucoside; *iP9G*: isopentenyl adenine-9-glucoside.



Supplementary Figure C.6. Evaluation of the ABA-related compounds from both control and mite-infested samples. Principal Component Analysis (PCA) biplot of control and mite infested samples at 30 min, 24 h, 4 d, 7 d, 12 d and 15 d of infestation. PCA was performed on the matrix of hormonal levels of ABA-related compounds. Samples are identified using a specific colour code for different treatment, and specific shape for different time point. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Results come from five biological independent replicates of four individual plants.



Supplementary Figure C.7. Transition of Col-0 plants to the flowering state is delayed during mite infestation conditions. Three-week-old Col-0 plants were infested with 20 mites of *T. urticae*. Percentage of plants with floral button (FB), flower (F) and siliques (S) was checked each day after mite infestation. Eleven and 18 independent replicates were evaluated for mite-infested and control plants, respectively.



Supplementary Figure C.8. Leaf cuticular lipids in *RGL3* mutant and overexpressing lines in control and after 4 d of mite infestation. Adaxial cuticular lipids of *Arabidopsis* were stained with Auramine O. Stacks of xz planes along the y-axis were collected using a confocal microscope at 40X magnification. The leaves are from mite infestation and control treatments for Col-0, *rgl3*, *Ler* and *35S:RGL3* plants. Images show a representative cell of the leaf epidermis for each genotype.

Annex D. Supplementary Tables

Supplementary Table D.1. Oligonucleotide sequences for RT-qPCR analysis.

Gene ID	Gene name	Forward primer sequence	Reverse primer sequence
AT5G25760	<i>UBQ</i>	5'-GCTCTTATCAAAGGACCTTCGG-3'	5'-CGAACTTGAGGAGGTTGCAAAG-3'
AT4G25420	<i>GA20OX1</i>	5'-ACTGATCTCTGAGGCCTGTAA-3'	5'-AAGAAGCGGCTCGTGTATTC-3'
AT1G02400	<i>GA2OX6</i>	5'-CCCTCAGATCTTGACGGTGT-3'	5'-TGGCTTCTTTGCTGTGTTTG-3'
AT1G14920	<i>GAI</i>	5'-CACCTCGGCTTGGAAGTCTC-3'	5'-TGACAAAGGGAAAAACAGTAGGATTT-3'
AT2G01570	<i>RGA</i>	5'-AATAGTGGCCAAGGTTATCGTG-3'	5'-AGTGTGCCAACCCAACATC-3'
AT1G66350	<i>RGL1</i>	5'-CAAGCATGTTGTTGGCACTT-3'	5'-GCAACAAACAACCTTCATTCTCT-3'
AT3G03450	<i>RGL2</i>	5'-GCGGATAAGAAGAAGGCTGATG-3'	5'-GCTTCTGTGCTACTTCAGCCATC-3'
AT5G17490	<i>RGL3</i>	5'- TCGAGACCCGAACCGAATC-3'	5'- CGCCTTAACCGTCGCTAACA-3'
AT2G46970	<i>PIL1</i>	5'-AGCTGTTGGTAGATCCACGGAATTG-3'	5'-GTTCTCGCATGAACCTGTGTCTTCG-3'
AT2G40610	<i>EXPA8</i>	5'-TCAACCATCACCGTCACAGCTACA-3'	5'-ATGCTGAAGAGGAGGATTGCACCA-3'
AT1G74710	<i>ICS1</i>	5'-CAAATCTCAACCTCCGTCGT-3'	5'-AATCAATTGCTCCGATTTGC-3'
AT1G64280	<i>NPR1</i>	5'-TGCATCAGAAGCAACTTTGG-3'	5'-GGCCTTTGAGAGAATGCTTG-3'
AT2G14610	<i>PR1</i>	5'-TCAGTGAGACTCGGATGTGC-3'	5'-CGTTCACATAATTCCCACGA-3'
AT1G17420	<i>LOX3</i>	5'-CGCCAATCAACAGTTTCTGA-3'	5'-CTCGTCTCGTGGCACATACA-3'
AT5G42650	<i>AOS</i>	5'-CGTTAGGAAGCTCCGTTAATTTCTC-3'	5'-TTCACGAACTGGAACAAGAAAACA-3'
AT1G32640	<i>MYC2</i>	5'-TCCGAGTCCGGTTCATTCT-3'	5'-TCTCGGGAGAAAGTGTTATTGAA-3'
AT5G24770	<i>VSP2</i>	5'-ATGCCAAAGGACTTGCCCTA-3'	5'-CGGGTCGGTCTTCTCTGTTC-3'
AT5G44420	<i>PDF1.2A</i>	5'-GTTCTCTTTGCTGCTTTCGAC-3'	5'-GCAAACCCCTGACCATGT-3'
AT4G29740	<i>CKX4</i>	5'-TTCCCACTCTCCGATCAAAC-3'	5'-TAATGGGAAGGGTGTTCTG-3'
AT5G38970	<i>BR6OX1</i>	5'-TCAGTGGAATGAGCTTGAGG-3'	5'-AGTTCCCAGCGATTTGTGTC-3'
AT1G65310	<i>XTH17</i>	5'-CTTTGGGAAGCCGAGCATTG-3'	5'-ATGGGGAATTCGCGGAACAA-3'
AT5G52400	<i>CYP715A1</i>	5'-GGTGGCAACAAGAATACGGGAAAG-3'	5'-ATTGAGGATCGGCCACGTACA-3'
AT3G25180	<i>CYP82G1</i>	5'-GCTCCACGAACATCTCAAG-3'	5'-CCTGTTAATGTGAGAGCCAAAA-3'
AT3G23250	<i>MYB15</i>	5'-GGTGCGGATATCGATGAAAG-3'	5'-CATTATTAGCGGAGCCCAAG-3'
AT1G80840	<i>WRKY40</i>	5'- TCTCACTATTGGCGTTACTCG-3'	5'- CGAGAGCTTCTTGTCTCAGC-3'
AT1G04180	<i>YUC9</i>	5'-TTCTCGCCACCGGTTATCGTAG-3'	5'-AGCGATGTTAACGGCGTCTACTG-3'

Supplementary Table D.2. RNA-seq data quality of fastqc files.

Sample name	Raw reads	Clean reads	Raw bases	Clean bases	Error rate(%)	Q20(%)	Q30(%)	GC content(%)
C_30min_R1	21497444	21409687	6.4	6.4	0.03	97.77	93.4	46.46
C_30min_R2	23767232	23663209	7.1	7.1	0.03	97.24	92.37	46.36
C_30min_R3	19643453	19554646	5.9	5.9	0.03	97.19	92.44	46.35
C_30min_R4	21069067	20930072	6.3	6.3	0.03	97.44	92.93	46.43
C_24h_R1	20055701	19966975	6	6	0.03	97.2	92.48	46.33
C_24h_R2	21811535	21728414	6.5	6.5	0.03	97.79	93.61	46.16
C_24h_R3	22469659	22291261	6.7	6.7	0.03	97.68	93.41	46.31
C_24h_R4	20452814	20295887	6.1	6.1	0.02	98.08	94.27	46.37
C_4d_R1	20214480	20108574	6.1	6	0.03	97.4	92.88	46.24
C_4d_R2	19732613	19632945	5.9	5.9	0.03	97.79	93.67	46.22
C_4d_R3	22016734	21898107	6.6	6.6	0.03	97.82	93.66	46.3
C_4d_R4	22687371	22468748	6.8	6.7	0.02	98.03	94.16	46.27
C_7d_R1	26158187	25991327	7.8	7.8	0.03	97.39	92.82	46.26
C_7d_R2	21556764	21422762	6.5	6.4	0.03	97.74	93.52	45.88
C_7d_R3	23315208	23152033	7	6.9	0.03	97.61	93.23	45.92
C_7d_R4	19883122	19580032	6	5.9	0.03	97.74	93.6	46.2
C_12d_R1	21670903	21554982	6.5	6.5	0.03	97.02	92.06	45.75
C_12d_R2	23984641	23853489	7.2	7.2	0.03	96.93	91.87	45.74
C_12d_R3	20699466	20572444	6.2	6.2	0.03	97.81	93.65	45.85
C_12d_R4	22381555	22088468	6.7	6.6	0.03	97.56	93.09	45.76
C_15d_R1	19583892	19483472	5.9	5.8	0.03	97.75	93.57	45.7
C_15d_R2	20001028	19892171	6	6	0.03	97.09	92.21	45.75
C_15d_R3	21894013	21731249	6.6	6.5	0.03	97.56	93.11	45.57
C_15d_R4	22996046	22778748	6.9	6.8	0.02	98.04	94.17	45.9
M_30min_R1	22707009	22610090	6.8	6.8	0.03	97.32	92.64	44.09
M_30min_R2	19945076	19864979	6	6	0.03	97.44	92.82	44.2
M_30min_R3	20460883	20362008	6.1	6.1	0.03	97.2	92.4	44.12
M_30min_R4	21267979	21150453	6.4	6.3	0.03	97.66	93.3	44.41
M_24h_R1	20874505	20767634	6.3	6.2	0.03	97.09	92.18	45.52
M_24h_R2	19627219	19531289	5.9	5.9	0.03	96.93	91.81	45.59
M_24h_R3	20385644	20308884	6.1	6.1	0.03	97.65	93.24	45.55
M_24h_R4	20764556	20497400	6.2	6.1	0.02	98.15	94.47	45.59
M_4d_R1	22812722	22645371	6.8	6.8	0.03	97.64	93.25	45.83
M_4d_R2	23769554	23565991	7.1	7.1	0.03	97.48	92.7	45.86
M_4d_R3	24629072	24402724	7.4	7.3	0.03	97.15	92.02	45.85
M_4d_R4	22777211	22354407	6.8	6.7	0.02	98.05	94.22	46.03

M_7d_R1	20511805	20396690	6.2	6.1	0.03	97.26	92.55	46.18
M_7d_R2	20842707	20742556	6.3	6.2	0.03	97.72	93.45	46.22
M_7d_R3	20609013	20447588	6.2	6.1	0.03	97.21	92.49	46.22
M_7d_R4	20859616	20615059	6.3	6.2	0.02	98.21	94.61	46.27
M_12d_R1	20089580	19988835	6	6	0.03	97.66	93.34	46.03
M_12d_R2	21307273	21126164	6.4	6.3	0.03	97.3	92.67	46
M_12d_R3	19934796	19776273	6	5.9	0.03	97.67	93.4	45.99
M_12d_R4	22421878	22217469	6.7	6.7	0.03	97.63	93.29	46.22
M_15d_R1	20795237	20710521	6.2	6.2	0.03	97.07	92.18	45.98
M_15d_R2	21879756	21761646	6.6	6.5	0.03	97.01	92.08	46.05
M_15d_R3	23077990	22916251	6.9	6.9	0.03	97.51	93	45.75
M_15d_R4	22087603	21874364	6.6	6.6	0.03	97.52	93.08	45.78

Supplementary Table D.3. RNA-seq quality mapping.

Sample name	Total reads	Total mapped reads	Uniquely mapped reads	Multiple mapped reads	Total mapping rate	Uniquely mapping rate	Multiple mapping rate
C_30min_R1	42819374	42247878	41272173	975705	98.67%	96.39%	2.28%
C_30min_R2	47326418	46578621	45557103	1021518	98.42%	96.26%	2.16%
C_30min_R3	39109292	38405430	37567447	837983	98.20%	96.06%	2.14%
C_30min_R4	41860144	41189749	40272791	916958	98.40%	96.21%	2.19%
C_24h_R1	39933950	38966980	38086725	880255	97.58%	95.37%	2.20%
C_24h_R2	43456828	42324231	41337416	986815	97.39%	95.12%	2.27%
C_24h_R3	44582522	43477973	42425396	1052577	97.52%	95.16%	2.36%
C_24h_R4	40591774	39651380	38710266	941114	97.68%	95.36%	2.32%
C_4d_R1	40217148	39502012	38593306	908706	98.22%	95.96%	2.26%
C_4d_R2	39265890	38591699	37666956	924743	98.28%	95.93%	2.36%
C_4d_R3	43796214	43263002	42155480	1107522	98.78%	96.25%	2.53%
C_4d_R4	44937496	44167568	43071787	1095781	98.29%	95.85%	2.44%
C_7d_R1	51982654	51156066	49900219	1255847	98.41%	95.99%	2.42%
C_7d_R2	42845524	42279749	41285265	994484	98.68%	96.36%	2.32%
C_7d_R3	46304066	45616375	44492878	1123497	98.51%	96.09%	2.43%
C_7d_R4	39160064	38391966	37428226	963740	98.04%	95.58%	2.46%
C_12d_R1	43109964	42239927	41334730	905197	97.98%	95.88%	2.10%
C_12d_R2	47706978	46737477	45720218	1017259	97.97%	95.84%	2.13%
C_12d_R3	41144888	40618094	39755085	863009	98.72%	96.62%	2.10%
C_12d_R4	44176936	43384976	42383326	1001650	98.21%	95.94%	2.27%
C_15d_R1	38966944	38436633	37549695	886938	98.64%	96.36%	2.28%
C_15d_R2	39784342	39030228	37297753	1732475	98.10%	93.75%	4.35%
C_15d_R3	43462498	42837004	41946916	890088	98.56%	96.51%	2.05%
C_15d_R4	45557496	44959603	42575043	2384560	98.69%	93.45%	5.23%
M_30min_R1	45220180	31840429	31149844	690585	70.41%	68.88%	1.53%
M_30min_R2	39729958	27610988	26662982	948006	69.50%	67.11%	2.39%
M_30min_R3	40724016	28833210	28225572	607638	70.80%	69.31%	1.49%
M_30min_R4	42300906	31412830	30758223	654607	74.26%	72.71%	1.55%
M_24h_R1	41535268	38205796	37320061	885735	91.98%	89.85%	2.13%
M_24h_R2	39062578	36159812	35305472	854340	92.57%	90.38%	2.19%
M_24h_R3	40617768	37281042	36420705	860337	91.79%	89.67%	2.12%
M_24h_R4	40994800	37676023	36756786	919237	91.90%	89.66%	2.24%
M_4d_R1	39977670	39357951	38445507	912444	98.45%	96.17%	2.28%
M_4d_R2	45290742	42734419	41689806	1044613	94.36%	92.05%	2.31%
M_4d_R3	47131982	44996601	43898975	1097626	95.47%	93.14%	2.33%

M_4d_R4	48805448	46299837	45167175	1132662	94.87%	92.55%	2.32%
M_7d_R1	44708814	42597421	41477041	1120380	95.28%	92.77%	2.51%
M_7d_R2	40793380	40034480	39037352	997128	98.14%	95.70%	2.44%
M_7d_R3	41485112	40864981	39878780	986201	98.51%	96.13%	2.38%
M_7d_R4	40895176	39970773	39025873	944900	97.74%	95.43%	2.31%
M_12d_R1	41230118	40676384	39630493	1045891	98.66%	96.12%	2.54%
M_12d_R2	42252328	41210624	40293793	916831	97.53%	95.36%	2.17%
M_12d_R3	39552546	38955848	38102382	853466	98.49%	96.33%	2.16%
M_12d_R4	44434938	43606407	42520734	1085673	98.14%	95.69%	2.44%
M_15d_R1	41421042	40587654	39656090	931564	97.99%	95.74%	2.25%
M_15d_R2	43523292	42613862	41630856	983006	97.91%	95.65%	2.26%
M_15d_R3	45832502	44702308	43635436	1066872	97.53%	95.21%	2.33%
M_15d_R4	43748728	42742815	41734156	1008659	97.70%	95.40%	2.31%

Supplementary Table D.4. Reference list for studies included in the meta-analysis.

N°	Publications
1.	Agrawal, A. A., Strauss, S. Y., & Stout, M. J. (1999). Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. <i>Evolution</i> , 53 :1093-1104.
2.	Aldea, M., Hamilton, J. G., Resti, J. P., Zangerl, A. R., Berenbaum, M. R., Frank, T. D., & DeLucia, E. H. (2006). Comparison of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood saplings. <i>Oecologia</i> , 149 :221-232.
3.	Bardner, R. (1968). Wheat bulb fly, <i>Leptohylemyia coarctata</i> Fall., and its effect on the growth and yield of wheat. <i>Annals of Applied Biology</i> , 61 :1-11.
4.	Blasi, É. A., Buffon, G., Rativa, A. G., Lopes, M. C., Berger, M., Santi, L., Lavallé-Adam, M., Yates III, J. R., Schwambach, J., Beys-da-Silva, W. O., & Sperotto, R. A. (2017). High infestation levels of <i>Schizotetranychus oryzae</i> severely affects rice metabolism. <i>Journal of Plant Physiology</i> , 219 :100-111.
5.	Bownes, A., Hill, M. P., & Byrne, M. J. (2010). Evaluating the impact of herbivory by a grasshopper, <i>Cornops aquaticum</i> (Orthoptera: Acrididae), on the competitive performance and biomass accumulation of water hyacinth, <i>Eichhornia crassipes</i> (Pontederiaceae). <i>Biological Control</i> , 53 :297-303.
6.	Buffon, G., Blasi, É. A. dos R., Lamb, T. I., Adamski, J. M., Schwambach, J., Ricachenevsky, F. K., Bertolazi, A., Silveira, V., Lopes, M. C. B., & Sperotto, R. A. (2021). <i>Oryza sativa</i> cv. Nipponbare and <i>Oryza barthii</i> as unexpected tolerance and susceptibility sources against <i>Schizotetranychus oryzae</i> (Acari: Tetranychidae) mite infestation. <i>Frontiers in Plant Science</i> , 12 : 613568.
7.	Câmara, T., Arnan, X., Barbosa, V. S., Wirth, R., Iannuzzi, L., & Leal, I. R. (2020). Disentangling the effects of foliar vs. floral herbivory of leaf-cutting ants on the plant reproductive success of <i>Miconia nervosa</i> (Smith) Triana (Family Melastomataceae). <i>Bull. Entomol. Res.</i> 110 :77-83.
8.	Collins, C. M., Rosado, R. G., & Leather, S. R. (2001). The impact of the aphids <i>Tuberolachnus salignus</i> and <i>Pterocomma salicis</i> on willow trees. <i>Annals of Applied Biology</i> , 138 :133-140.
9.	Delaney, K. J., Haile, F. J., Peterson, R. K. D., & Higley, L. G. (2008). Impairment of leaf photosynthesis after insect herbivory or mechanical injury on common milkweed, <i>Asclepias syriaca</i> . <i>Environmental Entomology</i> , 37 :1332-1343.
10.	Delaney, K. J. (2012). <i>Nerium oleander</i> indirect leaf photosynthesis and light harvesting reductions after clipping injury or <i>Spodoptera eridania</i> herbivory: high sensitivity to injury. <i>Plant Science</i> , 185 :218-226.
11.	Donovan, M. P., Nabity, P. D., & DeLucia, E. H. (2013). Salicylic acid-mediated reductions in yield in <i>Nicotiana attenuata</i> challenged by aphid herbivory. <i>Arthropod-Plant Interactions</i> , 7 :45-52.
12.	Gianoli, E., & Niemeyer, H. M. (1997). Lack of costs of herbivory-induced defenses in a wild wheat: integration of physiological and ecological approaches. <i>Oikos</i> , 80 :269-275.
13.	Gore, J., Cook, D. R., Catchot, A. L., Musser, F. R., Stewart, S. D., Leonard, B. R., Lorenz, G., Studebaker, G., Akin, D. S., Tindall, K. V., & Jackson, R. E. (2013). Impact of twospotted spider mite (Acari: Tetranychidae) infestation timing on cotton yields. <i>Journal of Cotton Science</i> , 17 :34-39.
14.	Haile, F. J., Higley, L. G., Ni, X., & Quisenberry, S. S. (1999). Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. <i>Environmental Entomology</i> , 28 :787-794.
15.	Haile, F. J., & Higley, L. G. (2003). Changes in soybean gas-exchange after moisture stress and spider mite injury. <i>Environmental Entomology</i> , 32 :433-440.
16.	Halitschke, R., Hamilton, J. G., & Kessler, A. (2011). Herbivore-specific elicitation of photosynthesis by mirid bug salivary secretions in the wild tobacco <i>Nicotiana attenuata</i> . <i>New Phytologist</i> , 191 :528-535.
17.	Harper, A. M., & Kaldy, M. S. (1982). Effect of the pea aphid, <i>Acyrtosiphon pisum</i> (Homoptera: Aphididae), on yield and quality of forage alfalfa. <i>The Canadian Entomologist</i> , 114 :485-489.
18.	Heng-Moss, T., Macedo, T., Franzen, L., Baxendale, F., Higley, L., & Sarath, G. (2006). Physiological responses of resistant and susceptible buffalograsses to <i>Blissus occiduus</i> (Homoptera: Blissidae) feeding. <i>Journal of Economic Entomology</i> , 99 :222-228.
19.	Hutchison, W. D., & Campbell, C. D. (1994). Economic impact of sugarbeet root aphid (Homoptera: Aphididae) on sugarbeet yield and quality in southern Minnesota. <i>Journal of Economic Entomology</i> , 87 :465-475.

20. **Jing, J., Raaijmakers, C., Kostenko, O., Kos, M., Mulder, P. P., & Bezemer, T. M.** (2015). Interactive effects of above-and belowground herbivory and plant competition on plant growth and defence. *Basic and Applied Ecology*, **16**:500-509.
21. **Kessler, A., & T. Baldwin, I.** (2004). Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco *Nicotiana attenuata*. *The Plant Journal*, **38**:639-649.
22. **Kleinjan, C. A., Edwards, P. B., & Hoffmann, J. H.** (2004). Impact of foliage feeding by *Zygina* sp. on tuber biomass and reproduction of *Asparagus asparagoides* (L.): relevance to biological control in Australia. *Biological Control*, **30**:36-41.
23. **Kozel, A. V., Zvereva, E. L., & Kozlov, M. V.** (2017). Impacts of root herbivory on seedlings of three species of boreal forest trees. *Applied Soil Ecology*, **117**:203-207.
24. **Lamp, W. O., Nielsen, G. R., Fuentes, C. B., & Quebedeaux, B.** (2004). Feeding site preference of potato leafhopper (Homoptera: Cicadellidae) on alfalfa and its effect on photosynthesis. *Journal of Agricultural and Urban Entomology*, **21**:25-38.
25. **Liu, J., Legarrea, S., & Kant, M. R.** (2017). Tomato reproductive success is equally affected by herbivores that induce or that suppress defenses. *Frontiers in Plant Science*, **8**:2128.
26. **Macedo, T. B., Bastos, C. S., Higley, L. G., Ostlie, K. R., & Madhavan, S.** (2003). Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *Journal of Economic Entomology*, **96**:188-193.
27. **Macedo, T. B., Peterson, R. K., Weaver, D. K., & Ni, X.** (2009). Impact of *Diuraphis noxia* and *Rhopalosiphum padi* (Hemiptera: Aphididae) on primary physiology of four near-isogenic wheat lines. *Journal of Economic Entomology*, **102**:412-421.
28. **Mundim, F. M., Alborn, H. T., Vieira-Neto, E. H., & Bruna, E. M.** (2017). A whole-plant perspective reveals unexpected impacts of above-and belowground herbivores on plant growth and defense. *Ecology*, **98**:70-78.
29. **Park, Y. L., & Lee, J. H.** (2005). Impact of twospotted spider mite (Acari: Tetranychidae) on growth and productivity of glasshouse cucumbers. *Journal of Economic Entomology*, **98**:457-463.
30. **Peschiutta, M. L., Scholz, F. G., Goldstein, G., & Bucci, S. J.** (2020). Lagged effects of sawfly leaf herbivory on reproductive organs in cherry trees: Overcompensation in flower production reduces quality of fruits and seeds. *Basic and Applied Ecology*, **45**:22-30.
31. **Pincebourde, S., Frak, E. L. A., Sinoquet, H., Regnard, J. L., & Casas, J.** (2006). Herbivory mitigation through increased water-use efficiency in a leaf-mining moth–apple tree relationship. *Plant, Cell & Environment*, **29**:2238-2247.
32. **Poveda, K., Steffan-Dewenter, I., Scheu, S., & Tschardt, T.** (2003). Effects of below-and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia*, **135**:601-605.
33. **Preisser, E. L., Gibson, S. E., Adler, L. S., & Lewis, E. E.** (2007). Underground herbivory and the costs of constitutive defense in tobacco. *Acta Oecologica*, **31**:210-215.
34. **Reddall, A., Sadras, V. O., Wilson, L. J., & Gregg, P. C.** (2004). Physiological responses of cotton to two-spotted spider mite damage. *Crop Science*, **44**:835-846.
35. **Retuerto, R., Fernandez-Lema, B., & Obeso, J. R.** (2004). Increased photosynthetic performance in holly trees infested by scale insects. *Functional ecology*, **18**:664-669.
36. **Roubířková, A., Mudrák, O., & Frouz, J.** (2012). The effect of belowground herbivory by wireworms (Coleoptera: Elateridae) on performance of *Calamagrostis epigejos* (L) Roth in post-mining sites. *European Journal of Soil Biology*, **50**:51-55.
37. **Sanford, L. L., & Webb, R. E.** (1977). Loss of yield in potato selections infested with potato leafhoppers. *American potato journal*, **54**:581-586.
38. **Santamaría, M. E., Martínez, M., Arnaiz, A., Ortego, F., Grbic, V., & Diaz, I.** (2017). MATI, a novel protein involved in the regulation of herbivore-associated signaling pathways. *Frontiers in Plant Science*, **8**:975.
39. **Santamaría, M. E., Diaz, I., & Martínez, M.** (2018). Dehydration stress contributes to the enhancement of plant defense response and mite performance on barley. *Frontiers in Plant Science*, **9**:458.
40. **Schmidt, L., Schurr, U., & Roese, U. S.** (2009). Local and systemic effects of two herbivores with different feeding mechanisms on primary metabolism of cotton leaves. *Plant, Cell & Environment*, **32**:893-903.

41. **Sotelo, P., Pérez, E., Najar-Rodriguez, A., Walter, A., & Dorn, S.** (2014). Brassica plant responses to mild herbivore stress elicited by two specialist insects from different feeding guilds. *Journal of Chemical Ecology*, **40**:136-149.
 42. **Tang, J. Y., Zielinski, R. E., Zangerl, A. R., Crofts, A. R., Berenbaum, M. R., & DeLucia, E. H.** (2006). The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *Journal of Experimental Botany*, **57**:527-536.
 43. **Velikova, V., Salerno, G., Frati, F., Peri, E., Conti, E., Colazza, S., & Loreto, F.** (2010). Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. *Journal of Chemical Ecology*, **36**:629-641.
 44. **Vranjic, J. A., & Ash, J. E.** (1997). Scale insects consistently affect roots more than shoots: the impact of infestation size on growth of eucalypt seedlings. *Journal of Ecology*, **85**:143-149.
 45. **Watanabe, T., & Kitagawa, H.** (2000). Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). *Journal of Economic Entomology*, **93**:1192-1198.
 46. **Zangerl, A. R., Arntz, A. M., & Berenbaum, M. R.** (1997). Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis, and growth. *Oecologia*, **109**:433-441.
-

Supplementary Table D.5. Publication bias risk assessment. Fail-safe numbers obtained using the Rosenberg approach ($\alpha < 0.05$) and Egger's test p -values for each analysis. Fail-safe numbers greater than $5N+10$ indicate that the data are robust. N = number of experiments included in the meta-analysis. P -values higher than 0.05 suggest that no publication bias are present in the data. When trim and fill procedure was applied, fail-safe number and Egger's test p -values shown represent the values obtained after the procedure was used. An asterisk indicates robust data rejecting publication bias.

Parameter		Fail-safe number	N	Egger test (p -value)	Trim and fill procedure
Growth		1105*	62	0.00023	No
	Variables				
	Feeding guild				
	Phloem-feeding	227*	16	0.02763	No
	Chewing	7	17	0.20972*	No
	Cell-content feeder	153*	11	0.83805*	No
	Gall-forming	0	7	0.76674*	Yes
	Plant host				
	Crop	175*	19	0.42328*	No
	Herbaceous	8	18	0.11396*	No
	Woody	26	10	0.62628*	Yes
	Plant stage				
	Early vegetative stage	294*	28	0.0762*	No
	Late vegetative stage	85*	10	0.17195*	No
	Reproductive stage	0	9	0.37161*	No
	Infestation lenght				
	Short-term	0	10	0.95847*	No
	Long-term	466*	33	0.00113	No
	Infestation magnitude				
	Light	0	15	0.86523*	No
	Medium	47	19	0.42526*	No
	Heavy	235*	11	0.01249	No
Photosynthesis		2587*	88	0.00005	No
	Variables				
	Feeding guild				
	Phloem-feeding	197*	28	0.00086	No
	Chewing	29	14	0.11082*	No
	Cell-content feeder	345*	20	0.00005	No
	Gall-forming	0	5	0.00271	Yes
	Leafminer	44*	3	0.18925*	No
	Plant host				
	Crop	618*	49	0.0003	No
	Herbaceous	110*	9	0.03665	No
	Woody	0	11	0.08038*	No
	Plant stage				
	Early vegetative stage	464*	29	0.00287	No
	Late vegetative stage	156*	23	0.00132	No
	Reproductive stage	7	6	0.8468*	Yes
	Infestation length				
	Short-term	448*	39	0.04352	No
	Long-term	487*	23	0.00044	No
	Infestation magnitude				
	Light	0	33	0.85929*	No
	Medium	352*	17	0.00212	No
	Heavy	162*	18	0.00323	No

Reproduction		449*	42	0.00417	No
	Variables				
	Feeding guild				
	Phloem-feeding	5	3	0.76814*	Yes
	Chewing	67	14	0.84094*	No
	Cell-content feeder	91*	11	0.00241	No
	Plant host				
	Crop	112*	15	0.01226	No
	Herbaceous	33	8	0.68816*	Yes
	Woody	6	7	0.34419*	Yes
	Plant stage				
	Early vegetative stage	65	13	0.59653*	No
	Late vegetative stage	55	16	0.93976*	No
	Infestation length				
	Short-term	9	4	0.9379*	Yes
	Long-term	195*	22	0.22279*	No
	Infestation magnitude				
	Light	0	6	0.00607	Yes
	Medium	120*	17	0.16589*	No
	Heavy	94*	11	0.04939	No
Carbohydrate content		0	17	0.64227*	No

Supplementary Table D.6. Standards errors for both manual and automated approaches for each of the *Arabidopsis* accessions tested. Statistical analysis was performed on nine biological replicates for each accession.

	Standard error
--	----------------

Ecotype	Manual	Automated
<i>Ler</i>	0.0668	0.0570
Gr-1	0.0629	0.0561
Col-0	0.0872	0.0805
Sha	0.1056	0.0824
Can-0	0.1395	0.1362
Rub-1	0.1940	0.1832

Supplementary Table D.7. List of transcriptomic studies included in the transcriptomic meta-analysis for GA pathway evaluation.

Organism	Source database	Study type (RNA-seq/ Microarray)	Conditions			Reference
			Plant growth stage	Infestation time	No. of mites	
<i>Arabidopsis thaliana</i> (Col-0)	ArrayExpress (E-MTAB-9448)	RNA-seq	3-week-old	30 min	20	Santamaría <i>et al.</i> , 2021
				1 h		
				3 h		
				24 h		
	NCBI GEO (GSE255811)	RNA-seq	3-week-old	4 d	20	García <i>et al.</i> , 2024
				7 d		
				12 d		
<i>Cucumis sativa</i> (Cucumber)	Springer	RNA-seq	4-week-old	15 d	50	He <i>et al.</i> , 2020
				24 h		
				48 h		
				72 h		
<i>Hordeum vulgare</i> (Barley)	NCBI GEO (GSE83676)	RNA-seq	4-week-old	2h	400	Bui <i>et al.</i> , 2018
	ArrayExpress (E-MTAB-6565)	RNA-seq	1-week-old	24h	200	Santamaría <i>et al.</i> , 2018
				7 d	20	
<i>Lycopersicon esculentum</i> (Tomato)	ArrayExpress (E-MEXP-3888)	Microarray	5-week-old	7 d	45	Nachappa <i>et al.</i> , 2013
<i>Manihot esculenta</i> (Cassava)	Springer	RNA-seq	---	5 d	32	Yang <i>et al.</i> , 2019b
<i>Solanum lycopersicum</i> (Tomato)	NCBI GEO (GSE116827)	Microarray	3-week-old	7 d	45	Schimmel <i>et al.</i> , 2018
	NCBI GEO (GSE70375)	Microarray	4-week-old	24 h	200	Wybouw <i>et al.</i> , 2015
	Google Scholar	RNA-seq	5-week-old	72 h	60	Weinblum <i>et al.</i> , 2021
<i>Vitis vinifera</i>	Google Scholar	RNA-seq	~1-week-old (2-leaf stage)	24 h	50	Díaz-Riquelme <i>et al.</i> , 2016
<i>Zea mays</i> (Maize)	NCBI GEO (GSE100121)	RNA-seq	3-week-old	2 h	400	Bui <i>et al.</i> , 2018
				24 h	200	

REFERENCES

Bui, H., Greenhalgh, R., Ruckert, A., Gill, G. S., Lee, S., Ramirez, R. A., & Clark, R. M. (2018). Generalist and specialist mite herbivores induce similar defense responses in maize and barley but differ in susceptibility to benzoxazinoids. *Frontiers in Plant Science*, **9**: 1222.

Díaz-Riquelme, J., Zhurov, V., Rioja, C., Pérez-Moreno, I., Torres-Pérez, R., Grimplet, J., Carbonell-Bejerano, P., Bajda, S., Van Leeuwen, T., Martínez-Zapater, J. M., Grbic, M., & Grbic, V. (2016). Comparative genome-wide transcriptome analysis of *Vitis vinifera* responses to adapted and non-adapted strains of two-spotted spider mite, *Tetranychus urticae*. *BMC Genomics*, **17**: 1-15.

- He, J., Bouwmeester, H. J., Dicke, M., & Kappers, I. F.** (2020). Transcriptional and metabolite analysis reveal a shift in direct and indirect defences in response to spider-mite infestation in cucumber (*Cucumis sativus*). *Plant Molecular Biology*, **103**: 489-505.
- Martel, C., Zhurov, V., Navarro, M., Martinez, M., Cazaux, M., Auger, P., Migeon, A., Santamaría, M. E., Wybouw, N., Diaz, I., Van Leeuwen, T., Navajas, M., Grbic, M., & Grbic, V.** (2015). Tomato whole genome transcriptional response to *Tetranychus urticae* Identifies divergence of spider mite-induced responses between tomato and Arabidopsis. *Molecular Plant-Microbe Interactions*, **28**: 343–361.
- Nachappa, P., Margolies, D. C., Nechols, J. R., Whitfield, A. E., & Rotenberg, D.** (2013). Tomato spotted wilt virus benefits a non-vector arthropod, *Tetranychus urticae*, by modulating different plant responses in tomato. *PLoS One*, **8**: e75909.
- Santamaría, M. E., Diaz, I., & Martinez, M.** (2018). Dehydration stress contributes to the enhancement of plant defense response and mite performance on barley. *Frontiers in Plant Science*, **9**: 458.
- Santamaría, M. E., Garcia, A., Arnaiz, A., Rosa-Diaz, I., Romero-Hernandez, G., Diaz, I., & Martinez, M.** (2021). Comparative transcriptomics reveals hidden issues in the plant response to arthropod herbivores. *Journal of Integrative Plant Biology*, **63**: 312-326.
- Schimmel, B. C., Alba, J. M., Wybouw, N., Glas, J. J., Meijer, T. T., Schuurink, R. C., & Kant, M. R.** (2018). Distinct signatures of host defense suppression by plant-feeding mites. *International Journal of Molecular Sciences*, **19**: 3265.
- Weinblum, N., Cna'Ani, A., Yaakov, B., Sadeh, A., Avraham, L., Opatovsky, I., & Tzin, V.** (2021). Tomato cultivars resistant or susceptible to spider mites differ in their biosynthesis and metabolic profile of the monoterpenoid pathway. *Frontiers in Plant Science*, **12**: 630155.
- Wybouw, N., Zhurov, V., Martel, C., Bruinsma, K. A., Hendrickx, F., Grbić, V., & Van Leeuwen, T.** (2015). Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. *Molecular Ecology*, **24**: 4647-4663.
- Yang, J., Wang, G. Q., Zhou, Q., Lu, W., Ma, J. Q., & Huang, J. H.** (2019b). Transcriptomic and proteomic response of *Manihot esculenta* to *Tetranychus urticae* infestation at different densities. *Experimental and Applied Acarology*, **78**: 273-293.