TOMÁS THORMANN ABRANCHES DE MAGALHÃES

Understanding host preference of *Trioza erytreae* (Del Guercio) by a multi-omics approach

UNIVERSIDADE DE ÉVORA

Universidade do Algarve
Faculdade de Ciências e Tecnologia

2025

TOMÁS THORMANN ABRANCHES DE MAGALHÃES

Understanding host preference of *Trioza erytreae* (Del Guercio) by a multi-omics approach

Doutoramento em Ciências Agrárias e Ambientais

Trabalho efetuado sob orientação de:

Natália Tomás Marques
Professora Auxiliar da Universidade do Algarve e Investigadora do Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & Instituto para as Alterações Globais e Sustentabilidade (MED & CHANGE)

Amílcar Manuel Marreiros Duarte
Professor Auxiliar da Universidade do Algarve e Investigador do Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento & Instituto para as Alterações Globais e Sustentabilidade (MED & CHANGE)

José Alberto Cardoso Pereira
Professor Coordenador Principal do Instituto Politécnico de Bragança e Investigador do Centro de Investigação da Montanha & Laboratório para a Sustentabilidade e Tecnologia em Regiões de Montanha (CIMO & SusTEC)

UNIVERSIDADE DE ÉVORA

Universidade do Algarve
Faculdade de Ciências e Tecnologia

2025

Declaração de autoria de trabalho

Understanding host preference of *Trioza erytreae* (Del Guercio) by a multi-omics approach

Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

_____

(Tomás Thormann Abranches de Magalhães)

Acknowledgments

These four years have been an incredible journey which allowed me to get to know and to collaborate with great minds, great scientists and great people. To whom I'm deeply grateful.

I would like to express my deepest gratitude to my supervisors:

Prof. Dr. Natália Tomás Marques, who trusted in an agronomist with scarce laboratory skills to embark in this endeavour. For all the guidance, coaching and laboratory skill development. For all the ideas, improvements and writing help. For the opportunity and access. And of course, for all the time, patience, readiness to help and for the friendship. For all the talks about life in the greenhouse and the lab. Her trust and tireless help have significantly improved my molecular biology and scientific skills, for all that I am immensely grateful.

Prof. Dr. Amílcar Manuel Marreiros Duarte for his mentoring, for the focus on the holistic and critical thinking, for the search for the important questions. For his openness to share citriculture and life experience knowledge. For the long and insightful lunchbreak or after hours talks, that motivated me to learn more and become better. Pela amizade, inclusão, oportunidades, partilha e todos os ensinamentos, como o da importância da língua portuguesa e o nosso dever de a defendermos. Tornei-me um melhor pensador crítico e melhor cidadão por tua causa. Muito obrigado por tudo.

Prof. Dr. José Alberto Cardoso Pereira for his knowledge, for his productivity focus and organisation. For making me feel at home in the other side of the country, including me as if I was there for years. For the objective view and problem-solving orientation. For all the contacts and opportunities. For the great food, times shared and friendship. His passion for analysing scientific data and for connecting people have significantly improved my researcher and connector skills, for all that I am immensely grateful.

A special thanks for my colleague and friend Susana Anahi Dandlen for all her insights and for being my lab mentor. For the time, patience and mentoring. For teaching me all those lab techniques and important details to keep an eye out for. For your relaxed way to deal with any problem that arose and for your friendship thank you.

A particular acknowledgement for three specific teams, the "proteomics team", namely Dr Liliana Anjos and Prof. Dr. Deborah Power from CCMAR, Universidade do Algarve, for all the shared knowledge in the field, for the help on the experimental design, protocol design and scientific writing. The "volatiles" team from CIMO, Instituto Politécnico de Bragança, namely Dr. Nuno Rodrigues, Daniela Ruano and Dr. Ana Rodrigues, for all the teachings and for the lemon volatile madness help, and for the good times in the lab. The "Insetos" team from CIMO namely Dr. Isabel Rodrigues, Dr. Jacinto Benhadi-Marín, and Diogo Félix Oliveira for all the helpful insights, for all the hard work, readiness to help, entomology teachings and fun times. I unofficially add to the "Insetos" team from the

Universidade do Algarve Beatriz Duarte, for her readiness to help, entomology insights and fun personality that helped my motivation in this thesis.

I thank the opportunity to collaborate with and to work with Dr Andreia Bento-Silva, Prof. Dr Maria Rosário Bronze and Prof. Dr Noélia Duarte from iMed.ULisboa, Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, and with Prof. Dr. Rui Guerra and Dr. Ana Cavaco from Centro de Eletrónica, Optoeletrónica e Telecomunicações, Universidade do Algarve whose contributions were crucial to this thesis.

These four years also showed me that it's not all science and I would really like to thank all my outside support that came in many different ways:

I would like to thank the sea and the incredible and unique feeling I get from exploring and riding the waves you provide. My soul washer.

All the colleagues in MED that have made this a fun journey, Beatriz Duarte, Ana Trindade, Pedro Matias, Luísa Coelho and many others. For all the fun trips and shared trials. All of my northern CIMO "family" for the fun times and shared finos, Isabel Rodrigues, Diogo Felix, Maria Eliza, Jacinto, Ketrin Kubiak, Marta Madureira, Amir Bzaina, Nuno Ferreiro and many others.

My fellow Fariceirenses, now Mafrolhanenses or Olhafrenses for the help in *Trioza erytreae* monitoring in Mafra lemon orchards. And more importantly for the amazing friendship. Tiago and Irina you are an amazing couple, with parenting superpowers for your little pair of lights Aurora and Lucas. Bora bora!

My childhood friends Flavio, Sascha, Michel and Tomás Tigchelaar and their growing families. For all the amazing times, for being there every time and for making me grow into a better man. My Back to Anfi group, Wemans, Veríssimo, Valter, Bioxala, Dacach, Simolho, Dorge, Varalonga, Flores and Al for all the incredible experiences and gatherings along the way.

My grandparents from both sides who all have been my idols in their own kind of way, I'll try to perpetuate your incredible knowledge sharing and calmness the best I can. Obrigado, Avô Eduardo, por seres a mente mais brilhante que conheci, e avó Nita por todas as boas lembranças deixadas. Vielen Dank Oma ich habe von dir und Opa so viel gelernt, wie zu leben und alle zu lieben. Immer besser ein bisschen Verrückt als stink normal.

My parents and sister Sara, firstly for all the love. For all the opportunities, education, motivation and life teachings. Pai, muito obrigado por tudo, por mostrares a importância da família e amigos, por me fazeres ser melhor e saber amar. Mama, danke für alles, du bist die beste Person, die ich kenne, das am meisten hilf bereits ist und dass immer die andere als erste Priorität hat. If I get to be anything like both of you, I'll be a great person. Sara is a perfect example of your great influence and she's the best sister, and an amazing Mom, hardworker and great friend. Proud of and thankful for all of you.

Of course, I would like to extend this thanks to my modern family all my extra parents and siblings, Lena for all the love and family magic, Nikolaus for the curious

mind and love for the land, and João Pedro, Rodrigo, Ricardo, Sofia and Manu for all the love, good times and support.

And finally, to my wife and whole world Cláudia. For all the incredible moments, all the love and support. For making life seem so easy, even in difficult times. For all the energy boosts, for breaking barriers and pushing me to be better. For guidance and passion, I strive to emulate. For your heart that is the size of the world. For all the funny sounds of my quirky music box. Basically, for being the amazing lightful person you are, and for sharing your greatness with me.

Funding

Tomás Thormann Abranches de Magalhães was supported by the Portuguese Foundation for Science and Technology (FCT) through the PhD scholarship 2020.07798.BD (https://doi.org/10.54499/2020.07798.BD) financed by national funds from MCTES, via FCT, and co-funded by the European Union (EU) through the European Social Fund.". This work was supported by national funds through FCT/MCTES (PIDDAC): CIMO, UIDB/00690/2020 (DOI: 10.54499/UIDB/00690/2020) and UIDP/00690/2020 (DOI: 10.54499/UIDP/00690/2020); and SusTEC, LA/P/0007/2020 (DOI: 10.54499/LA/P/0007/2020), MED, UIDB/05183/2020 (https://doi.org/10.54499/UIDB/05183/2020) and UIDP/05183/2020 https://doi.org/10.54499/UIDP/05183/2020) and CHANGE (https://doi.org/10.54499/LA/P/0121/2020), CEOT UIDB/00631/2020 and UIDP/00631/2020. Additionally, by the "European Union Horizon 2020" research program, under the grant agreement ID: 817526 Pre-HLB "Preventing HLB epidemics for ensuring citrus survival in Europe.

Abstract

The citriculture industry is significantly challenged by the devastating bacterial disease Huanglongbing (HLB). There is currently no effective cure for HLB, its management is based on inoculum elimination, and on the control of its vectors, namely the psyllids *Trioza erytreae* and *Diaphorina citri*. While Europe is currently free of HLB, *T. erytreae* is present in Spain and Portugal, two of Europe's main citrus producers. The present study aimed to analyse the mechanisms underpinning *T. erytreae*'s interaction with its citrus hosts. The plant hosts used in this study were the highly suitable lemon (*Citrus ×limon*) and the less suitable sweet orange *(Citrus ×sinensis*). Both hosts were infested with *T. erytreae* adults, and three times more nymphs developed on lemon than on sweet orange plants. A multi-omics analysis on the plant's enriched vascular sap of both citrus hosts revealed heightened primary and secondary metabolisms activity of sweet orange plants in response to infestation, including jasmonic acid (JA)-related defence mechanisms. A proteomic analysis of the nymphs revealed that the diet provided by lemon plants induced growth and energy pathways in *T. erytreae*. Based on these results, a subsequent experiment was conducted to stimulate the defence responses of lemon plants, analysing its effect on *T. erytreae* infestation and on the plants' volatile organic compounds (VOC). Lemon plants were sprayed with JA to activate herbivory-related defences. The JA treatment resulted in a substantial reduction in the number of *T. erytreae* eggs and nymphs. Furthermore, the VOC profile was found to be affected by infestation and JA treatment, with high emission of (Z)-3-hexenol acetate, 2-hexen-1-ol and carveol. This research provides knowledge that can be used to develop novel methods for *T. erytreae* control, enhancing the array of responses to the HLB challenge and promoting a sustainable citriculture.

Keywords: African citrus psyllid; huanglongbing; metabolomics; plant–insect interaction; proteomics; volatiles

Resumo

No contexto da produção frutícola mundial, a citricultura ocupa uma posição de destaque, representando 17,7% da produção global de fruta no período entre 2019 e 2023. A produção de citrinos é afetada por diversos inimigos, que incluem pragas e doenças, sendo que a proteção contra estes inimigos representa uma fatia muito importante dos custos de produção desta cultura. O Huanglongbing (HLB), causado pelas bactérias *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus e *Candidatus* Liberibacter asiaticus, é uma das doenças mais devastadoras dos citrinos, afetando gravemente a sua produção. Estas bactérias desenvolvem-se nos vasos condutores floémicos, afetando o transporte e a distribuição de nutrientes na planta. Os sintomas associados ao HLB incluem cloroses assimétricas nas folhas, amarelecimento dos ramos, frutos pequenos e deformados, inversão de cor dos frutos e um definhamento geral da planta. Atualmente não existe um tratamento curativo eficaz para esta doença, e o seu controlo assenta essencialmente na eliminação de plantas e ramos infetados, assim como no controlo dos insetos vetores. Os vetores conhecidos da doença são a psila-africana-dos-citrinos *Trioza erytreae* (Del Guercio, 1918), já identificada em Portugal e em Espanha, e a psila-asiática-dos-citrinos *Diaphorina citri* (Kuwayama, 1908), identificada no Chipre.

Apesar da presença dos vetores no continente europeu, ainda não foi identificada a presença da doença HLB. No entanto, a presença dos insetos vetores causa uma enorme preocupação entre citricultores europeus. A presença de *T. erytreae* causa particular preocupação pois a sua distribuição geográfica engloba dois dos maiores produtores de citrinos da Europa, nomeadamente Espanha, o maior produtor, e Portugal, o quarto maior produtor. O controlo efetivo destes vetores requer uma elevada frequência de tratamentos fitossanitários, implicando elevados custos económicos, ambientais e de saúde pública. Uma elevada intensidade e frequência de tratamentos pode também levar ao desenvolvimento de fenómenos de resistência dos insetos vetores aos compostos químicos aplicados, o que já foi observado em *D. citri*. O estado atual da citricultura europeia leva à necessidade de desenvolver e melhorar as estratégias de proteção contra

psilídeos vetores de HLB, de modo a prevenir a difusão da doença HLB e manter a produtividade do sector.

O estudo da interação planta–inseto, é crucial na exploração de vias alternativas para a proteção das plantas hospedeiras contra as pragas e na redução do seu impacto na produção. A interação planta–inseto é complexa e multifacetada, e requer uma análise multidisciplinar, que englobe conhecimentos de entomologia, fisiologia vegetal, química e biologia molecular. As abordagens ómicas, por incluírem técnicas analíticas de elevado rendimento e resolução, têm demonstrado ser ferramentas de elevada utilidade na análise da interação bioquímica e molecular do complexo planta–inseto. Estudos que incorporaram a utilização de ferramentas ómicas referem a identificação de compostos específicos (péptidos, fito-hormonas e voláteis) importantes na interação tripartida entre os citrinos hospedeiros, os psilídeos vetores e as bactérias causadoras de HLB. A título de exemplo pode referir-se a identificação de um transcrito de um péptido prevalente em cultivares de citrinos menos suscetíveis ao HLB, identificado através de uma abordagem transcriptómica, nomeadamente o homólogo do péptido "109-aa Arabidopsis heat-stable protein HS1". Este péptido, aplicado por injeção no tronco, demonstrou ser eficaz no tratamento e prevenção da doença HLB. Dois estudos de abordagem metabolómica demonstraram que a infestação com *D. citri* afetou a regulação das fito-hormonas ácido salicílico e ácido jasmónico na laranjeira. A aplicação via foliar destas fito-hormonas em citrinos demonstrou efeitos de repelência nos insetos adultos e de redução na reprodução de *D. citri*.

O presente trabalho teve como objetivo o estudo da interação de *T. erytreae* com os citrinos seus hospedeiros, através de abordagens ómicas, de modo a identificar as proteínas, metabolitos e/ou vias metabólicas relevantes para esta interação planta-inseto. Para o ensaio inicial foram escolhidos dois hospedeiros, o limoeiro [*Citrus ×limon* (L.) Burm. f.], que apresenta elevada suscetibilidade à *T. erytreae*, e a laranjeira-doce [*Citrus ×sinensis* (L.) Osbeck] com menor suscetibilidade. Em laboratório, ambos os hospedeiros foram infestados com adultos de *T. erytreae*, e as posturas efetuadas foram acompanhadas continuamente até ao quarto e quinto instar ninfal (estados N4 e N5). Os estados

ninfais N4 e N5 foram considerados como objeto de estudo por apresentarem uma elevada intensidade de alimentação e uma reduzida locomoção. Características que possibilitam a análise da resposta das plantas hospedeiras em órgãos nos quais é possível verificar, com segurança, a presença e alimentação contínua do psilídeo. Quando as ninfas de *T. erytreae* atingiram os estados N4 e N5 foram recolhidas e guardadas a –80 °C. Recolheu-se subsequentemente amostras da seiva vascular enriquecida (composta pela seiva do floema e do xilema, incluindo o possível conteúdo de algumas células adjacentes a estes tecidos) de cada uma das plantas hospedeiras.

O conjunto de proteínas e compostos e/ou vias metabólicas identificadas na análise proteómica e metabolómica permitiram o desenho do último ensaio associado a esta tese. Este ensaio teve como objetivo a análise do efeito da pulverização foliar de ácido jasmónico em plantas de limoeiro, sendo estas posteriormente infestadas com *T. erytreae*. Em paralelo foi analisado o perfil de voláteis das plantas de limoeiro antes e após a aplicação do ácido jasmónico e a infestação do psilídeo, a tempos diferentes. A infestação pelo psilídeo foi avaliada até ao desenvolvimento dos estados N4 e N5 das ninfas.

A tese encontra-se estruturada em sete capítulos dos quais o capítulo 1 constitui a introdução geral e o capítulo 7 a discussão geral. Os resultados obtidos no presente estudo estão descritos nos capítulos 2 a 6.

O capítulo 2 explana o conhecimento atual sobre os hospedeiros e potenciais hospedeiros de *T. erytreae*. A maioria dos hospedeiros suscetíveis ao psilídeo pertence à subfamília Aurantioideae da família Rutaceae, na qual se insere o género *Citrus*. Dentro deste género existem espécies em que a suscetibilidade difere muito entre cultivares, como as tangerineiras (*Citrus reticulata* Blanco), enquanto outras espécies mantêm uma suscetibilidade constante, independentemente da cultivar, como os limoeiros. Neste capítulo foi referida a importância da existência de rebentos e folhas jovens para o desenvolvimento de *T. erytreae*. Foi igualmente salientado que há uma escassez de estudos da componente bioquímica e molecular no estudo da interação de *T. erytreae* com os seus hospedeiros.

Os resultados das análises ómicas usadas no estuda da interação inseto–hospedeiro estão descritas nos capítulos 3 a 5. Nestes ensaios observou-se que o número de ninfas que se desenvolveram nos limoeiros foi três vezes superior ao número de ninfas nas laranjeiras.

No capítulo 3 é descrito o resultado da avaliação do proteoma da seiva vascular enriquecida de plantas de limoeiro e laranjeira infestadas e não infestadas com *T. erytreae*. Os resultados obtidos no ensaio inicial indicam que o número de ninfas desenvolvido no limoeiro foi três vezes superior ao observado na laranjeira. O proteoma foi analisado através da técnica de cromatografia líquida de nanoescala acoplada a espectrometria de massa em tandem (nanoLC-MS/MS). O desenvolvimento de *T. erytreae* foi menos eficaz nas laranjeiras, tendo-se observado uma maior prevalência de ninfas incapazes de atingir instares mais avançados em comparação com as ninfas que se desenvolveram nas plantas de limoeiro. A resposta do proteoma da laranjeira infestada revelou modificações no metabolismo primário. Ocorreu também a ativação da via de sinalização do ácido jasmónico, para além de outras respostas de defesa da planta.

No capítulo 4 é descrito o resultado de uma nova análise do proteoma (nanoLC-MS/MS) e do metaboloma da seiva vascular enriquecida, esta última realizada através de cromatografia líquida de alto rendimento acoplada a espectrometria de massa em tandem (HPLC-MS/MS). Como resposta à infestação de *T. erytreae*, nos limoeiros observou-se uma indução da biossíntese de fenilpropanoides e na laranjeira observou-se uma resposta metabolómica comum à resposta proteómica, nomeadamente a indução da síntese de ácido jasmónico. Constatou-se também que a integração de diferentes análises bioquímicas e moleculares foi crucial para uma caracterização aprofundada da complexidade multifacetada da interação planta–inseto.

No capítulo 5 é descrito o resultado da análise proteómica ao às ninfas de *T. erytreae*. Realizou-se a análise proteómica, via nanoLC-MS/MS, das ninfas que se desenvolveram em limoeiro e laranjeira. Paralelamente, analisou-se o proteoma das ninfas quando estas foram transferidas para uma dieta de 24h composta apenas por sacarose. Nas ninfas que se desenvolveram na laranjeira observou-se

um aumento de proteínas associadas a insetos com fenótipos subdesenvolvidos e inférteis. Nas ninfas que se desenvolveram no limoeiro observou-se a indução de vias metabólicas associadas ao desenvolvimento e ao metabolismo da energia. As ninfas desenvolvidas nos limoeiros apresentaram uma maior alteração do proteoma quando transferidas para a dieta de sacarose, a qual é pobre em nutrientes, indicando que a dieta fornecida pelo limoeiro é mais favorável para o crescimento de *T. erytreae* que a dieta fornecida pelas laranjeiras.

Os resultados dos capítulos 3, 4 e 5 levaram ao desenho da experiência analisada no capítulo 6. Neste capítulo descreve-se a aplicação foliar de ácido jasmónico e de como este induziu a defesa do limoeiro contra a *T. erytreae*. Após a aplicação do ácido jasmónico infestaram-se os limoeiros com *T. erytreae*. O número de posturas de *T. erytreae* foi três vezes menor e o número de ninfas desenvolvidas no quarto e quinto instar foi cinco vezes inferior em plantas pulverizadas com ácido jasmónico em comparação com plantas controlo. O efeito da infestação e da aplicação de acido jasmónico foi avaliado através da fração volátil emitida pelas folhas dos limoeiros, analisado por -microextração em fase sólida, com cromatografia gasosa acoplada a espetrometria de massa (HS-SPME-GC/MS). Adicionalmente, analisou-se o nível endógeno de ácido jasmónico na planta através de imunoabsorção enzimática (ELISA). Observou-se uma maior modificação do perfil de voláteis e maior indução de ácido jasmónico endógeno pela oviposição e pela alimentação dos adultos de *T. erytreae* nas plantas que não foram pulverizadas com ácido jasmónico exógeno. A pulverização de ácido jasmónico levou a uma resposta mais ativa por parte dos limoeiros no período subsequente. Esta resposta foi evidenciada por um aumento dos níveis de ácido jasmónico endógeno e da intensidade da emissão de voláteis em resposta à alimentação contínua das ninfas de *T. erytreae*. Os resultados obtidos demonstraram que a infestação por *T. erytreae* causou a indução de voláteis no limoeiro que atraem insetos predadores e parasitoides, nomeadamente a 6-methyl-5-hepten-2-ona e o (Z)-3-hexenol. Simultaneamente, observou-se uma indução de voláteis relacionados com a defesa da planta, como o fenol e o acetato de 2-hexen-1-ol. Adicionalmente, observou-se uma indução do carveol que tem um efeito

repelente para insetos. A indução destes compostos em resposta à infestação de *T. erytreae* indica que os mesmos poderão ter um papel importante nesta interação planta–inseto.

Os estudos desta tese identificaram um conjunto de compostos químicos, do metabolismo primário e secundário, com possível impacto nos insetos. Determinou-se que a aplicação foliar de ácido jasmónico afeta negativamente a infestação de *T. erytreae* em limoeiros. A aplicação foliar de ácido jasmónico em citrinos hospedeiros de *T. erytreae* como método de proteção contra este psilídeo deve ser testado em trabalhos futuros, de modo a avaliar a sua eficácia e viabilidade económica em condições de produção comercial. O conhecimento obtido nesta tese poderá servir como base para o desenvolvimento de novas ferramentas e estratégias de proteção contra *T. erytreae* enquanto psilídeo vetor de HLB, promovendo assim uma citricultura próspera, sustentável e livre de HLB.

Palavras-chave: Psila-africana-dos-citrinos; huanglongbing; metabolómica; interação planta inseto; proteómica; voláteis.

Table of contents

List of abbreviations and acronyms

ACN	Acetonitrile
AGC	Automatic gain control
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
Bt	*Bacillus thuringiensis* Berliner
CkIIbeta	Casein kinase II beta subunit
CLaf	*Candidatus* Liberibacter africanus
CLam	*Candidatus* Liberibacter americanus
CLas	*Candidatus* Liberibacter asiaticus
CoA	Coenzyme A
"Control"	Lemon plants sprayed with control spray mix and without *T. erytreae* infestation (Chapter 6)
DAI	Days after infestation
DAMs	Differentially abundant metabolites
DAPs	Differentially abundant proteins
DAS	Days after spray
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum associated degradation system
EurekaLemonCon	'Eureka' Lemon control (Chapter 3)
EurekaLemonInf	Infested 'Eureka' lemon (Chapter 3)
FA	Formic acid
FDR	False discovery rate
HLB	Huanglongbing
HMO	Petroleum-based horticultural mineral oils
HPLC	high-performance liquid chromatography
HPLC-DAD-MS/MS	high-performance liquid chromatography coupled with a diode array detector and tandem mass spectrometry
HPLC-MS/MS	high-performance liquid chromatography coupled with tandem mass spectrometry
HS-SPME-GC/MS	Headspace solid phase microextraction gas chromatography coupled to mass spectrometry)
JA	Jasmonic acid
JAsp	Lemon plants sprayed with JA spray mix and without *T. erytreae* infestation (Chapter 6)

JAsp_Psyll	Lemon plants sprayed with JA spray mix and infested with *T. erytreae* (Chapter 6)
KEGG	Kyoto Encyclopedia of Genes and Genomes
"Lemon"	Treatment group formed by *Trioza erytreae* nymphs developed exclusively on lemon plants (Chapter 5)
LemonCon	'Eureka' Lemon control (Chapter 4)
LemonInf	Infested 'Eureka' lemon (Chapter 4)
LemonSuc	Treatment group comprised by *Trioza erytreae* nymphs developed on lemon plants that underwent a 24 h sucrose feeding treatment (Chapter5)
MRM	multiple reaction monitoring
MS	mass spectrometry
nanoLC-MS/MS	nanoscale liquid chromatography coupled to tandem mass spectrometry
PCA	Principal component analysis
PhD	Doctor of philosophy
Psyll	Lemon plants sprayed with control spray mix and infested with *T. erytreae* (Chapter 6)
ROS	Reactive oxygen species
SA	Salicylic acid
SDM	standard deviation of the mean
SEM	standard error mean
SwO	Sweet orange, *C. ×sinensis*
"SwO"	Treatment group formed by *Trioza erytreae* nymphs developed exclusively on sweet orange plants (Chapter 5)
SwOCon	'Valencia' sweet orange control (Chapter 4)
SwOInf	Infested 'Valencia' sweet orange (Chapter 4)
SwOSuc	Treatment group comprised by *Trioza erytreae* nymphs developed on sweet orange plants that underwent a 24 h sucrose feeding treatment (Chapter 5)
TCA	Tricarboxylic acid
TIC	Total ion chromatogram
ValenciaSwOCon	'Valencia' sweet orange control (Chapter 3)
ValenciaSwOInf	Infested 'Valencia' sweet orange (Chapter 3)
VOC	Volatile organic compound

Chapter 1. General introduction

1.1. Scope of the Thesis

Citriculture, one of the main fruit production industries in the world, is confronted nowadays with a multitude of challenges, including the management of diseases and pests. The most important problem at the moment is Huanglongbing (HLB), a devastating bacterial disease transmitted by the psyllid vectors *Trioza erytreae* (Del Guercio, 1918) and *Diaphorina citri* (Kuwayama, 1908). The effective control of these vectors is imperative for the prevention and management of HLB. Although Europe is currently free of HLB, the vector *Trioza erytreae* is present in Portugal and Spain, two of Europe's most important citrus producing countries. Consequently, the development and implementation of novel strategies to manage this vector and prevent the incursion of HLB into Europe is imperative. The present thesis focused on studying the interaction of *T. erytreae* with its citrus hosts using a proteomics, metabolomics and volatolomics approach, aiming to contribute to the development of new methods to control this insect.

1.2. The importance of citriculture in the world

Citrus is one of the most important fruit crops on a global scale, accounting for 17.67% of the total fruit production between 2019 and 2023 (FAO, 2025) (Fig. 1.1).

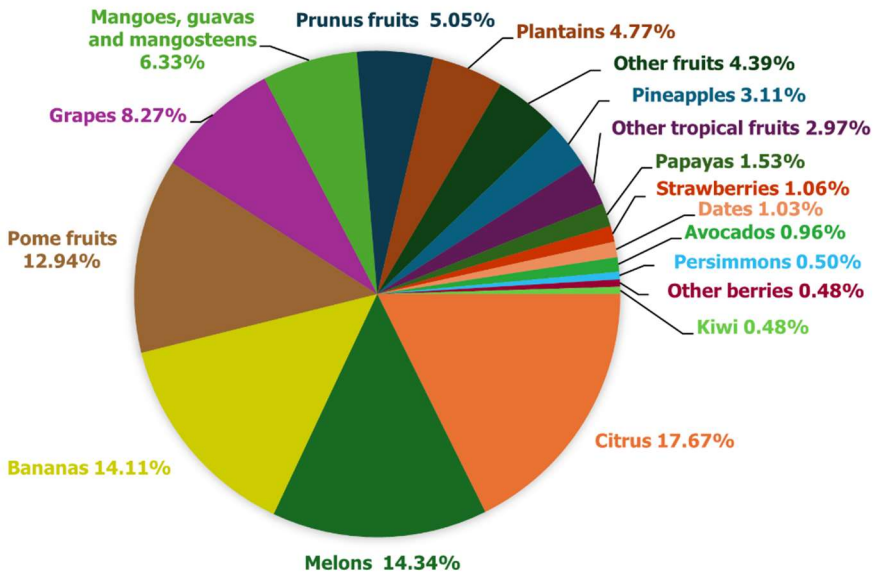

Figure 1.1. Percentage of worldwide total fruit production in the period of 2019-2023 (FAO, 2025).

Between 2019 and 2023 an average of 163.0 million tonnes of citrus were produced per year, and sweet oranges [*Citrus* ×*sinensis* (L.) Osbeck] had the highest representation with 68.6 million tonnes, followed by mandarins (*Citrus reticulata* Blanco), with 49.8 million tonnes, and lemons [*Citrus* ×*limon* (L.) Burm. f.] and limes (*Citrus* spp.), with 21.4 million tonnes (Figure 1.1). China and Brazil, with 45.6 and 19.6 million tonnes per year respectively, were the main citrus producers in the world. Within Europe, Spain, with 6.2 million tonnes, was the biggest producer (FAO, 2025) (Fig. 1.2). Portugal produces a total of 409.3 thousand tonnes of citrus per year, with the Algarve region accounting for about 85% of this production (INE, 2024).

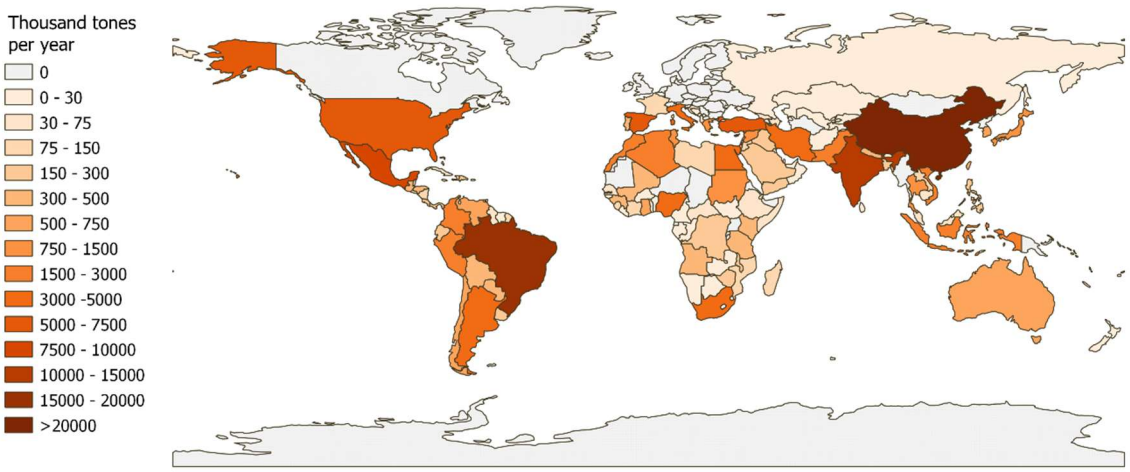

Figure 1.2. Representation of total average annual citrus production per country, in the period of 2019-2023. Map created using the free and open source QGIS (QGIS Development Team, 2020). World map layer source: Natural Earth, free vector and raster map data (naturalearthdata.com). Citrus production data source: (FAO, 2025)

1.3. Huanglongbing (HLB) and its agents

Huanglonging (HLB), also known as “citrus greening”, is a disease caused by a phloem-limited bacterium that affects citrus and other Rutaceae. Symptoms of this disease include non-symmetrical mottled chlorosis of leaf blades, yellow shoots, stunted growth, small and deformed fruits with colour inversion (Fig 1.3).

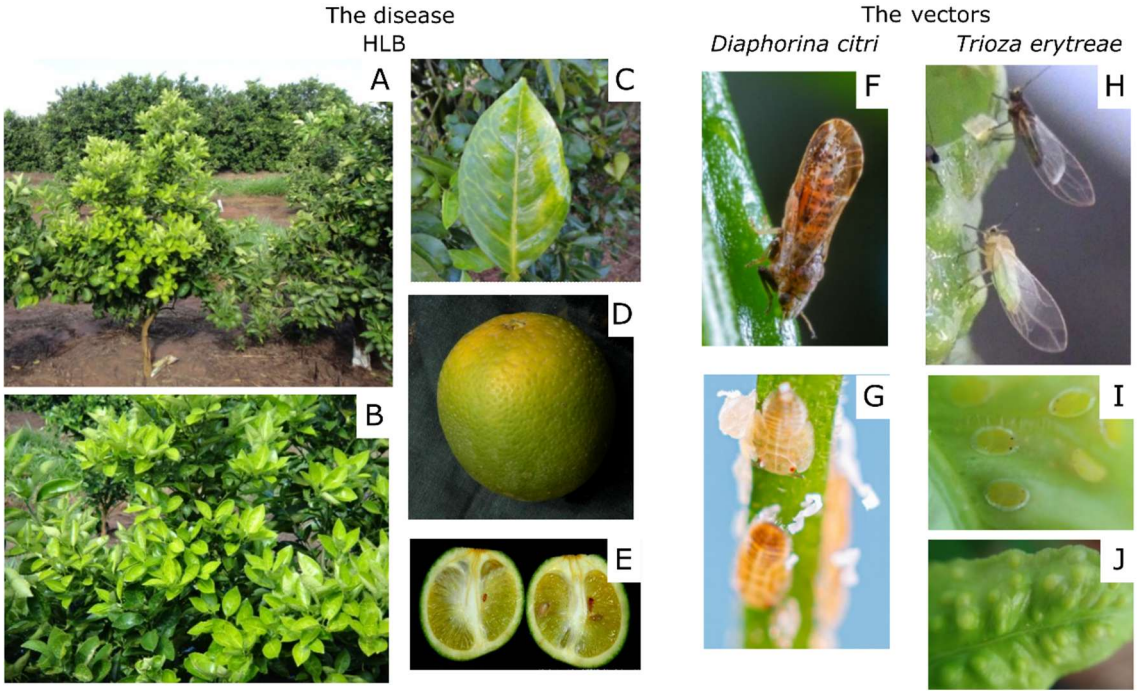

Figure 1.3. Typical symptoms of Huanglongbing (HLB), and HLB vectors. A- Citrus tree with yellow shoots; B- Detail of detail yellow shoots; C- Leaf with non-symmetrical mottled chlorosis; D- Fruit exhibiting colour inversion; E- Deformed fruits with aborted brown seeds; F- *Diaphorina citri* adult; G- *D. citri* nymphs; H- *Trioza erytreae* adults, immature adult with green colouration and mature adult with dark colouration ; I- *T. erytreae* nymphs, ; J- Upper side of a leaf with pit gall formations caused by *T. erytreae*. Photo credits: A, B and C, Amílcar Duarte; D and E, Joseph M. Bové; F and G, Fundecitrus; H, José A. Pereira; I and J, Tomás Magalhães

The bacteria responsible for HLB are known to cause the plugging of the phloem, thereby restricting the flow of nutrients and consequently impacting plant growth and productivity (Achor et al., 2010; Bové, 2006). This disease has a significant economic impact, as evidenced by the 74% decrease in citrus production in Florida (Singerman and Rogers, 2020). The disease has also led to costly changes in orchard and nursery management in Brazil (Ayres et al., 2015; Bassanezi et al., 2020). The causal agents of HLB are *Candidatus* Liberibacter africanus (CLaf), *Candidatus* Liberibacter americanus (CLam) and *Candidatus* Liberibacter asiaticus (CLas) (Bové, 2006). The distribution of CLas encompasses Asia, America and Africa, while CLaf is distributed in Africa and the Middle East, and CLam is present solely in Brazil (EPPO, 2025) (Fig. 1.4). The psyllids *T. erytreae* (African citrus psyllid) and *D. citri* (Asian citrus psyllid) have been identified as the known vectors of this bacteria (Aubert, 1987; Bové, 2006). The distribution of *D. citri*

overlaps with the distribution of CLas and includes additional countries in Africa and the Middle East adjacent to CLas distribution (Fig. 1.4). The distribution of *T. erytreae* overlaps with the distribution of CLaf, and includes additional adjacent countries in Africa, as well as Portugal and Spain (EPPO, 2025) (Fig. 1.4). Despite both psyllids having demonstrated the ability to successfully vector both CLas and CLaf (Lallemand et al., 1986; Reynaud et al., 2022; Roberts et al., 2017), *T. erytreae* is predominantly associated with CLaf and *D. citri* is predominantly associated with CLas and CLam, which aligns with their overlapping distribution (Fig. 1.4). *Trioza erytreae* nymphs develop on the leaves of their hosts, producing pit galls, which are circular or oval-shaped depressions on the underside of the leaf, that are visible on the upper side of the leaf as convex bulges (Annecke and Cilliers, 1963; Van Der Merwe, 1923) (Fig 1.3). *Trioza erytreae* demonstrates preferences for specific hosts, with lemon plants being the preferred host; less preferred are the common hosts such as sweet orange plants, and on the lower end of attractiveness are the occasional hosts such as the trifoliate orange (*Citrus trifoliata* L.) (Aubert, 1987; Hernández-Suárez et al., 2021).

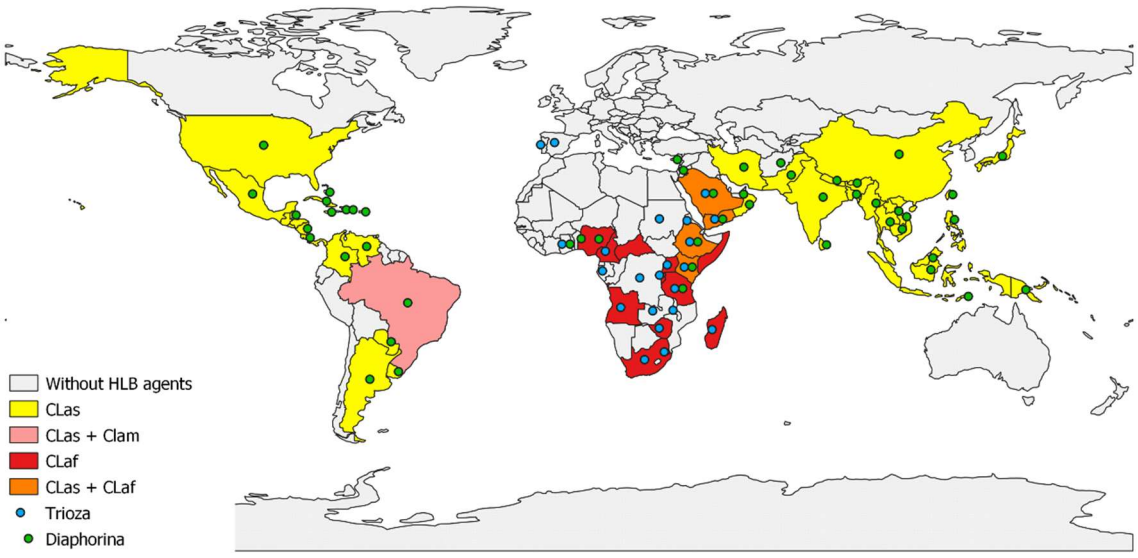

Figure 1.4. Distribution of Huanglongbing (HLB) agents and their vectors on a global scale. "Clas" represents countries with *Canditatus* Liberibacter asiaticus, "Clas + CLam" represents countries with both *Canditatus* Liberibacter asiaticus and *Canditatus* Liberibacter americanus, "Claf" represents countries with *Canditatus* Liberibacter africanus, "Clas + CLaf" represents countries with both *Canditatus* Liberibacter asiaticus and *Canditatus* Liberibacter africanus, "Trioza" represents countries with the presence of *Trioza erytreae,* "Diaphorina" represents countries with the presence of *Diaphorina citri*. Map created using the free and open source QGIS (QGIS Development Team, 2020). World map layer source: Natural Earth, free vector and raster map data (naturalearthdata.com). HLB agents' distribution data source: EPPO (2025).

The recent introduction of the HLB vectors into the Mediterranean basin – *T. erytreae* (Portugal and Spain) and *D. citri* (Israel and Cyprus) (EPPO, 2023, 2022, 2021, 2015) – has raised concern among the citriculture stakeholders in this HLB-free area. It has been demonstrated that the introduction of the vector frequently precedes subsequent outbreaks of HLB, and that the time between the two events has been progressively reduced (Alquézar et al., 2022; Bové, 2006). Moreover, the presence of scattered citrus trees in Mediterranean landscapes has the potential to further complicate the control of this vector (Nunes et al., 2025). In Portugal *T. erytreae* has reached the Algarve region, although not yet spreading to the areas of Algarve with the highest citrus production densities (Duarte et al., 2024) (Fig. 1.5). Nevertheless, it is of critical importance to avoid further spread of *T. erytreae* to protect both Iberian and European citriculture from HLB.

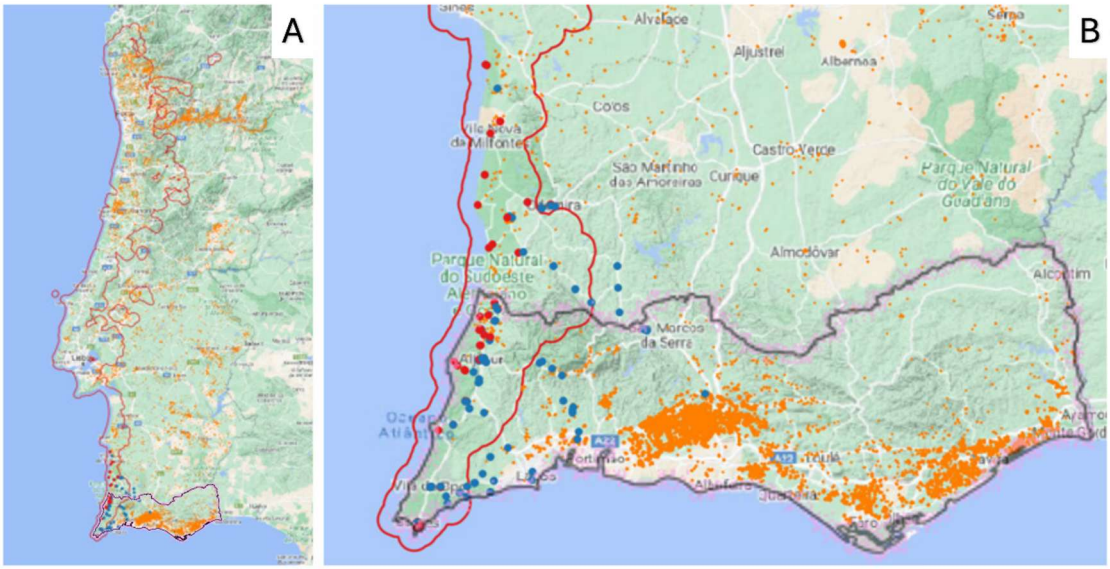

Figure 1.5. Map of the distribution of *Trioza erytreae* and the citrus production areas in 2022. A- Inland Portugal *T. erytreae* distribution and citrus production areas; B- Algarve region (Portugal) *T. erytreae* distribution and citrus production areas. Algarve region demarcated in purple. The orange spots and areas indicate the location of the citrus orchards. The red line delineates the area officially infested with *T. erytreae* with a 3 km wide buffer zone. Red dots correspond to points where symptom or signs of the pest have been identified in 2022 (positive points). Conversely, blue dots denote locations where no symptoms of the pest were observed (negative points). The figure was adapted from Duarte et al. (2024).

1.3.1. The importance of psyllid vector control

The citriculture sector has demonstrated a significant commitment to counteracting the HLB disease, with numerous stakeholders identifying HLB as a priority and a key challenge. Various curative management techniques have been evaluated and are currently under development. These include trunk injection with antibiotics and defence elicitors (Li and Nangong, 2022), spraying with leaf extracts from other plants (Pitino et al., 2020), and integrated practices using thermotherapy and preventive measures (Li et al., 2019). However, to this date, no effective long-term cure has been designed or implemented (Alquézar et al., 2022). The most widely implemented HLB management strategy at present involves the use of certified healthy plant material, the eradication of infected plant material (inoculum) and the control of vector populations (Belasque et al., 2010). In HLB-free areas, the primary preventive measures entail the obstruction of the entry of HLB-infected material, in conjunction with the oversight and control and/ or eradication of the psyllid vectors (Alquézar et al., 2022; Pérez-Hedo et al., 2025). This emphasises the pivotal role of vector control in the effective management of HLB.

The control of HLB psyllid vectors predominantly involves the application of conventional phytosanitary sprays. However, this approach may result in the development of resistance in psyllids to the active compounds employed. This phenomenon has been evidenced in *D. citri* (Chen et al., 2022; García-Méndez et al., 2019). Nevertheless, alternative control methods and cultural practices have demonstrated potential. The biological control with *Tamarixia* spp. parasitoids has proven to be one of the most successful alternatives for psyllid control. This has been especially relevant for *T. erytreae* control by the parasitoid *Tamarixia dryi* Waterston (1922), resulting in the near eradication of the psyllid from Reunion and Mauritius (Aidoo, 2023; Alquézar et al., 2022; Berg and Greenland, 2000). Furthermore, systematic releases of *T. dryi* in Portugal and Spain, combined with the sub-optimal climatic conditions for *T. erytreae*, has led to a considerable decrease in psyllid population to residual levels (EPPO, 2021; Paiva et al., 2020). In Portugal, during the course of two consecutive years of prospection (2022 to 2024), no symptoms were observed and the presence of *T. erytreae* was not detected in the

Algarve region (DGAV, 2024). Additional alternative control measures that have demonstrated promising outcomes encompass the utilisation of predators and entomopathogenic fungi to lower psyllid populations, alongside the application of kaolin to deter *T. erytreae* (Aidoo et al., 2021; Juan-Blasco et al., 2012; Oliveira et al., 2022). However, the implementation of these solutions is constrained by limitations and difficulties associated with their integration with existing management practices in commercial orchards and nurseries. Consequently, research on innovative and alternative methods for the control of *T. erytreae* is of pivotal importance.

1.4. Perspectives of novel control methods and the potential from plant–insect interactions studies

The biological molecules and metabolic pathways that are activated in plant defence mechanisms against insects have been refined and optimised over the course of centuries of evolution. The exploration of these biological molecules and pathways holds immense potential as novel methods to affect and limit insect development. It is imperative to ascertain the most effective manner to induce these molecules and pathways in order to successfully affect the insect. Subsequent to this, larger-scale studies are required to assess the viability of the potential innovative control techniques and enhance its efficacy.

A notable illustration of this process was the identification of a protein in *Bacillus thuringiensis* Berliner that exhibited insecticidal properties against lepidopteran insects (Estruch et al., 1996). This knowledge was subsequently employed in the production of transgenic maize (*Zea mays* L.) varieties to confer resistance to lepidopteran pests, which has been patented and is now commercially available (Nykoll et al., 2012). *Bacillus thuringiensis* strains have also been tested for their effect on *D. citri*, along with other non-conventional insecticides, such as the petroleum-based horticultural mineral oils (HMO) (Dorta et al., 2020; Tansey et al., 2015). These non-conventional insecticides were applied to *C. ×sinensis*, with *B. thuringiensis*, through drenching and HMO through foliar spray. The findings from these studies indicated that HMO treatments resulted in a

reduction of *D. citri* populations (Tansey et al., 2015), whereas Bt treatments caused an increase in *D. citri* mortality rates (Dorta et al., 2020). These findings illustrate potential novel control methods for the psyllid vectors of HLB. However, further research and optimisation are required to ascertain their viability in field application and its effect on *T. erytreae*.

Alternative methods to genome editing, such as the exploitation of plant semiochemicals, peptides and secondary metabolites have also shown great potential for the development of novel control methods. Some of these molecules have shown to elicit plant defence responses, repel or attract the insect, and even directly hinder the insect's development (Abd El-Ghany, 2019; Tlak Gajger and Dar, 2021; Zhang et al., 2023). In order to identify these potential targets within the plant–insect interaction, it is necessary to undertake an exploratory analysis and broad scan of the biochemical and molecular interactions between the plant and the insect. Of particular interest is understanding how the insect affects the plant's gene expression, transcription factors, proteins, metabolism and volatiles and vice-versa (Dyer et al., 2018). The employment of high-throughput omics technologies has been demonstrated to facilitate the identification of multiple biological molecules, thereby underscoring their status as a potent instrument for the exploration of the biochemical and molecular interplay inherent to plant–insect interactions (Barah and Bones, 2015). This methodological approach has resulted in the identification of several potential novel control methods for different pests. For example, through transcriptomics and volatolomics a transcript involved in terpenoid biosynthesis, namely 5-epi-aristolochene synthase (EAS), was found to be induced in *Nicotiana tabacum* L. by whitefly (*Bemisia tabaci* Gennadius). Consequently, the effect of exogenous application of terpenoids on *N. tabacum* was investigated, resulting in a significant hindrance to whitefly infestation (Luan et al., 2013). Proteomic studies have shown that plants produce lectins in response to insect infestation and that lectins have an insecticidal effect, suggesting that these proteins should be further investigated as natural insecticides (Paul and Das, 2021; Roy et al., 2014; Zeng et al., 2017).

Several omics studies have identified potential candidates for novel control methods for HLB, and its associated agents. For instance, the influence of host plant volatiles on the attraction of HLB psyllid vectors has been demonstrated by volatolomics (Antwi-Agyakwa et al., 2019; Coutinho-Abreu et al., 2014; Zanardi et al., 2019). These volatiles have been incorporated into field traps for the psyllids and tested in field conditions, yielding encouraging results for *D. citri,* and less encouraging results for *T. erytreae* (Coutinho-Abreu et al., 2014; Khadka et al., 2020; Pullock et al., 2024). Two metabolomic analyses showed that *D. citri* infestation influenced phytohormone homeostasis in *C.* ×*sinensis* (Ibanez et al., 2019; Nehela et al., 2018). Exogenous applications of two phytohormones, namely salicylic acid (SA) and jasmonic acid (JA), affected *D. citri* feeding behaviour, and JA treatments also showed to be detrimental for the psyllid reproduction (Gao et al., 2023). Gene expression analysis identified genes related to the pathogen-associated molecular patterns-triggered immunity (PTI) as an important component of citrus resistance to canker (Shi et al., 2015). The induction of PTI has been shown to function as an antifeedant for *D. citri* (Shi et al., 2019). A transcriptome study on *C.* ×*sinensis* response to *D. citri* infestation highlighted the importance of kinases, phytohormones, defence related transcription factors (WRKY), phenylpropanoid and flavonoid biosynthesis, indicating a need for further studies to ascertain their effect on the psyllid vector (Sun et al., 2022).

A transcriptomics approach has also been applied for the HLB disease itself, by screening HLB-tolerant and HLB-sensitive plant hosts (Huang et al., 2021b). The authors identified a transcript of the peptide homologous to a 109-aa Arabidopsis heat-stable protein HS1 with antimicrobial and antifungal activity, as a potential target for a novel control method. This peptide was then applied via trunk injection in HLB-infected plants and via foliar spray in healthy plants that were subsequently infected. These treatments showed efficacy in treating HLB-positive trees and in inhibiting the emergence of new HLB infections in healthy trees. Field trials are currently being undertaken (Huang et al., 2021a).

A non-omics study was conducted to evaluate the efficacy of seven compounds as alternatives to conventional phytosanitary sprays to control *T.*

erytreae. One of these compounds was the oil extracted from the peel of *C.* ×*sinensis* fruit (orange oil). The compounds were applied by foliar spray onto *C.* ×*sinensis*. The most effective compounds in reducing the initial infestation rates were diatomaceous earth and kaolin. Notably, orange oil treatment caused the highest nymph mortality in a field experiment (Hernández-Suárez et al., 2023), underscoring the potential efficacy of *T. erytreae* control methods that use compounds found in host plants.

1.5. Objectives and Thesis structure

It is imperative to investigate new strategies to control or prevent the spread of the psyllid vector *T. erytreae* that transmits the bacterial disease HLB. The study of the plant host–vector interactions holds significant potential for identifying crucial mechanisms to affect the psyllid vector. *Trioza erytreae* has been rapidly spreading towards the densest productive citrus area in Portugal and poses an impending threat for the European citriculture. It is imperative to understand how *T. erytreae* interacts with the citrus plant, and how the citrus host plant responds to *T. erytreae*. The present study employed two important citrus species with different rates of suitability as hosts of *T. erytreae*, namely *C.* ×*limon* plants with the highest suitability and the less suitable *C.* ×*sinensis* plants.

The primary objective of this study was to identify proteins and metabolites involved in the interactions between the two citrus hosts and *T. erytreae* from a systems biology perspective. The proteome and metabolome of the enriched vascular sap of the two hosts were analysed in response to *T. erytreae* infestation. In addition, the proteome of *T. erytreae* developing on these two hosts was analysed. Furthermore, the impact of the exogenous application of jasmonic acid (JA) on the volatile profile of *C.* ×*limon* and on the infestation by *T. erytreae* was characterised.

This PhD thesis was organised in chapters 1 to 7, described below. Chapters 3, 4 and 5 describe analyses that stemmed from a common experimental trial that led to the design of the experimental trial performed for Chapter 6.

- **Chapter 1** the current chapter, which comprises an overview of citriculture, HLB, *Trioza erytreae*, plant–insect interaction and the importance of innovative pest management strategies for this vector.
- **Chapter 2** which comprises a systematic review of *T. erytreae* and the current knowledge of its interaction with its hosts.
- **Chapter 3** characterises the proteome response of *C.* ×*sinensis* and *C.* ×*limon* hosts to *T. erytreae* infestation.
- **Chapter 4** characterises the interplay of metabolome and proteome responses of *C.* ×*sinensis* and *C.* ×*limon* hosts to *T. erytreae* infestation.
- **Chapter 5** characterises the proteome differences between *T. erytreae* nymphs when developing on *C.* ×*sinensis* and *C.* ×*limon* hosts.
- **Chapter 6** characterises the effect of the exogenous application of jasmonic acid on *C.* ×*limon* volatile profile, and on *T. erytreae* infestation on this host.
- **Chapter 7** Compiles and discusses the findings of the studies on the interactions of *C.* ×*sinensis* and *C.* ×*limon* hosts with *T. erytreae,* including the potential of the identified molecules on the control of the psyllid.

Chapter 2. *Trioza erytreae* (Del Guercio, 1918) and the interaction with its hosts: a review

This chapter has been adapted from the review article published in:

Magalhães, T., Duarte, A., Pereira, J. A., & Marques, N. T. (2025). *Trioza erytreae* (Del Guercio, 1918) and the interaction with its hosts: a review. *Agriculture*, 15(1), 101. **https://doi.org/10.3390/agriculture15010101**

2.1. Abstract

The cultivation of citrus in the Mediterranean region is of considerable economic importance. The viability of this industry is contingent upon a number of factors, with adequate phytosanitary management being of particular significance. During the last decade, the geographical range of the invasive psyllid, *Trioza erytreae* (Del Guercio, 1918), has expanded to the mainland territories of Portugal and Spain. *Trioza erytreae* acts as a vector for the Huanglongbing disease (HLB). This review presents the current knowledge about the hosts of the psyllid and their attractiveness and suitability. A classification of the hosts according to their suitability, as assessed in the literature, is provided. The attributes of the hosts and the methods used to assess their suitability are described, as well as the climatic factors that affect the psyllid–host interaction. The review emphasises the importance of a comprehensive evaluation of the interactions between the psyllids and their hosts to develop and implement more effective strategies for controlling *T. erytreae*.

2.2. Abbreviations

HLB (huanglongbing); RH (relative humidity)

2.3. Introduction

The citrus industry is the most important fruit sector in the world, with an annual production exceeding 166 million tonnes (FAO, 2021). The industry is currently being confronted with an incredibly devastating bacterial disease that is rapidly disseminating globally: the Huanglongbing (HLB) disease. HLB is caused by *Candidatus* Liberibacter spp. bacteria, which clog the phloem and limit the flow of nutrients in the tree, thereby affecting its development, fruit production and quality. The two functional vectors that transmit *C*. Liberibacter are the psyllids *Diaphorina citri* (Kuwayama, 1908) (Hemiptera: Liviidae), mainly present in the Asian and American continents, and *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae), mainly present in the African continent. The two vectors have recently reached the European continent, posing a threat to Mediterranean citriculture. *Diaphorina citri* is spreading from the East, having been detected in Israel and Cyprus (EPPO, 2023, 2022) while *T. erytreae* is spreading from the West with observations in the Iberian Peninsula dating back to 2014 in the north-western region near the coastline (Duarte et al., 2024; Pérez-Otero et al., 2024).

The Mediterranean basin is one of the few citrus-producing regions that has not been affected by HLB. To date, no positive results have been obtained from the HLB tests conducted in Europe on both vectors (Alquézar et al., 2022; EPPO, 2023, 2021). The potential spread of these harmful insects in the Mediterranean region represents a substantial concern for citrus growers as their presence could facilitate the rapid dissemination of the HLB disease. The spread of *D. citri* carrying *C*. Liberibacter has been rapid in the citrus-producing regions on both American continents over the 15-year period since HLB was first identified. This has resulted in a 74% reduction in citrus production and a 62% decline in the number of citrus producers in Florida (Singerman and Rogers, 2020), which serves to demonstrate the destructive capacity of this disease. The management of HLB requires the elimination of infected plants (Ayres et al., 2015) and a significant alteration in cultural practices (Bassanezi et al., 2020). Several control strategies for this disease have been tested, such as the injection of antibiotics into the stem, thermotherapy and application of endophytes. However, no treatment has been shown to be

effective in controlling HLB (Li and Nangong, 2022; Limayem et al., 2024; Munir et al., 2021).

The control of psyllid vectors represents a primary concern for the citrus industry. The main management strategies were comprehensively outlined by Aidoo (2023). These strategies encompass the use of chemical control, applying insecticides during peak flushing periods (Urbaneja-Bernat et al., 2020), and biological control, with the release of the parasitoids *Tamarixia dryi* (Waterston, 1922) (Benhadi-Marín et al., 2022; Urbaneja-Bernat et al., 2019), and *T. radiata* (Aidoo, 2023). The release of *Tamarixia dryi* has been undertaken in France, Spain, and Portugal, encompassing all territories that have been infested, and contingent on the relevant government authorities (Aubert, 1987; Aubert, B. Quilici, 1984; Duarte et al., 2024; EPPO, 2021, 2015; Etienne and Aubert, 1980; Reynaud et al., 2022). Furthermore, novel strategies based on entomopathogens (Aidoo et al., 2021), and kaolin applications (Oliveira et al., 2022) are currently under investigation.

The presence of *T. erytreae* in the Iberian Peninsula represents a significant challenge to the protection of Europe's main citrus producers. A multitude of factors, including climatic conditions (Van den Berg et al., 1991a), the presence of natural enemies (Catling, 1970), chemical treatments, and host plants, exert a substantial influence on the survival and development of *T. erytreae*. The insect exhibits a high degree of dependency on its hosts, as they play a pivotal role in its establishment, development, and dissemination. Moreover, the adult psyllid has a limited lifespan (85 h) when deprived of its hosts (Gottwald, 2010; Van den Berg and Deacon, 1988; Van den Berg et al., 1990). In the absence of the bacteria, the psyllid's direct damage to citrus hosts is considered negligible (Carvalho and Aguiar, 1997; Van Der Merwe, 1923). Nevertheless, nurserymen have reported it to be of significant consequence, as the pit gall symptoms that form in citrus leaves during nymph development impact the plants' eligibility for commercialisation (Carvalho and Aguiar, 1997; Van Der Merwe, 1923). A deeper understanding of the *T. erytreae* host range may facilitate the design of more efficacious control strategies, and the advancement of psyllid epidemiological studies, given the pivotal role of the

interactions between the vector, the plant, and the pathogen. This review provides an update on the current knowledge regarding the interaction between *T. erytreae* and its hosts, with a particular focus on the factors influencing its establishment, oviposition, and development.

2.4. *Trioza erytreae* hosts

Trioza erytreae feeds and develops mainly on Rutaceous plants (Carvalho and Aguiar, 1997; Van Der Merwe, 1923). *Trioza erytreae* lays its eggs on the tips of young shoots. The nymphs hatch and settle on the underside of the developing leaves, where they complete their five-instar development before a new flying adult emerges. During its development, the nymphs form “pit galls” which are circular or oval-shaped depressions on the underside of the leaf. The pit galls are perfectly fitted to the nymph and, on the upper side of the leaf they are visible as convex bulges (Annecke and Cilliers, 1963; Van Der Merwe, 1923).

The Aurantioideae subfamily, which is part of the Rutaceae family, comprises 33 genera. The *Citrus* genus, which is part of this subfamily, comprises the preferred *T. erytreae* hosts (Annecke and Cilliers, 1963). Three species from the Rutaceae, namely the two Aurantioideae, *Clausena anisata* (Willd.) Hook.fil., De Wild. & Staner and *Citrus* ×*limon* (L.) Burm, along with *Vepris lanceolata* (Lam.) G.Don (non Aurantioideae), have been historically linked with *T. erytreae*, as they were the first psyllid hosts to be documented in the literature (Aubert, 1987; Bové, 2014; Carvalho and Aguiar, 1997; Moran, 1968a, 1968b; Van Der Merwe, 1923). The first host to be recorded was *Citrus* ×*limon*, which was formally described in 1918 in Eritrea (Del Guercio, 1918). Almost all species and varieties within the *Citrus* genus serve as hosts, including those used as rootstocks (Hernández-Suárez et al., 2021). The term ‘suitability’ is used to refer to the host’s ability to support all stages of psyllid development until the emergence of a new generation. The suitability of some citrus species remains inconclusive, as is the case of *C. australasica* F. Muell (Aubert, 1987), or even contradictory, as observed in *C. trifoliata* L. (Aubert, 1987; Hernández-Suárez et al., 2021) and *C. japonica* Thunb. (Aubert, 1987; Van den Berg et al., 1991a).

Plants from other genera of the subfamily Aurantioideae outside the *Citrus* genus were described as suitable hosts. These included *Murraya paniculata* (L.) Jacq. and *Glycosmis pentaphylla* (Retz.) Corrêa, which are commonly used as ornamentals (Barkley and Beattie, 2008). There is a lack of consensus regarding the suitability of certain Aurantioideae hosts for the growth of the psyllid. For instance, *C. anisata* has been reported as a suitable host in some studies (Aidoo et al., 2019a; Aidoo et al., 2019c; Aubert, 1987; Van Der Merwe, 1923), while in others it has been identified as unsuitable due to the absence of oviposition (Moran, 1968a). Similarly, *Calodendrum capense* (L.fil.) Thunb., which has been described as a suitable host (Aidoo et al., 2019a), has been deemed unsuitable in other studies (Aubert, 1987; Moran, 1968b; Van Der Merwe, 1923). Suitable Rutaceae hosts identified outside the Aurantioideae subfamily, were *V. lanceolata* and *Zanthoxylum capense* (Thunb.) Harv (Aubert, 1987; Moran, 1968b; Van Der Merwe, 1923).

Possible host species outside the Rutaceae family are *Ficus* spp. L. (Kalyebi et al., 2016), including *Ficus sycomorus* L. (Moraceae family) (Abate, 1988), *Pygeum africanum* Hook.fil. (Rosaceae family) (Abate, 1988), *Stephania abyssinica* (Dill. & A.Rich.) Walp. (Menispermaceae family) (Abate, 1988; Kalyebi et al., 2016), and *Diospyros mespiliformis* Hochst. ex A.DC. (Ebenaceae family) (Kalyebi et al., 2016). *Trioza erytreae* has only been documented to feed on these hosts and to be the likely cause of leaf pit gall symptoms. However, there is no evidence to suggest that nymphal development or the emergence of a new generation of psyllids has occurred (Abate, 1988; Kalyebi et al., 2016). Therefore, in light of the current knowledge, these species can only be considered as non-reproductive hosts or as feeding hosts (Table 2.1). Carrot plants [*Daucus carota* subsp. sativus (Hoffm.) Schübl. & Martens] were also evaluated as possible hosts for the transmission of '*Candidatus* Liberibacter solanacearum' to sour orange plants *C. aurantium* L.. However, despite oviposition, *T. erytreae* was unable to complete its life cycle (Quintana-González De Chaves et al., 2020).

As outlined in the preceding paragraphs, the available data supports the classification of *T. erytreae* as oligophagous. The collected data indicate that hosts outside the Rutaceae family are non-viable hosts. Some of these non-viable hosts

may have the potential to be used in psyllid control measures. For instance, researchers have suggested using *Nicotiana tabacum*, a non-viable host, near citrus orchards as a control measure for *D. citri* (Zheng et al., 2023). Table 2.1 provides a concise overview of the *T. erytreae* host suitability data that have been tested to date. The hosts were classified according to their attractiveness to the psyllid and their suitability as hosts, based on an evaluation of the results described in the literature. With regard to the *Citrus* genus, which comprises more than 30 species, only 17 have been evaluated as potential hosts for *T. erytreae*. Ten of the species have been classified as having high to highest suitability for *T. erytreae*. The highest suitability was identified for lemon (*C.* ×*limon*), citron (*C. medica*) and lime (*C.* ×*aurantiifolia*) (Table 2.1). The two most widely cultivated citrus trees (FAO, 2021), sweet orange (*C.* ×sinensis) and mandarin (*C. reticulata*), were classified as having good host suitability (Table 2.1). Additionally, *Clausena anisata* and *M. koenigii* L. Spreng., two additional species within the Aurantioideae subfamily, were classified as having high and highest suitability, respectively (Table 2.1).

It appears that species belonging to the Rutaceae family that are not part of the Aurantioideae subfamily are less suitable for *T. erytreae*. Only two members of the *Vepris* genus exhibited a suitability classification above the medium level (Table 2.1).

In view of the paucity of studies on hosts outside the Rutaceae family, it is not yet possible to conclude that they are unsuitable. However, current evidence suggests that the hosts examined so far are unsuitable (Table 2.1).

While the majority of citrus hosts exhibited comparable levels of attractiveness and suitability for oviposition (Table 2.1), two hosts, namely citrange (*C. trifoliata* × *C.* ×*sinensis*) (Hernández-Suárez et al., 2021) and *Vepris bilocularis* (Wight & Arn.) Engl. (Aidoo et al., 2019a), exhibited a low level of attraction and a high oviposition rate. This suggests that these hosts possess a quality that allows for optimal oviposition without attracting *T. erytreae* (Table 2.1).

Table 2.1. Summary of *T. erytreae* hosts, including the host name and common name, as well as host suitability classification in the following categories: "Attraction", "Survival", "Oviposition", "Nymphal Development", "Adult Emergence", and "Host Classification". Where "+ + + + +" represents the highest suitability, "+ + + +" high suitability, "+ + +" good suitability, "+ +" medium suitability, "+" low suitability, "−" almost no suitability, "− −" no suitability. The "Host Category" indicates host support for the *T. erytreae* life cycle; "R" represents the "reproductive hosts", where the full life cycle is supported by the host; "NR" represents the "non-reproductive hosts", where oviposition is observed but the full life cycle cannot be completed; "F" represents the solely "feeding hosts", where oviposition was tested but not supported. The "NR/R" combination represents hosts where oviposition was supported, but the following stages were not analysed (reproductivity was not ascertained); the "F/NR" combination represents hosts where feeding was observed, and oviposition was not analysed (oviposition support was not ascertained). The designation "NT" indicates that the host comparison studies have not been conducted, while "NC" indicates that they are not classifiable.

Host; Common Name	Attraction	Survival	Oviposition	Nymphal Development	Adult Emergence	Host Classification	Host Category	References
Family: Rutaceae; Subfamily: Aurantioideae; Genus: *Citrus*								
Citrus ×*limon* (L.) Burm. f.; **Lemon**	+++++	+++++	+++++	+++++	+++++	+++++	R	[1-19]
Citrus ×*latifolia* Yu. Tanaka; **Tahiti lime**	+++++	NT	+++++	NT	NT	+++++*	R	[9,10]
Citrus medica L.; **Citron**	+++++	+++++	+++++	+++++	NT	+++++	R	[3]
Citrus ×*aurantiifolia* (Christm.) Swingle; **Lime**	++++	+++	++++	+++	NT	++++	R	[3,9,10,13,20]
Citrus trifoliata × *Citrus* ×*sinensis*; **Citrange**	+	+++++	+++++	NT	NT	++++**	NR/R	[6]
Citrus macrophylla Wester	+++++	+	+++++	NT	NT	++++**	NR/R	[6]
Citrus ×sinensis (L.) Osbeck; **Sweet orange**	+++	+++	+++	++	+++	+++	R	[2,3,5,6,9–12,19–31]
Citrus reticulata Blanco; **Mandarin**	+++	++	+++	+++	NT	+++	R	[3,6,9–11,13,19,20,24]
Citrus reticulata × *Citrus* ×*sinensis*; **Tangor**	+++	+++	+++	+++	+	+++	R	[3,9,10,19,20,27]
Citrus reticulata × *Citrus* ×*paradisi*; **Tangelo**	+++	NT	+++	NT	NT	+++*	R	[9,10,12,28]
Citrus ×*paradisi* Macfadyen; **Grapefruit**	++	+++	++	+++	NT	++	R	[3,10,19,20,27]
Citrus maxima (Burm.) Merril; **Pomelo**	+	+++	+	+++	NT	++	R	[3,9]
Citrus reshni (Engl) Yu. Tanaka; **Cleopatra mandarin**	+	+++	+	NT	NT	+**	NR/R	[6]
Citrus japonica Thunb.; **Kumquat**	++	+	+	−−	NT	+	NR	[3,20]

Host; Common Name	Attraction	Survival	Oviposition	Nymphal Development	Adult Emergence	Host Classification	Host Category	References
Citrus trifoliata × *Citrus* reticulata; **Citrandarin**	+	+	++	NT	NT	+**	NR/R	[6]
Citrus trifoliata L.	+	+	−−	−−	NT	−	F	[3,6,20]
Citrus australasica F.Muell.; **Caviar lime**	+	+	−−	−−	NT	−	F	[3]
Citrus ×*aurantium* L.; **Sour orange**	+	++***	++***	++***	++***	++***	R	[11,14,20,24,32]
Citrus ×*jambhiri* Lush.; **Rough lemon**	+++	NT	NT	NT	NT	NC	R	[24,33,34]
Citrus ×*paradisi* x *Citrus trifoliata* ;**Citrumelo**	NT	NT	NT	NT	+++++	NC	R	[12]
Family: Rutaceae; Subfamily: Aurantioideae; Genus: Other than *Citrus*								
Murraya koenigii (L.) Spreng.	+++++	NT	+++++	+++++	++++	+++++	R	[12,35]
Clausena anisata (Willd.) Hook.fil., De Wild. & Staner	+++	++++	++++	+++++	++	++++	R	[3,7,8,12,21–23,29,35–38]
Murraya paniculata (L.) Jacq.	+	+++	+	+	NT	+	R	[3]
Glycosmis pentaphylla (Retz.) Corrêa	NT	NT	NT	NT	NT	NC	F/NR	[36]
Triphasia trifolia (Burm.fil.) P.Wilson	NT	NT	NT	NT	NT	NC	F/NR	[36]
Family: Rutaceae; Subfamily: Other than Aurantioideae								
Vepris lanceolata (Lam.) G.Don; **White ironwood**	+++++	+++++	+++++	+++++	+++	+++++	R	[3,7,8,23,36,38]
Vepris nobilis (Delile) Mziray	+++	NT	+++	+++++	+++	+++	R	[35]
Zanthoxylum capense (Thunb.) Harv.	+	+++	++	++	+	++	R	[3,7,8,36,38,39]
Vepris bilocularis (Wight & Arn.) Engl.	+	NT	+++++	+	+++	++	R	[35]
Calodendrum capense (L.fil.) Thunb.;**Cape chestnut**	++	+	+	−	−	−	R	[3,7,8,35]
Zanthoxylum asiaticum (L.) Appelhans,Groppo & J.Wen	+	+	+	−−	NT	−	NR	[3]
Vepris Comm. ex A.Juss.	NT	NT	NT	NT	NT	NC	R	[3,40]
Ruta graveolens L.; **English rue**	NT	NT	NT	NT	NT	NC	NR	[36]

Host; Common Name	Attraction	Survival	Oviposition	Nymphal Development	Adult Emergence	Host Classification	Host Category	References
Agathosma ciliaris (L.) Druce	NT	NT	NT	NT	NT	NC	NR	[36]
Choisya ternata Kunth; **Mexican orange**	NT	NT	NT	NT	NT	NC	F/NR	[41]
Family: Other than Rutaceae								
Morus alba L.; **Mulberry**	+	NT	NT	NT	NT	NC	F/NR	[4]
Tropaeolum majus L.; **Garden nasturtium**	+	NT	NT	NT	NT	NC	F/NR	[4]
Daucus carota subsp. *sativus* (Hoffm.) Schübl. & Martens; **Carrot**	+	+	+	−−	NT	−/****	NR	[32]
Stephania abyssinica (Dill. & A.Rich.) Walp.	NT	NT	−−	NT	NT	NC	F	[24,35,37]
Ficus sycomorus L.	NT	NT	−−	NT	NT	NC	F/NR	[35,37]
Ficus thonningii Blume	NT	NT	−−	NT	NT	NC	F	[35]
Diospyros mespiliformis Hochst. ex A.DC.	NT	NT	NT	NT	NT	NC	F/NR	[24]
Pygeum africanum Hook.fil.	NT	NT	NT	NT	NT	NC	F/NR	[37]

NC denotes that the classification is not applicable; * classification based only on two tested categories; ** comparison study limited to rootstocks; *** the only comparison made regarding the oviposition was for sour orange and a non-Rutaceae host (a more comprehensive analysis involving additional hosts would be necessary to classify this host) (Quintana-González De Chaves et al., 2020); **** In the case of carrot, classification has been applied despite the species not being a viable host. However, it is important to note that this is based on a single study (Quintana-González De Chaves et al., 2020). References in this table are as follows: 1 - (Ameline et al., 2023); 2 - (Magalhães et al., 2024); 3 - (Aubert, 1987); 4 - (Moran and Brown, 1973); 5 - (Carvalho and Aguiar, 1997); 6 - (Hernández-Suárez et al., 2021); 7 - (Moran, 1968a); 8 - (Moran, 1968b); 9 - (Tamesse, 2000); 10 - (Tamesse and Messi, 2004); 11 - (Cook et al., 2014); 12 - (Aidoo et al., 2019c); 13 - (Tamesse and Messi, 2002); 14 - (Benhadi-Marín et al., 2021); 15 - (Moran and Buchan, 1975); 16 - (Moran and Blowers, 1967); 17 - (Aidoo et al., 2022); 18 - (Urbaneja-Bernat et al., 2020); 19 - (Quintana-González de Chaves et al., 2024); 20 - (Van den Berg et al., 1991a); 21 - (Van den Berg et al., 1990); 22 - (Van den Berg et al., 1991d); 23 - (Van den Berg and Deacon, 1988); 24 - (Kalyebi et al., 2016); 25 - (Catling, 1969); 26 - (Catling, 1971); 27 - (Samways and Manicom, 1983); 28 - (Catling and Atkinson, 1974); 29 - (Van den Berg, 1992); 30 - (Catling and Annecke, 1968); 31 - (Catling, 1972); 32 - (Quintana-González De Chaves et al., 2020); 33 - (Antwi-Agyakwa et al., 2021); 34 - (Antwi-Agyakwa et al., 2019); 35 - (Aidoo et al., 2019a); 36 - (Van Der Merwe, 1923); 37 - (Abate, 1988); 38 - (Hollis, 1984); 39 - (Van den Berg et al., 1991c); 40 - (Van den Berg, 1990); 41 - (Cocuzza et al., 2017).

2.5. Host characteristics and their influence on *Trioza erytreae* development

The suitability of a host for *T. erytreae* depends on intrinsic and extrinsic factors. The following aspects related to young flushes are particularly important in determining host suitability: the intensity of flushing, the timing of flushing, leaf length, shoot length, and tissue softness/succulence. Additionally, the nutritional status, age and phytosanitary condition of the host plant are of considerable importance. The significance of these host traits resides in the requirement of young leaves and shoots for successful oviposition and nymph development (Catling, 1972, 1969; Van den Berg, 1990, 1986; Van den Berg et al., 1991d, 1990) (Fig. 2.1).

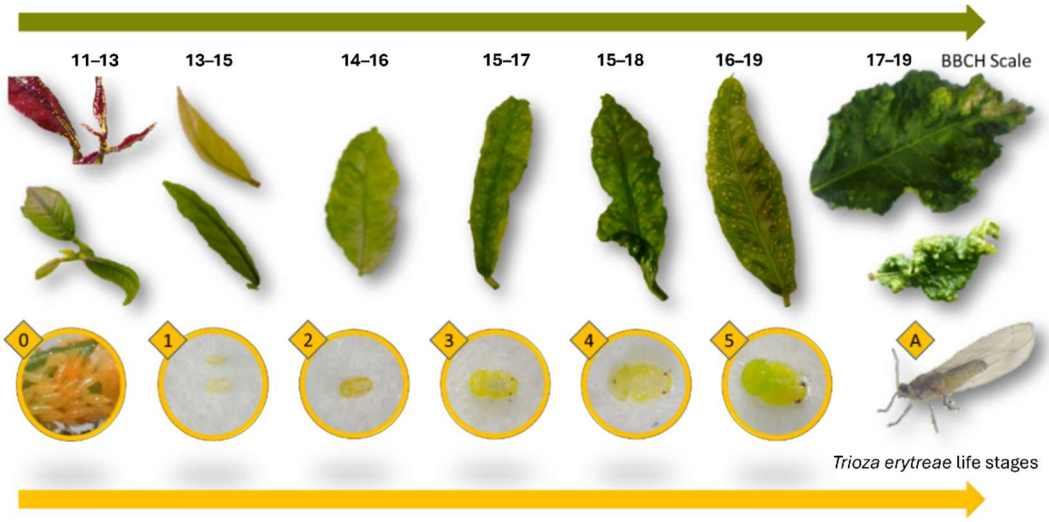

Figure 2.1. The various stages of leaf development and the symptoms of infestation by *Trioza erytreae* along with the developmental stages of the psyllid. The upper half of the figure depicts the leaf appearance and phenological stages of *Citrus ×limon* according to the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) scale (Agustí et al., 1995), when infested with the *T. erytreae* stages. The lower portion of the figure depicts the developmental stages of *Trioza erytreae*: stage 0 - eggs; stage 1 - first instar nymph; stage 2 - second instar nymph; stage 3 - third instar nymph; stage 4 - fourth instar nymph; stage 5- fifth instar nymph, and stage A- adult.

The phytosanitary status of the host plant exerts an influence on the flushing rhythm and the nutritional status of the shoots and leaves, which in turn affects the growth and development of psyllids. For example, a decrease in leaf nitrogen levels substantially hampered psyllid development (Catling, 1971). Additionally, in a *C. ×sinensis* orchard, the presence of chlorotic young shoots caused a high mortality rate of *T. erytreae*. Furthermore, the surviving nymphs were observed to exhibit reduced size and flattened morphology, along with an extended period of

developmental stages (Catling, 1971), contrary to a more rapid spread of *T. erytreae* observed in young, healthy and vigorous trees (Catling and Atkinson, 1974).

Trioza erytreae shows a tendency to transit between suitable hosts in the vicinity of citrus orchards, contingent upon the availability of fresh flush growth. The prevalence of psyllids is higher when citrus orchards undergo alternate flushes with out-of-season flushes in hosts situated outside the orchards (Catling, 1969; Van den Berg, 1992; Van den Berg et al., 1991c). Therefore, the development of *T. erytreae* is facilitated when a host plant produces young flushes throughout the year (Van den Berg, 1990). *Citrus ×limon* is a highly attractive host for psyllids due to the continuous formation of young flushes throughout the year, which provides consistent opportunities for settlement and growth (Catling, 1969; Matias et al., 2023).

The nymphs of *T. erytreae* nymphs can move over a distance of 300 mm in search of optimal feeding spots, which are characterised by the presence of young flushes and soft tissues (Van den Berg et al., 1991d). The greater the hardness of the tissue, the longer the nymphs will spend searching, thereby increasing the probability that the nymphs will become dehydrated, preyed upon, or parasitised (Van den Berg et al., 1991d). In comparison to nymphs developing on either side of leaves (softer tissues), fewer nymphs complete their development on branches (harder tissues), which act as a deterrent to oviposition (Moran and Buchan, 1975; Van den Berg et al., 1991d). It was observed that longer shoots, which are characteristic of lemon and satsuma mandarin trees (Matias et al., 2023), tend to attract a higher number of psyllids (Samways and Manicom, 1983). As the leaf matures and grows, a reduction in the hatching rate of the eggs and the survival of the nymphs is observed. Therefore, mature, longer and larger leaves are detrimental to *T. erytreae* development (Catling, 1971; Samways and Manicom, 1983). This may be attributed to the hardness of the leaves, which impairs the eggs' ability to absorb water, a vital requirement for their survival (Catling, 1971; White, 1968).

Trioza erytreae is able to extend its longevity and the pre-oviposition period in the absence of young flushes. However, this phenomenon has only been observed to occur for a limited duration of time (Catling, 1969). In citrus orchards in

South Africa, a high flushing intensity rendered the plant highly attractive to *T. erytreae*, while a low number of young flushes resulted in a high mortality rate for the psyllid. This indicates that the number of flushes is an important factor in the psyllid's attraction to the host plant (Catling, 1972; Catling and Atkinson, 1974).

The timing of flushing is of pivotal relevance, as when it coincides with optimal climatic conditions for *T. erytreae* development, significant population peaks are observed (Catling, 1969). The efficacy of parasitoids in targeting *T. erytreae* is enhanced when psyllid population levels are high (Mc Daniel and Moran, 1972).

Previous studies have demonstrated that plants infected with HLB flush at different periods in comparison to their healthy counterparts (Cifuentes-Arenas et al., 2022). Having both healthy and HLB-infected plants, exhibiting asynchronous flush periods, provides more favourable conditions for psyllid proliferation (Catling, 1969). This aspect should be considered in the management of HLB-affected orchards.

To ensure its own survival and reduce conspecific competition for resources, *T. erytreae* avoids flushes with high levels of infestation. This behaviour contributes to insect dispersal, leading to the search of new, uncolonised shoots. In their study Van den Berg et al. (1991b) observed a positive correlation between the number of eggs, nymphs, and adults on the host and the dispersion rate.

Pruning is a cultural practice that regulates the growth of new shoots (Matias et al., 2023). Heading cuts stimulate the development of new shoots (Jacinto et al., 2024) and make citrus trees more attractive to psyllids. Topping is an operation that consists of multiple heading cuts applied to the top of the trees (Matias et al., 2023), thereby inducing the development of numerous new shoots and attracting psyllids (de Carvalho et al., 2024). This operation has already been tested to control *D. citri* in conjunction with the application of insecticides to the pruned trees (de Carvalho et al., 2024). Furthermore, deficit irrigation has been shown to extend the period during which the citrus plant exhibits no flushes. This was evidenced in lemon orchards subjected to deficit irrigation over a 12-week period during the winter,

although the same procedure did not affect sweet orange trees (Van den Berg, 1986). It can be reasonably deduced that the cultivation of sweet oranges and lemons in the same orchard, or in adjacent orchards is not recommended, given that lemon trees exhibit continuous flushing (Matias et al., 2023). During the flushing period of the sweet orange tree, an influx of insects migrating from the lemon trees to the orange trees has been observed, leading to an increase in the psyllid population (Catling, 1969). A comprehensive understanding of citrus flushing cycles and effective management techniques is essential for the implementation of cultural practices that mitigate *T. erytreae* populations.

2.6. The influence of climatic conditions on *Trioza erytreae* and its hosts

The climatic variables exert an influence on the duration of insect development (Annecke and Cilliers, 1963; Van den Berg, 1990; Van Der Merwe, 1923), as well as on the intensity and timing of host flushing (Primo-Millo and Agustí, 2020). The two main studies described in this section were conducted in climatically controlled conditions, and both used *C.* ×*limon* as the *T. erytreae* host (Aidoo et al., 2022; Pérez-Otero et al., 2024). Other studies do not specify the citrus host (Annecke and Cilliers, 1963; Moran and Blowers, 1967; Van Der Merwe, 1923), or are based on field studies on sweet orange (*C.* ×*sinensis*) orchards (Catling, 1972; Catling and Annecke, 1968). It has been established that the duration of the developmental process, from the egg stage to the adult stage, is significantly influenced by temperature (Aidoo et al., 2022; Pérez-Otero et al., 2024). The optimal temperature range for the growth of the *T. erytreae* population is between 18 °C and 24 °C (Aidoo et al., 2022; Pérez-Otero et al., 2024). This aligns with the spring average daily temperature range of 12 °C to 20 °C in subtropical regions, where citrus trees produce a considerable number of short shoots (Primo-Millo and Agustí, 2020).

Temperatures above 27 °C or below 10 °C severely delay or prevent the completion of *T. erytreae* life cycle, and if the temperature is constant at 10 °C, 27 °C, or 30 °C, the life cycle is not completed (Pérez-Otero et al., 2024). It is well documented that citrus hosts enter a state of dormancy when the daily average

temperatures fall below 12 °C (Primo-Millo and Agustí, 2020). Therefore, temperatures below 10 °C will restrict the development of both the host and the psyllid. While temperatures above 30 °C appear to exert a deleterious effect on the psyllid, the same does not occur with regard to host flushing, given that citrus hosts produce long new shoots at daily average temperature ranges between 25 °C and 35 °C (Primo-Millo and Agustí, 2020). The specific conditions required for each developmental stage have been the subject of considerable research. The pre-oviposition period, at 25 °C, lasts between 3.4 and 10.5 days (Pérez-Otero et al., 2024). The viability of eggs is compromised when temperatures are below 8 °C and above 33 °C (Pérez-Otero et al., 2024). The successful development of eggs and nymphs is contingent upon a temperature range of 15 °C to 24 °C (Aidoo et al., 2022; Pérez-Otero et al., 2024). Aidoo et al. (2022), observed that the mortality rate of the first nymphal instar was highest at 15 °C and lowest at 18 °C, whereas the third instar exhibited the highest survival rates at 20 °C.

The duration of the *T. erytreae* life cycle is also subject to the influence of humidity. Recent studies conducted under controlled conditions showed that at a relative humidity of 65% and at a temperature of 15 °C, the development period is 56.23 days, whereas at 24 °C, it is reduced to 19.95 days. Similarly, at a temperature of 15 °C and a relative humidity of 70%, the developmental period is 46.7 days, whereas at 25 °C, it is reduced to 23.9 days (Aidoo et al., 2022; Pérez-Otero et al., 2024). With respect to nymphal development, no development was observed at a constant temperature of 25 °C, when both 40% RH and 90% RH were maintained. In these conditions, the psyllid only reached the third instar stage, and the time taken for pre-oviposition and egg hatching was extended. However, at 70% RH, the entire life cycle of the psyllid was completed in 23.9 days (Pérez-Otero et al., 2024).

The influence of climatic conditions on insect development times also has an indirect impact on the overall population size of *T. erytreae* (Catling and Annecke, 1968; Cocuzza et al., 2017; Tamesse and Messi, 2004). High mortality rates have been attributed to hot and dry summer days (Van Der Merwe, 1923). A 100% mortality rate of eggs and first-instar nymphs was observed when temperature and humidity parameters, reported as the saturation deficit index (SD), were 45 mbar or

higher. At 35 mbar, the mortality rate was 70%, while at 15 mbar, it decreased to 10% (Aubert, 1987). As a result, Catling (1972) introduced the term “lethal days” to describe periods when values exceeded 34,6 mbar, which had a significant impact on egg viability and the first instar stage of development (Aidoo et al., 2022; Catling, 1972).

Studies carried out before 1970 utilising citrus branches (the species of citrus is not specified) have demonstrated that the requisite duration for egg hatching is 7-9 days, with a range of 5-17 days during summer and winter conditions, respectively. The nymphal stage lasted, on average, 20-27 days, with summer conditions requiring 18 days and winter conditions requiring 34 days (Annecke and Cilliers, 1963; Moran and Blowers, 1967; Van Der Merwe, 1923). Additionally, the lifespan of the adult insect during periods of warm weather ranges from 26 to 36 days (Annecke and Cilliers, 1963). More recent studies conducted under controlled conditions showed a similar trend to the aforementioned branch studies, with slight differences. The eggs hatched, on average, between 7.2 and 13.5 days after oviposition, while the development of nymphs lasted from 16.4 to 33.4 days (Pérez-Otero et al., 2024). The specified timeframes are specific to constant temperatures of 15 °C and 25 °C, respectively. Pérez-Otero et al. (2024) additionally observed that female adults outlive male adults across all tested temperatures. The mean survival of female specimens was 44.2 days at 15 °C, while the mean survival of male specimens was 17.2 days at 25 °C. In citrus plants cultivated in temperate climates, the duration from bud break to complete leaf development is 60 days when daily average temperatures exceed 13 °C (Micheloud et al., 2018). This timeframe enables *T. erytreae* to generate at least one generation. Under optimal climatic conditions, two generations may occur per flushing season (Catling, 1969).

It is necessary to evaluate the climatic conditions in relation to the region’s orography, as well as the presence or absence of suitable hosts, to identify the geographic regions where *T. erytreae* can thrive. This systematic approach enables the delineation of regions where *T. erytreae* may be able to establish and proliferate, thereby facilitating the prompt implementation of protective measures (Benhadi-Marín et al., 2022, 2020; Paiva et al., 2020).

2.7. Methods to study *T. erytreae* host attraction and suitability and their applications

Several methodologies have been employed in order to facilitate a comprehensive understanding of insect–host interaction and host suitability. These include population surveys, choice tests, no-choice tests, studies of insect development and morphometrics, and chemical and molecular interaction studies. Some studies focus on the factors that guide *T. erytreae* to the host, while others focus on the factors that affect the insect's survival and growth subsequent to the selection of a host (Fig. 2.2).

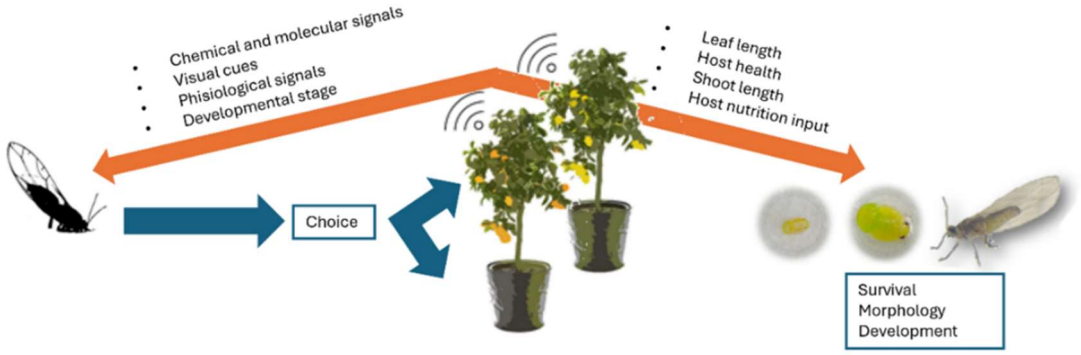

Figure 2.2. Host effect on *Trioza erytreae*. Orange arrows represent host cues/signals; blue arrows represent insect decision pathways.

In population surveys, hosts are sampled within a defined geographical area and time range to record the presence and/or symptoms of *T. erytreae*. This approach enables the determination of the psyllid's natural preference. Such surveys may be designed at a national level or at the level of individual orchards.

Extensive population surveys constitute a valuable initial step in identifying the hosts of the psyllids and discerning their preferences (Aidoo et al., 2019b; Duarte et al., 2024; Kalyebi et al., 2016). To illustrate, a national-level survey conducted in Uganda documented the proportion of each host species in the total number of identified infested plants. The findings demonstrated that mandarins (*C. reticulata*) were the preferred host (66.7%), followed by sour orange (*C. aurantium*) and rough lemon *C.* ×*jambhiri* Lush. (both with 13.3%), in addition to the least-attractive sweet orange variety "Washington Navel" (6.7%) (*C. sinensis*) (Kalyebi et

al., 2016). In the same study, three non-Rutaceae plants were identified as bearing galls and *T. erytreae* adults, namely *Stephania abyssinica* (Dill. & A. Rich) Walp. var. *tomentella* (Oliv.) Deils (Menispermaceae family), *Diospyros mespiliformis* (Ebenaceae family), and *Ficus* spp. (Moraceae family). However, the absence of the observation of nymphs (Kalyebi et al., 2016), indicates the necessity for further investigation to ascertain the suitability of these hosts for *T. erytreae*.

The study of psyllid populations at the orchard level offers considerable advantages in terms of the information they provide regarding host attractiveness and their suitability to *T. erytreae*. This is primarily due to the reduced edaphoclimatic, and orographic variability observed in such studies. The results of studies conducted in multi-species orchards have provided valuable insights into host attractiveness for *T. erytreae*. The findings of Samways and Manicom (1983), demonstrated that the 'Valencia' cultivar (*C.* ×*sinensis*) exhibited a higher mean number of branches with eggs and of *T. erytreae* adults in comparison to the 'Navel' orange (*C.* ×*sinensis*), the 'Ortanique' tangor (*C. reticulata* × *C.* ×*sinensis*), and the grapefruit [*C.* ×*paradisi* (Macfadyen)]. Conversely, a comparable study conducted by Van den Berg et al. (1991a) found that the 'Navel' orange was more attractive than the 'Valencia' orange, which demonstrates how variables inherent to the experimental field and methodology may influence the results.

A considerable number of mandarin cultivars (*C. reticulata*), along with their hybrids tangor (*C. reticulata* × *C.* ×*sinensis*) and tangelo (*C. reticulata* × *C.* ×*paradisi*), were evaluated for their attractiveness for *T. erytreae* in different multi-species orchards (Tamesse, 2000; Tamesse and Messi, 2002; Van den Berg et al., 1991b). The findings of these studies indicate that mandarins have a high degree of intra-species variability in terms of attractiveness for *T. erytreae*. The "Satsuma" subgroup of cultivars, including 'Owari', 'Saigon' and 'Wase', was found to be highly attractive to the psyllid. In contrast, the cultivars 'Dancy', 'Fortune' (a 'Dancy' hybrid), and tangelo hybrid cultivars, such as 'Page' and 'Osceola' show a low attractiveness for *T. erytreae* (Table 2.2) (Tamesse, 2000; Tamesse and Messi, 2002; Van den Berg et al., 1991b).

Table 2.2. Host attractiveness and effect on different stages of *Trioza erytreae* infestation.

Host: Common Name; *Species*; Group; Subgroup; 'Cultivar'/ Variety		Attractiveness	Oviposition	Survival	Nymph Development	Adult Emergence
Family: Rutaceae; Subfamily: Aurantioideae; Genus: *Citrus*						
Citrus reticulata Blanco; **Mandarin**						
Mandarin hybrid (Clementine × Ponkan); 'Fremont'		**1H** [1], **1M** [2]	**1M** [1], **1L** [2]	NA	NA	NA
Mandarin hybrid (Satsuma × King) 'Kara'		**1H** [1]	**1H** [1]	NA	NA	NA
Satsuma	'Saigon'	**1H** [1]	**1H** [1]	NA	NA	NA
	'Wase'	**1H** [1]	**1H** [1]	NA	NA	NA
	'Owari'	**1H** [1]	**1H** [1]	NA	NA	NA
	'Saint Jean'	**1M** [1]	**1M** [1]	NA	NA	NA
	'Kowano'	**1M** [1]	**1L** [1]	NA	NA	NA
Ponkan		**1M** [1]	**1H** [1]	NA	NA	NA
Clementines	Clementine	**1M** [1]	**1M** [1]	NA	NA	NA
	Clemenules	**1M** [3]	**1M** [3]	**1M** [3]	NA	NA
Willowleaf mandarin		**1M** [4]	**1M** [4]	**1M** [4]	**1M** [4]	NA
Madagascar		**1H** [1]	**1M** [1]	NA	NA	NA
King of Siam		**1M** [1]	**1L** [1]	NA	NA	NA
Green Rind		**1L** [5]	NA	NA	NA	NA
Mandarin hybrid (Clementine × Tangelo)	'Fairchild'	**1M** [1]	**1M** [1]	NA	NA	NA
	'Osceola'	**1M** [1]	**1L** [1]	NA	NA	NA
	'Page'	**1L** [1]	**1L** [1]	NA	NA	NA
Mandarin 'Dancy'		**1L** [1]	**1L** [1]	NA	NA	NA
Mandarin hybrid (Clementine × Dancy) 'Fortune'		**1L** [1]	**1L** [1]	NA	NA	NA
Mandarin 'Emperor'		**1L** [5]	NA	NA	NA	NA
Total		**7H** [1,6], **11M** [1-5], **6L** [1,5]	**4H** [1], **9M** [1,3,4,7], **7L** [1,2]	**3M** [3,4]	**2M** [4]	NA

Host: Common Name; *Species*; Group; Subgroup; 'Cultivar'/ Variety		Attractiveness	Oviposition	Survival	Nymph Development	Adult Emergence
Citrus × *sinensis* (L.) Osbeck; **Sweet orange**						
Common oranges	'Valencia'	**1H** [8], **1M** [5], **4L** [1,3,6]	**1H** [8], **2L** [1,3]	**1M** [3]	NA	**1M** [9]
	'Hamlin'	**1M** [1]	**1M** [1]	NA	NA	NA
	'Pineapple'	**1M** [1]	**1L** [1]	NA	NA	NA
	'Mid Season'	**1M** [5]	NA	NA	NA	NA
	'Mouton'	**1L** [5]	NA	NA	NA	NA
	'Oom Louis'	**1L** [5]	NA	NA	NA	NA
	'Pera'	**1L** [5]	NA	NA	NA	NA
Navel oranges	'Navel'	**1H** [5], **1M** [8]	**1M** [8]	NA	NA	NA
	'Navelina'	**1H** [3]	**1M** [3]	**1M** [3]	NA	NA
Total		**3H** [3,5,8], **7M** [1,4,5,8], **5L** [1,3,5]	**1H** [8], **4M** [1,3,4,8], **3L** [1,3]	**3M** [3,4]	**1M** [4]	**1M** [9]
Citrus × *limon* (L.) Burm. f.; **Lemon**						
	'Lisbon	**1H** [1]	**1H** [1]	NA	NA	NA
	'Eureka'	**1H** [1]	**1H** [1]	NA	NA	NA
	'Villafranca'	**1H** [2]	**1M** [2]	NA	NA	NA
	'Fino 49'	**1M** [3]	**1L** [3]	**1M** [3]	NA	NA
Total		**8H** [1,2,4,10-13], **1M** [3]	**4H** [1,4,10], **1M** [2], **1L** [3]	**2H** [4-14], **1M** [3]	**1H** [4], **1M** [14]	**2H** [9,14]

Host: Common Name; *Species*; Group; Subgroup; 'Cultivar'/ Variety		Attractiveness	Oviposition	Survival	Nymph Development	Adult Emergence
Citrus ×*aurantiifolia* (Christm.) Swingle; **Lime**						
	'Mexican'	**1H** [2], **1L** [1]	**1H** [2], **1L** [1]	NA	NA	NA
	'Likeland'	**1H** [1]	**1H** [1]	NA	NA	NA
Total		**2H** [1,2], **2M** [4,5], **1L** [1]	**2H** [1,2], **1M** [4], **1L** [1]	**1M** [4]	**1M** [4]	NA
Citrus ×*paradisi* Macfadyen; **Grapefruit**						
	'Red Blush'	**1M** [1]	**1M** [1]	NA	NA	NA
	'Shambar'	**1M** [1]	**1M** [1]	NA	NA	NA
	'Marsh'	**1L** [1]	**1L** [1]	NA	NA	NA
	'Star Ruby'	**1M** [3]	NA	NA	NA	NA
Total		**4M** [4,3,5,8], **2L** [1,8]	**3M** [4,8], **2L** [1,8]	**1M** [4]	**1M** [4]	NA
Citrus reticulata × *Citrus* ×*sinensis*; **Tangor**						
	'Ortanique'	**2H** [1,3], **1M** [8]	**1H** [3], **2M** [1,8]	**1M** [3]	NA	NA
	'Murcott'	**1L** [5]	NA	NA	NA	NA
Total		**2H** [1,3], **2M** [4,8], **1L** [5]	**1H** [3], **2M** [1,8]	**2M** [3,4]	**1M** [4]	NA
Citrus ×*aurantium* L.; **Sour orange**		**1H** [15], **1M** [6], **3L** [5,12,13]	**1H** [15]	**1H** [15]	**1H** [15]	NA
Citrus maxima (Burm.) Merril; **Pomelo**		**1L** [4]	**1L** [4]	**1M** [4]	**1L** [4]	NA

Host: Common Name; *Species*; Group; Subgroup; 'Cultivar'/ Variety		Attractiveness	Oviposition	Survival	Nymph Development	Adult Emergence
Citrus reticulata × *Citrus* ×*paradisi*; **Tangelo**						
	Minneola	**1H** [1]	**1H** [1]	NA	NA	**1L** [9]
	Orlando	**1L** [1]	**1L** [1]	NA	NA	NA
Total		**1H** [1], **1L** [1]	**1H** [1], **1L** [1]	NA	NA	**1L** [9]
Citrus trifoliata × *Citrus reticulata;* **Citrandarin**						
	Forner-Alcaide 5	**1L** [7]	**1M** [7]	**1L** [7]	NA	NA
	Forner-Alcaide 517	**1L** [7]	**1L** [7]	**1L** [7]	NA	NA
Total		**2L** [7]	**1M** [7], **1L** [7]	**2L** [7]	NA	NA
Citrus trifoliata L.						
	Flying Dragon	**1L** [7]	NA	**1L** [7]	NA	NA
Total		**3L** [4,5,7]	**1O** [4]	**2L** [4,7]	**1O** [4]	NA
Citrus japonica Thunb.; **Kumqua**t		**1M** [5], **1L** [4]	**1L** [4]	**1L** [4]	**1O** [4]	NA
Citrus ×*latifolia* Yu. Tanaka; **Tahiti lime**		**1H** [1]	**1H** [1]	NA	NA	NA
Citrus medica L., **Citron**		**1H** [4]	**1H** [4]	**1H** [4]	**1H** [4]	NA
Citrus macrophylla Wester		**1H** [7]	**1H** [7]	**1L** [7]	NA	NA
Citrus australasica F.Muell.; **Caviar lime**		**1L** [4]	**1O** [4]	**1L** [4]	**1O** [4]	NA
Citrus reshni (Engl) Yu.Tanaka; **Cleopatra mandarin**		**1L** [7]	**1M** [1]	**1L** [7]	NA	NA
Citrus trifoliata × *Citrus* ×*sinensis*; **Citrange**		**1L** [7]	**1H** [7]	**1H** [7]	NA	NA
Citrus L. **		**1H** [5]	NA	NA	NA	NA
Citrus × *jambhiri* Lush.; **Rough lemon**		**1M** [6]	NA	NA	NA	NA
Citrus ×*paradisi* × *Citrus trifoliata* ; **Citrumelo**		NA	NA	NA	NA	**1H** [9]

Host: Common Name; *Species*; Group; Subgroup; 'Cultivar'/ Variety	Attractiveness	Oviposition	Survival	Nymph Development	Adult Emergence
Family: Rutaceae; Subfamily: Aurantioideae; Genus: Other than *Citrus*					
Clausena anisata (Willd.) Hook.fil., De Wild. & Staner	**1H** [4], **1M** [16], **1L** [10]	**2H** [4,16], **1L** [10]	**1H** [4], **1M** [14]	**3H** [4,14,16]	**2M** [4,16], **1L** [9]
Murraya koenigii (L.) Spreng.	**1H** [16]	**1H** [16]	NA	**1H** [16]	**1H** [16], **1M** [9]
Murraya paniculata (L.) Jacq.	**1L** [4]	**1L** [4]	**1M** [4]	**1L** [4]	NA
Family: Rutaceae; Subfamily: Other than Aurantioideae					
Calodendrum capense (L.fil.) Thunb.; **Cape Chestnut**	**2M**[10,16], **1L**[4]	**1M**[10], **1L**[16], **1O**[4]	**2L** [14,16]	**2L**[14,16], **1O**[4]	**1L**[16], **1O**[14]
Vepris lanceolata (Lam.) G.Don; **White ironwood**	**2H** [4,10]	**2H** [4,10]	**2H** [4,14]	**2H** [4,14]	**1M** [14]
Zanthoxylum capense (Thunb.) Harv.	**2L** [4,10]	**1M** [10], **1L** [4]	**2M** [4,14]	**1M** [14], **1L** [4]	**1L** [14]
Vepris nobilis (Delile) Mziray	**1M** [16]	**1M** [16]	NA	**1H** [16]	**1M** [16]
Vepris bilocularis (Wight & Arn.) Engl.	**1L** [16]	**1H** [16]	NA	**1L** [16]	**1M** [16]
Zanthoxylum asiaticum (L.) Appelhans, Groppo & J.Wen	**1L** [4]	**1L** [4]	**1L** [4]	**1O** [4]	NA
Family: Other than Rutaceae					
Daucus carota subsp. *sativus* (Hoffm.) Schübl. & Martens; **Carrot**	**1L** [15]	**1L** [15]	**1L** [15]	**1O** [15]	NA
Tropaeolum majus L.; **Garden nasturtium**	**1L** [11]	NA	NA	NA	NA
Morus alba L.; **Mulberry**	**1L** [11]	NA	NA	NA	NA
Ficus thonningii Blume	NA	**1O** [16]	NA	NA	NA
Ficus sycomorus L.	NA	**1O** [16]	NA	NA	NA
Stephania abyssinica (Dill. & A.Rich.) Walp.	NA	**1O** [16]	NA	NA	NA

1“Total” represents the sum of all comparisons described for each host; it should be noted that the number of comparisons may exceed those specified in the aforementioned subgroups, as some studies do not specify the assayed cultivar or variety; 2 NA means “Not Applied”; 3 OLMH nomenclature: “O” represents a value of zero, “L” represents low comparative values, “M” represents intermediate comparative values, and “H” represents high comparative values. The number preceding the OLMH nomenclature represents the number of hosts classified. This number is sometimes higher than the number of references, as some studies compared more than one variety of the same species. * “Citrus” represents studies where the host was mentioned as “citrus miscellaneous crosses” References in this table are as follows: 1 - (Tamesse, 2000); 2 - (Tamesse and Messi, 2002); 3 - (Quintana-González de Chaves et al., 2024); 4 - (Aubert, 1987); 5 - (Van den Berg et al., 1991a); 6 - (Kalyebi et al., 2016); 7 - (Hernández-Suárez et al., 2021); 8 - (Samways and Manicom, 1983); 9 - (Aidoo et al., 2019c); 10 - (Moran, 1968a); 11 - (Moran and Brown, 1973); 12 - (Cook et al., 2014); 13 - (Benhadi-Marín et al., 2021); 14 - (Moran, 1968b); 15 - (Quintana-González De Chaves et al., 2020); 16 - (Aidoo et al., 2019a)

The attraction of *T. erytreae* to *Citrus* ×*limon* has been well documented (Bové, 2006). In an orchard of sour oranges (*C. aurantium*), a single *C.* ×*limon* tree was found to have twice the number of psyllids in yellow sticky traps compared to the other traps placed near sour oranges (Cook et al., 2014). In contrast to *C.* ×*sinensis* and *C. reticulata* cultivars, different *C.* ×*limon* cultivars have been observed to consistently exhibit high levels of attraction and oviposition rates (Tamesse, 2000; Tamesse and Messi, 2002). The 'Fino 49' lemon was the sole exception, as despite the high oviposition rate when grafted onto the Carrizo citrange (*C. trifoliata* × *C.* ×*sinensis*) rootstock, there was a low oviposition rate when grafted in other tested rootstocks (Quintana-González de Chaves et al., 2024) (Table 2.2). It can be inferred that for certain citrus species, the attraction and oviposition by the psyllid are less dependent on the cultivar, probably due to a lower genetic variability of these species (Uzun and Yesiloglu, 2012).

The host's characteristics and the signals they release to either attract or repel the psyllid can also be explored through choice experiments. The results of choice test studies, performed in a controlled environment have provided insights into the characteristics of psyllids that influence their preference and attraction. The study revealed that the sex of the psyllid may exert an influence on its attraction towards a host. In a dual-choice settlement assay, the probability of selecting a sour orange (*C. aurantium*) was 39% for males and 19% for females (Benhadi-Marín et al., 2021).

A choice experiment study showed that leaf softness affects the oviposition rates of *T. erytreae*, however it had no impact on the settling behaviour of the psyllid (Moran, 1968a; Moran and Buchan, 1975). Furthermore, choice experiment studies revealed that no oviposition occurred on leaves with a hardness rating exceeding 90 g/mm (Moran and Buchan, 1975). Hardness values represent the weight required for a 0.254 mm diameter flat-tipped pin to puncture 1 mm of leaf tissue (Moran and Buchan, 1975; Pollard, 1971).

A choice test and a no-choice test were used to compare the attraction and oviposition of *T. erytreae* on ungrafted rootstocks. The results showed *C. macrophylla* as the most appealing host, while 'Carrizo' citrange (*C. trifoliata* × *C.*

×*sinensis*) exhibited the highest oviposition rate (Hernández-Suárez et al., 2021). The lowest incidence of oviposition was observed in *C. trifoliata*, which was identified as the least attractive host (Hernández-Suárez et al., 2021). The available evidence indicates that citrus rootstocks may affect the volatile profiles of the host scion, which may, in turn, affect the attraction of psyllid pests to them (Jones and Killiny, 2021). It can also affect the suitability of the scion for *T. erytreae*, affecting both attractiveness and oviposition rate. Some cultivars appear to be more affected, as evidenced by the case of 'Fino 49' *C.* ×*limon* (Quintana-González de Chaves et al., 2024).

The use of no-choice experiments, in which the insect is presented with a single host option, enables the study of the host's suitability, as well as the survival and behaviour patterns of the insects after settlement. This approach provides valuable data for epidemiological studies, including the number of adults that form in a new generation (Moran, 1968b) and the number of generations that form in a year (Catling, 1969).

In a no-choice experiment, Aidoo et al. (2019a) observed oviposition differences among eight non-citrus hosts from the Rutaceae family. *Clausena anisata* had the highest percentage of flushes with eggs (52%), while *C. capense* had the lowest values (24%). *Ficus thonningii, F. sycomorus*, and *S. abyssinica* showed no oviposition. The carrot (*D. carota* subsp. *sativus*) was found to be unsuitable as no nymphs were able to reach the adult stage (Quintana-González De Chaves et al., 2020).

Studies on the morphometrics of *T. erytreae* developing in different hosts have provided insights into the host species' impact on the development of the psyllid. A comparative analysis was conducted on five non-citrus hosts from the Rutaceae family. The results showed that *C. capense* yielded the fewest and smallest adults of *T. erytreae*, *Clausena anisata* the second highest number and the largest adults, and *Murraya koenigii* the highest number of emerged adults with sizes similar to those formed in *Clausena anisata* (Aidoo et al., 2019a). Additionally, the morphology and size of *T. erytreae* wings (Aidoo et al., 2019c) also differed across

distinct host species, which could potentially influence the psyllid's ability for flight and dispersal.

The attraction of psyllids to hosts is influenced by plant volatiles. Valterová et al. (1997) conducted a study on the psyllid *Dyspersa apicalis* Foerster, which belongs to the Triozidae family, across a diverse range of host species. The study examined the psyllid's feeding and oviposition preferences in relation to the volatiles derived from the hosts and concluded that the least attractive host species exhibited a higher limonene content. In citrus, the concentration of volatiles in young leaves was higher than in mature leaves, despite the absence of any change in their attractiveness when assessed using a choice test (Antwi-Agyakwa et al., 2019). Nevertheless, these findings suggest that the higher concentration of volatiles in young leaves may increase the likelihood of *T. erytreae* detecting them in field settings. Among the volatiles produced by the hosts, the terpenes appear to play a role in their attractiveness to *T. erytreae* (Antwi-Agyakwa et al., 2019). The volatile profile of both young and mature leaves of *C.* ×*jambhiri*, specifically (S)-(−)-limonene, sabinene, and β-ocimene, was used as synthetic blends, isolated and in various ratios, in choice tests. These volatiles were more effective at attracting *T. erytreae* when combined with others from the plant's volatile profile, rather than when used alone (Antwi-Agyakwa et al., 2019). The leaves of *Vepris lanceolata* (Lam.) G. Don have a lemony scent similar to that of *C.* ×*limon* (L.) Burm. f., which may be linked to the high attractiveness and high rate of *T. erytreae* oviposition observed in this plant species (Aubert, 1987; Moran, 1968a) (Table 2.1). In light of these promising results, plant-based volatiles (acetic acid, (R)-(+)-limonene, sabinene, an ocimene isomer mix comprising cis-ocimene and ß-, myrcene, ethyl butyrate, methyl salicylate and p-cymene) have been incorporated into yellow-sticky traps to attract *T. erytreae*. However, in field conditions, this addition proved ineffective (Pullock et al., 2024).

An analysis of the volatiles of non-host plants revealed that *T. erytreae* avoided the volatiles of guava (*Psidium guajava* L.), garlic (*Allium sativum* L.) and lemongrass [*Cymbopogon citratus* (DC.) Stapf] (Antwi-Agyakwa et al., 2021). Studies suggested that the practice of interplanting citrus trees with guava (*P.*

guajava), in open fields decreased the populations of *D. citri* in the orchards. However, the effects were not evident under controlled greenhouse conditions (Gottwald et al., 2010; Hall et al., 2008). It would be interesting to study the impact of intercropping guava, garlic, and/or lemongrass in citrus orchards on *T. erytreae*, to ascertain the repelling effect in field conditions.

The majority of studies on the interaction of *T. erytreae* with its hosts have been focused on the analysis of plant volatiles. Nevertheless, a recent study performed by our research group that used a no-choice experimental design and proteomic analysis found that the proteomic response of lemon and sweet orange plants to *T. erytreae* was distinct. The proteomic response of sweet orange plants to the psyllid was more pronounced and extensive (Magalhães et al., 2024). This study suggests that citrus host plants adjust their proteome in response to *T. erytreae* infestation, which may be related to host suitability.

2.8. Final remarks and future perspectives

The objective of this review was to provide a synthesis of the existent knowledge regarding the hosts of *T. erytreae* and their interaction with the psyllid. Despite the extensive research conducted on *T. erytreae* further research is required to elucidate the host influence on nymphal development and adult emergence. This encompasses the analysis of the quantity and proportion of hatching eggs and nymphs, nymph development time, adult emergence, and morphometrics of emerged adults. Understanding insect–host interactions at the molecular level is essential for developing effective control strategies for the psyllid. The formulation of efficacious artificial diets for *T. erytreae*, and the improvement of diets already tested, such as the one proposed by Russell and Pelz-Stelinsk (2015) for *D. citri,* may facilitate the study of the effects of isolated diet compounds on the psyllids. This could potentially result in the development of an effective strategy for controlling psyllid populations.

A major challenge identified in this review was the gap in knowledge on the molecular aspect of this specific insect–host interaction. Omics-based approaches provide a comprehensive understanding of the interactions between insects and

plants (Barah and Bones, 2015). The application of omics approaches to the study of *D. citri* has facilitated a more profound understanding of the host's response to the psyllid infestation (Nehela et al., 2018; Sun et al., 2022). Furthermore, the identification of characteristic proteins related to psyllid phenotypes (Hosseinzadeh et al., 2021) and development stages has been made possible (El-Shesheny et al., 2016). And therefore, a meta-analysis of omics on the molecular profiles of citrus hosts would also be advantageous in identifying potential molecular correlations with *T. erytreae* preferences. This approach has been employed to study citrus hosts tolerant to HLB (Rawat et al., 2015).

Another major challenge identified in this review was the dispersed nature of the data and the diverse types of reporting on *T. erytreae* interaction with its hosts. Hence, the construction of an accurate database on potential *T. erytreae* hosts is of significant importance, as it facilitates informed decision-making regarding citrus management strategies, including control policies, breeding programs, research lines, and orchard management (Aidoo, 2023). In addition, the implementation of a standardised methodology for the reporting of *T. erytreae* populations to a centralised repository would contribute to a more complete and accessible knowledge base of the psyllid populations, behaviour and hosts, improving the precision of the prediction models, as was already developed for *D. citri* and HLB (Benhadi-Marín et al., 2022; Galvañ et al., 2023).

Chapter 3. Proteomic analysis may explain differences in *Citrus ×limon* and *Citrus ×sinensis* susceptibility to *Trioza erytreae*

This chapter has been adapted from an original research paper accepted in:

Magalhães, T., Anjos, L., Dandlen, S. A., Power, D. M., Pereira, J. A., Duarte, A., Marques, N. T. (2025). Proteomic analysis may explain differences in *Citrus ×limon* and *Citrus ×sinensis* susceptibility to *Trioza erytreae*. *Plant Signaling & Behavior*.

3.1. Abstract

The *Trioza erytreae* psyllid is a vector for Huanglongbing, a severe bacterial disease of citrus. *Citrus ×limon* is the preferred host, although the reason for this is unclear. This study compared the responses of *C. ×limon* 'Eureka' and *C. ×sinensis* 'Valencia' plants to *T. erytreae* infestation, specifically to nymph feeding. The number of successfully developed nymphs observed in 'Eureka' was three times that of 'Valencia' plants. The enriched vascular sap proteome of young leaves of infested and control plants was compared using nanoscale liquid chromatography coupled to tandem mass spectrometry. This study revealed 48 and 1,265 differentially abundant proteins (DAPs) in infested *C. ×limon* and *C. ×sinensis*, respectively. There was marked host specific response, with little overlap in proteomic features. Shared citrus host responses to the infestation were the downregulation of "Amino sugar and nucleotide sugar metabolism" and the upregulation of galactose, vitamin B6, and selenocompound metabolisms. The downregulation of photosynthesis-related proteins and the activation of defence-related pathways in *C. ×sinensis* suggest a robust response, which may explain the low success of nymph development on this host. The lower number of DAPs in *C. ×limon* infested with *T. erytreae*, may provide insights into the psyllids host preference. Further investigation exploring the identified candidate proteins and pathways will contribute to explain the interaction between *T. erytreae* and *C. ×limon*.

3.2. Abbreviations

ACN (acetonitrile); AGC (automatic gain control); CoA (coenzyme A); DAPs (differentially abundant proteins); DTT (dithiothreitol); ER (endoplasmic reticulum); ERAD (endoplasmic reticulum associated degradation system); EurekaLemonCon ('Eureka' Lemon control); EurekaLemonInf (infested 'Eureka' lemon); FA (formic acid); FDR (false discovery rate); HLB (huanglongbing); JA (jasmonic acid); KEGG (Kyoto Encyclopedia of Genes and Genomes), nanoLC-MS/MS (nanoscale liquid chromatography coupled to tandem mass spectrometry); ROS (reactive oxygen species); SwO (sweet orange, *C. ×sinensis*); ValenciaSwOCon ('Valencia' sweet orange control); ValenciaSwOInf (infested 'Valencia' sweet orange).

3.3. Introduction

Huanglongbing (HLB) is one of the most devastating diseases of the citrus industry and is associated with the presence of the phloem-limited bacterium *Candidatus* Liberibacter spp.. The primary vectors of this disease are the Asian citrus psyllid (*Diaphorina citri* Kuwayama) and the African citrus psyllid (*Trioza erytreae* Del Guercio), although it can also be transmitted by grafting (Bové, 2006). Huanglongbing has no viable treatment, so its management is based on vector control and the removal of infested plants (Bassanezi et al., 2013). The Mediterranean basin is one of the most important citrus growing areas in the world and, so far, has remained free of HLB (Alquézar et al., 2022). However, a major concern for the citrus industry is the recent introduction of *T. erytreae* into mainland Europe, and its spread along the north and west coasts of the Iberian Peninsula, despite not yet carrying the bacterium (Benhadi-Marín et al., 2022; Paiva et al., 2020). *Trioza erytreae* thrives on specific hosts, categorized as preferred hosts, common hosts, and occasional hosts (Aubert, 1987). Preferred hosts are the most attractive to the psyllid, facilitating optimal development. These include lemon plants [*Citrus* ×*limon* (L.) Burm. f.], white ironwood [*Vepris lanceolata* (Lam.) G. Don] and false horsewood [*Clausena anisata* (Willd.) Hook.f.] (Aidoo et al., 2019a; Aubert, 1987; Benhadi-Marín et al., 2021). Common hosts of intermediate attractiveness to *T. erytreae* include sweet orange [*C.* ×*sinensis* (L.) Osbeck], mandarin (*C. reticulata* Blanco) and grapefruit (*C.* ×*paradisi* Macfad.) (Aubert, 1987; Samways and Manicom, 1983; Tamesse, 2000). Trifoliate orange (*C. trifoliata* L.) and cape chestnut (*Calodendrum capense* Thunb.) are considered occasional hosts for the psyllids. These plants have limited attractiveness and provide inadequate support for psyllid growth and development (Aubert, 1987; Hernández-Suárez et al., 2021).

Young flushes are critical for *T. erytreae* oviposition and nymphal development (Catling, 1972). Oviposition occurs on young shoots, while nymphal development preferentially occurs on the underside of young leaves, resulting in pit gall formation (Moran and Blowers, 1967). The annual cycles of shoot growth and the volatile compounds (limonene, sabinene, and β-ocimene) emitted from the leaves of lemon trees are the principal factors explaining the psyllid´s preference

for lemon trees, which have been identified as the most suitable host (Antwi-Agyakwa et al., 2019). The oviposition rate of *T. erytreae* is high when the host is lemon (Moran, 1968a; Tamesse, 2000; Tamesse and Messi, 2002). A comparison of the growth and size of *T. erytreae* nymphs on six Rutaceae hosts showed that nymphs reached their largest size on lemon plants and citrumelo (*C.* ×*paradisi* × *C. trifoliata*), followed by sweet orange and *Murraya koenigii* (L.) Spreng plants. In contrast, nymphs developed poorly and were very small on false horsewood and tangelo (*C. reticulata* × *C.* ×*paradisi*) (Aidoo et al., 2019c).

The interaction between sap-sucking insects and their hosts is a complex process that alters the plants metabolism and distinguishes susceptible and resistant plant genotypes (Åhman et al., 2019). The role of the plant in *T. erytreae* host preference remains underexplored and seems to influence psyllid behaviour in terms of attractiveness, oviposition, and nymph development. Previous studies described the impact of plant hosts on insect development in resistant *Pisum sativum* L. (pea) cultivars infested with the phloem-feeding insect *Acyrthosiphon pisum* (Harris). In these instances, the shoot tips contained fewer photosynthesis-related proteins, which hindered insect development (Carrillo et al., 2014). The response of plants to insect infestation involves the vascular system, which is essential for long- and short-distance transport of nutrients and molecular signals (Dinant et al., 2010; Dinant and Lucas, 2012; Walker, 2022; Will et al., 2013). Adults and nymphs of *Trioza erytreae* feed on phloem sap and sometimes on xylem (Benhadi-Marín et al., 2021). The vascular system is a site of cross-talk between the psyllid and their host, particularly during the sedentary nymphal stage of development. This interaction modifies local plant responses making them beneficial to the psyllids (Åhman et al., 2019).

Phloem sap partitions photoassimilates and is rich in compounds such as ions, metabolites, RNA, immune signals, and proteins, some of which travel long distances in the plant and are part of signalling networks that determine the plants' response to herbivory (Carella et al., 2016; Dinant et al., 2010; Dinant and Lucas, 2012). Xylem vessels are responsible for the transportation of water and minerals absorbed by roots from the soil. Additionally, they facilitate the transport of amino

acids, carbohydrates, organic acids, and proteins, despite partitioning a significantly lower concentration of proteins than phloem sap (Aki et al., 2008; Kehr and Rep, 2007; Surano et al., 2024). Furthermore, in plants under stress, the xylem is also a route for the jasmonic acid (JA) signalling network (Farmer et al., 2020). The findings of Kienow et al. (2008), Koo et al. (2013), and Ruan et al. (2019) indicate that the JA signalling system affects how plants respond to insects. Outside of the plasma membrane, the apoplast, also known as the free diffusional region, is made up of the fluid inside the intercellular spaces and the cell wall matrix. The composition of the apoplast provides insight into the mechanisms that underlie the export and import of chemicals by individual cells. Additionally, it sheds light on the intricate interplay between the xylem and phloem compartments (Rodríguez-Celma et al., 2016). Knowledge about the composition of vascular sap proteins may reveal the influence of the host plant on nymphal development and contribute to explain the preference of *T. erytreae* for the specific *C.* ×*limon* host.

The present study took advantage of proteomics to compare two citrus hosts infested by *T. erytreae*: the preferred host, lemon (*C.* ×*limon* 'Eureka'), and the common host, sweet orange (*C.* ×*sinensis* 'Valencia Midknight Seedless'). The development of *T. erytreae* nymphs on these two hosts was monitored over time. The proteome of the enriched vascular sap, derived from the leaf and petiole midribs of young leaves of infested and control plants, was compared to analyse the response of the two citrus host species to *T. erytreae*. The extraction method, which employed centrifugation, resulted in the enrichment of the vascular sap proteome with small amounts of apoplast fluids and the contents of parenchyma and mesophyll cells. The proteome was analysed using nanoscale liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS). The results of the proteomic study revealed that there was minimal overlap in proteome features between the two citrus hosts infested with psyllids. Furthermore, the proteome of 'Valencia' SwO showed the most significant proteome change compared to the respective control.

3.4. Materials and methods

3.4.1. Plant material

The study employed a total of 32 two-year-old citrus plants, comprising 16 'Valencia Midknight Seedless' sweet orange [*C.* ×*sinensis* (L.) Osbeck] ('Valencia' SwO) and 16 'Eureka' lemon [*C.* ×*limon* (L.) Burm. f] ('Eureka' lemon) plants, grafted on 'Carrizo' citrange (*C. trifoliata* × *C.* ×*sinensis*) rootstock. The citrus plants were purchased from the certified nursery "Association of Nurserymen of the District of Coimbra" (Associação de Viveiristas do Distrito de Coimbra - AVDC) and were all from the same batch. This association is responsible for the propagation of certified material in accordance with European Union legislation (EU, 2008). The plants acquired for this trial had a phytosanitary passport and were categorised as "Certified" category. The plants were two years old and between 0.8 m and 1.0 m in height. The experiment was conducted over a period of four months, from May to July 2021. The plants were cultivated in 5 L tall pots (19 cm in diameter and 25 cm in height) in an artificial potting mix of pine bark and coconut fibre (50:50), fertilised in accordance with standard procedures, and maintained in a climate chamber at 23.5 ± 1°C, with a relative humidity of 79 ± 5% and a photoperiod of 14:10 h (L:D). Two treatment groups, each comprising eight plants, were established for each citrus species. One group was designated as the control, while the other was subjected to infestation by *T. erytreae*. Three weeks prior to infestation, all 32 plants were pruned in order to induce new shoot growth. The young plants were maintained in controlled conditions throughout the experiment, from the initial pruning stage to the leaf harvest, which occurred between 23 and 25 days after infestation. The number of new shoots exhibited by the plants selected for the experiment was similar. In the infested groups, each plant was infested with 10 *T. erytreae* adults, comprising five males and five females. Prior to leaf harvest, the total number of pit galls on each of the 16 infested plants was counted.

3.4.2. Insect origin and rearing

In 2021, nymphs and adults of *T. erytreae* were collected from pesticide-free lemon orchards in Caracoi (Porto district, Portugal. 41°18´46.4´´N 8°38'09.7''W).

Whenever *T. erytreae* was detected in a new area, the Portuguese official authorities proceeded to test for the presence of *Candidatus* Liberibacter spp.. A random sampling strategy was employed to test for the presence of *Candidatus* Liberibacter spp. in the Caracoi area and throughout Portugal. The tests were based on a polymerase chain reaction (PCR) utilising primers that were specific for *Candidatus* Liberibacter spp.. The PCR was performed on the insect and the plant samples that had been taken from the field. To date, no samples of *T. erytreae* have tested positive (EPPO, 2021, 2015). Adults of *T. erytreae* were collected using a hand-held aspirator and transferred to conical centrifuge tubes (50 mL). Colonies of *T. erytreae* were established on 'Eureka' lemon and 'Afín Verna 2' sour orange (*C.* ×*aurantium* L.) plants, which were purchased from the certified nursery AVDC with a phytosanitary passport. The infested plants were maintained in acrylic cages (40 x 30 x 43 cm) covered with insect-proof netting, within a climate chamber at 21 ± 1 °C, 50 ± 5% relative humidity, and under a photoperiod of 16:8 h light to dark (L:D). The infested plants used for *T. erytreae* rearing were irrigated according to their specific needs, approximately twice weekly. In order to prevent an overabundance of *T. erytreae* in the rearing plant, the insect populations were divided and transferred into new, non-infested plants when necessary, using a handheld aspirator.

3.4.3. Infestation and nymph development

Sexually mature adult psyllids reared in the acrylic cages were used to infest 'Valencia' SwO and 'Eureka' lemon plants. Ten adult *T. erytreae* specimens, comprising five males and five females, were aspirated from the rearing cages using a hand-held aspirator and collected in a conical centrifuge tube (50 mL). This tube was then used to introduce the psyllids to the netted citrus experimental hosts. A total of eight plants of each species were infested with adult *T. erytreae*, and a further eight plants of each species were used as controls. The experimental groups are hereafter referred to as EurekaLemonInf and ValenciaSwOInf for the infested plants and EurekaLemonCon and ValenciaSwOCon for the control plants (Fig. 3.1). Each plant was isolated within a net that was attached to the tree trunk above the pot to facilitate irrigation and was fixed above the canopy with a wooden frame. The adult psyllids were retained within the net for the duration of the experiment, which

spanned 23 to 25 days. The development of the psyllids was monitored over time, as were the numbers of fourth and fifth instar nymphs and the pit galls they formed on the leaves (Fig. 3.1). The total number of pit galls and nymphs was counted immediately prior to the leaf harvest in both the EurekaLemonInf and ValenciaSwOInf groups.

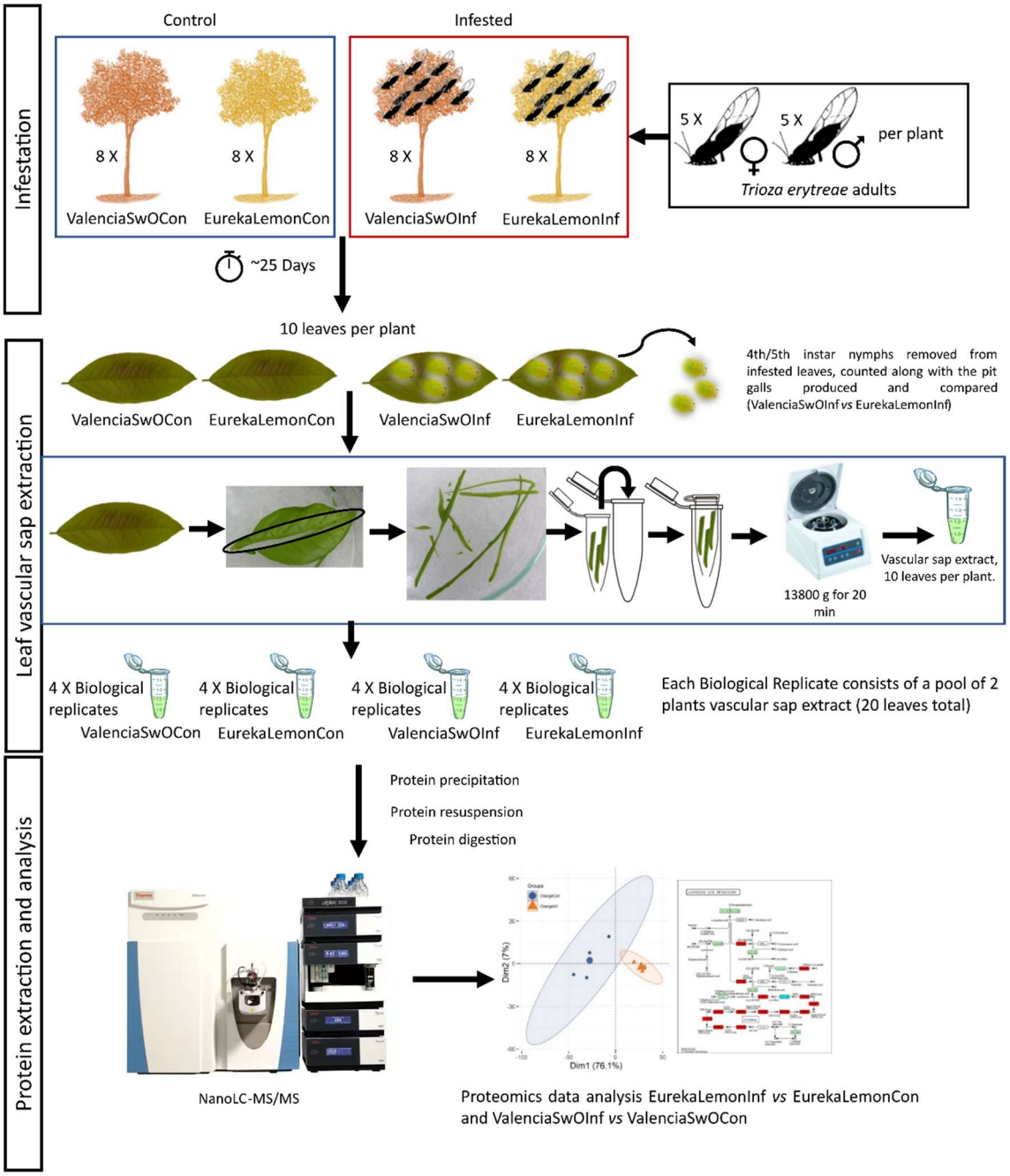

Figure 3.1. Experimental design and an overview of the workflow showing the main steps: infestation of citrus hosts with *Trioza erytreae*, counting of nymphs and pit galls, extraction of enriched vascular sap and proteomics analysis. EurekaLemonCon - control 'Eureka' lemon plants; EurekaLemonInf - 'Eureka' lemon plants infested with *Trioza erytreae*; ValenciaSwOInf – control 'Valencia' sweet orange (SwO) plants; ValenciaSwOInf – 'Valencia' SwO plants infested with *T. erytreae*.

3.4.4. Citrus enriched vascular sap protein extraction and protein profile analysis

Trioza erytreae nymphs were collected from each of the eight infested EurekaLemonInf and ValenciaSwOInf plants when they reached the fourth or fifth instar, which occurred 23–25 days after the infestation. Leaves in which the nymphs were found were collected together with their petioles intact. All the nymphs were counted and removed with the subsequent enriched vascular sap extraction process initiated without delay. Additionally, leaves of a similar size and developmental stage were collected on the same day as the infested groups from the non-infested control citrus plants EurekaLemonCon and ValenciaSwOCon. The vascular sap was extracted from all plants, with a total of 10 leaves being used from each plant for analysis. Leaves from the eight infested and eight non-infested plants of each species were collected and stored at 4 °C until the onset of the protein extraction procedure. Considering the technical constraints associated with the collection of pure vascular sap from young citrus leaves, the Hijaz and Killiny (2014a) protocol was adapted, to facilitate the extraction of enriched vascular sap from the leaf and petiole midribs by means of centrifugation (Fig. 3.1). The collection of enriched vascular sap for protein extraction was performed within one hour of the collection of the leaves. The midribs of leaves and petioles of young leaves were isolated and chopped into an appropriate size for a 0.5 mL perforated microtube using a sterilised scalpel. The perforated tube was placed in a 1.5 mL microtube and subjected to centrifugation at 4 °C and 13,800 × g (12,000 rpm) for 20 min. The extracted enriched vascular sap was collected at the bottom of the 1.5 mL microtube and stored at -80 °C. Protein extraction was performed on 10 leaves from each plant (Fig. 3.1). The centrifugation method, which employs the use of cut plant material, is primarily effective in the collection of vascular sap and apoplastic fluid. However, this process may also disrupt the cellular structure of younger cells, resulting in an enrichment of the vascular sap proteome with the contents of parenchyma cells, phloem companion cells, and mesophyll cells that were adjacent to the midrib vein. Accordingly, the extracted sap is hereafter designated as ‘enriched vascular sap’.

Acetone, Coomassie blue, trichloroacetic acid (TCA), thiourea, Tris-base, urea and the chemicals required for sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Merck KGaA (Darmstadt, Germany), while dithiothreitol (DTT) and hydrochloric acid were purchased from Thermo Fisher Scientific (Eindhoven, The Netherlands). The methodology for total protein extraction was based on a modified approach described by Zadražnik et al. (2013). The enriched vascular sap was extracted from 10 leaves of each sampled plant. Four pools were prepared for protein extraction from each experimental group (EurekaLemonInf, ValenciaSwOInf, EurekaLemonCon and ValenciaSwOCon), with each pool comprising two sampled plants (Fig. 3.1). A total of 100 µL of the extracted protein from each biological replicate was solubilised in 1.25 mL of extraction buffer [10% TCA, 60 mM dithiothreitol DTT in acetone], vortexed and stored at -80 °C overnight. Subsequently, the samples were centrifuged at 14,000 × g for 30 min at 4 °C. Thereafter, the pellet was washed three times in 1 mL of wash buffer (60 mM DTT in acetone), followed by a centrifugation at 14,000 × g for 5 min at 4 °C. Subsequently, the pellet was left to dry at room temperature and then resuspended in 40 µL of denaturing buffer (7 M urea, 2 M thiourea, 30 mM Tris-HCl, pH 8.5).

The total protein content of the extracted sap was quantified using a Quick Start™ Bradford Protein Assay Kit (Bio-Rad, Hercules, USA) in a Genesys 1Q-S spectrophotometer (Thermo Electron Corporation, Bremen, Germany), with bovine serum albumin (BSA) serving as the standard, in accordance with the manufacturer's instructions. To evaluate the quality of the protein extracts, 30 µg of total soluble protein from each sample was analysed by gel electrophoresis in a 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel (SDS-PAGE), according to the Laemmli method (Laemmli, 1970), and then stained with Coomassie blue. A total of 50 µg of protein from each sample was subjected to a solid-phase-enhanced sample-preparation (SP3) protocol as described by Hughes et al. (2018), followed by an enzymatic digestion with trypsin/LysC (2 µg) overnight at 37 °C and 1,000 rpm. The concentration of the resulting peptides was determined by fluorescence measurement.

3.4.5. Proteomic analysis of citrus enriched vascular sap

3.4.5.1. Proteomics data acquisition

The citrus enriched vascular sap proteome of the four experimental conditions (EurekaLemonInf, EurekaLemonCon, ValenciaSwOInf and ValenciaSwOCon, n = 4 samples per condition) was obtained as described in Osório et al. (2021). Protein identification and quantification were performed by nanoscale liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS) in an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany), with the assistance of an external service provider (Proteomics Scientific Platform of i3S, Ipatimup, Porto, Portugal). Five hundred nanograms of the trypsin/LysC digested samples were loaded onto a trapping cartridge (Acclaim PepMap C18 100 Å, 5 mm × 300 µm i.d., 160454, Thermo Scientific) in a mobile phase of 2% acetonitrile (ACN), 0.1% formic acid (FA) at a flow rate of 10 µL/min. Following a loading period of three minutes, the trap column was switched in-line to a 50 cm × 75 µm inner diameter EASY-Spray column (ES803, PepMap RSLC, C18, 2 µm, Thermo Scientific) at 250 nL/min. The separation was achieved by mixing the mobile phase, comprising A: 0.1% FA and B: 80% ACN, 0.1% FA, with the following gradient: 5 min (2.5% B to 10% B), 120 min (10% B to 30% B), 20 min (30% B to 50% B), 5 min (50% B to 99% B), and 10 min (hold 99% B). Subsequently, the column was equilibrated with 2.5% B for 17 min. The data acquisition process was controlled by Xcalibur 4.0 and Tune 2.9 software (Thermo Scientific). The mass spectrometer was operated in the data-dependent (dd) positive acquisition mode alternating between a full scan (*m/z* 380-1,580) and subsequent higher-energy collisional dissociation tandem mass spectrometry (HCD MS/MS). This was established for the 10 most intense peaks from a full scan (normalised collision energy of 27%). The electrospray ionisation (ESI) spray voltage was at 1.9 kV and the global settings were as follows: lock mass best (*m/z* 445.12003), lock mass injection, full MS and chrom peak width at a full width half maximum (FWHM) of 15 s. The full scan settings were as follows: 70 k resolution (*m/z* 200), automatic gain control (AGC) target 3×10^6, maximum injection time 120 ms; dd settings: minimum AGC target 8×10^3, intensity

threshold 7.3×10^4, charge exclusion: unassigned, 1, 8, >8, peptide matches preferred, exclude isotopes on, and dynamic exclusion 45 s. The MS2 settings were as follows: microscans - 1, resolution - 35 k(*m*/*z* 200), AGC target - 2×10^5, maximum injection time - 110 ms, isolation window - 2.0 *m*/*z*, isolation offset - 0.0 *m*/*z*, dynamic first mass and spectrum data type profile.

3.4.5.2. Data analysis, protein-label-free quantification and protein identification

The mass spectrometry (MS) raw data were processed using Proteome Discoverer 2.5.0.400 software (Thermo Scientific). Protein identification searches were performed against the UniProt protein sequence database for *C*. ×*sinensis* (taxon ID 2711, 44,601 entries) and a common contaminant database from MaxQuant (version1.6.2.6, Max Planck Institute of Biochemistry, Munich, Germany). The Sequest HT tandem mass spectrometry peptide database search program was used to identify tryptic peptides, with an ion mass tolerance of 10 ppm for precursors and 0.02 Da for fragmented ions and missing cleavage sites was set as 2. Cysteine carbamidomethylation was defined as a constant modification. Methionine oxidation, asparagine, and glutamine deamidation, peptide N-terminus Gln->pyro-Glut, protein N-terminus acetylation, and loss of methionine and Met-loss+Acetyl were defined as variable modifications. Peptide confidence was set to high and the Inferys rescoring node was considered for this analysis. The Percolator processing node was enabled with the following settings: maximum Delta Correlation (deltaCn) 0.05; decoy database search target False Discovery Rate (FDR) 1%; validation based on *q*-value.

Protein-label-free quantification was performed with the Minora feature detector node at the processing step. The following parameters were employed for precursor ion quantification: 1) peptides unique plus razor; 2) precursor abundance was based on intensity; 3) normalisation mode was based on the total peptide amount; 4) the minimum number of replicate files was set to 50% in each sample group; 5) the pairwise protein ratio calculation and hypothesis test were based on a *t*-test (background based). The Feature Mapper node from the Proteome Discoverer software was used to generate features from unique peptide-specific peaks within

a narrow retention time and mass range (mapping features from different sample files was permitted within a maximum shift of 10 min and 10 ppm of mass tolerance). For the purposes of feature linking and mapping, a signal to noise (S/N) threshold of 5 was employed for the minimum EurekaLemonInf *vs*. EurekaLemonCon comparison and ValenciaSwOInf *vs*. ValenciaSwOCon. The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner repository with the dataset identifier PXD043528 and 10.6019/PXD043528.

3.4.5.3. Bioinformatics analysis

The fold-change in protein abundance was calculated between the experimental groups EurekaLemonInf and EurekaLemonCon and between ValenciaSwOInf and ValenciaSwOCon. The peptide medians (log2-transformed) between relative protein abundances in the experimental groups were used as the basis for this calculation. The identification of differentially abundant proteins (DAPs) was undertaken. Functional analysis of DAPs was performed using *Arabidopsis thaliana* (L.) Heynh. orthologues of the identified proteins, obtained with the STRING web tool version 11 (https://string-db.org/) (Szklarczyk et al., 2021), with protein names and definitions obtained from the *Arabidopsis* Information Resource (Berardini et al., 2015). The enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/) (Kanehisa et al., 2022) and the KOBAS web-based tool (http://bioinfo.org/kobas/genelist/) (Bu et al., 2021). A hypergeometric test/Fisher's exact test with a Benjamin and Hochberg (Benjamini and Hochberg, 1995) FDR correction method was applied (adjusted *p*-value at <0.05). Bubble plots and Venn diagrams were created with R software (R Core Team, 2020) using the package ggplot2 (Wickham, 2016).

3.4.6. Statistical analysis

The mean number of nymphs and pit galls per plant was compared between infested plants, namely EurekaLemonInf and ValenciaSwOInf, using a Student's *t*-test performed in Rstudio software (R Core Team, 2020). For visualisation of data,

the package “ggstatsplot” (Patil, 2021) was used in Rstudio, and the standard error of the mean (SEM) is presented. To determine the DAPs enriched in vascular sap between infested and control treatments, the following filters were considered: (1) The minimum number of biological samples in which a protein was identified in an experimental group was set to 75% (e.g., 3 out of 4); (2) The presence of at least two unique peptides for protein assignment; and (3) Protein FDR set to a high q-value < 0.01. A Student’s t-test was performed on the log10 transformed protein abundance with the p-value adjusted using a permutation-based correction (with 250 randomisations) (FDR <0.05). Preprocessing and univariate hypothesis testing were performed in Perseus software version 2.0.7.0. (MaxQuant, Germany) (Tyanova et al., 2016).

Principal component analysis (PCA) was performed in Rstudio software (R Core Team, 2020) using the built-in “prcomp” function and autoscaled matrices, while the “factoextra” package was used for visualisation (Kassambra and Mundt, 2020). Two types of data were used for the PCA, one comprising all identified proteins in each experimental group, with the application of the filters previously mentioned for the DAP analysis, and the other including only the DAPs. Two permutations of the PCA were performed for the comparisons between the EurekaLemonInf *vs*. EurekaLemonCon groups and the ValenciaSwOInf *vs*. ValenciaSwOCon groups.

3.5. Results

3.5.1. *Trioza erytreae* nymphs developed better on ‘Eureka’ lemon than in ‘Valencia’ sweet orange plants

With regards to infestation, the number of new flushes exhibited no significant difference between the different host plants (Welch's *t*-test, p =0.15). The mean number of new flushes per plant was 4.63 for ‘Eureka’ lemon and 6.38 for the ‘Valencia’ SwO. The nymphs of *Trioza erytreae* developed to the fourth and fifth instar stage in both the ‘Eureka’ lemon and the ‘Valencia’ SwO plant hosts and induced the formation of pit galls at the feeding sites (see ‘Fig. 3.2.1, 3.2.2 and 3.2.3 for ‘Eureka’ lemon; Fig. 3.2.7 and 3.2.8 for ‘Valencia’ SwO). A more pronounced and darker colouration of the secondary and tertiary leaf vein was observed in a significant proportion of the infested ‘Valencia’ SwO leaves (ValenciaSwOInf, Fig. 3.2, zoom of 3.2.7), whereas the same symptom was rarely seen in infested ‘Eureka’ lemon leaves (EurekaLemonInf, Fig. 3.2, zoom of 3.2.1), and not at all in the leaves of control plants. The leaves were deformed when infested with a high number of nymphs (Fig. 3.2.2, 3.2.3 and 3.2.8).

The mean number of pit galls per plant for the eight infested ‘Eureka’ lemon plants (EurekaLemonInf) was 330.1 (± 47.5 SEM), whereas the infested ‘Valencia’ SwO plants (ValenciaSwOInf) had a mean number of 246.8 (± 30.8 SEM) pit galls, values that were not significantly different (p = 0.16) (Fig. 3.3). However, a significant difference was found in the mean number of nymphs (fourth and fifth instar) per host species (p< 0.05, Student’s *t*-test). The number of developing nymphs was found to be significantly lower in ValenciaSwOInf hosts, with a mean of 99.3 (± 27.6 SEM) nymphs per plant in comparison to EurekaLemonInf, where 318.5 (± 47.3 SEM) nymphs developed (Fig. 3.3).

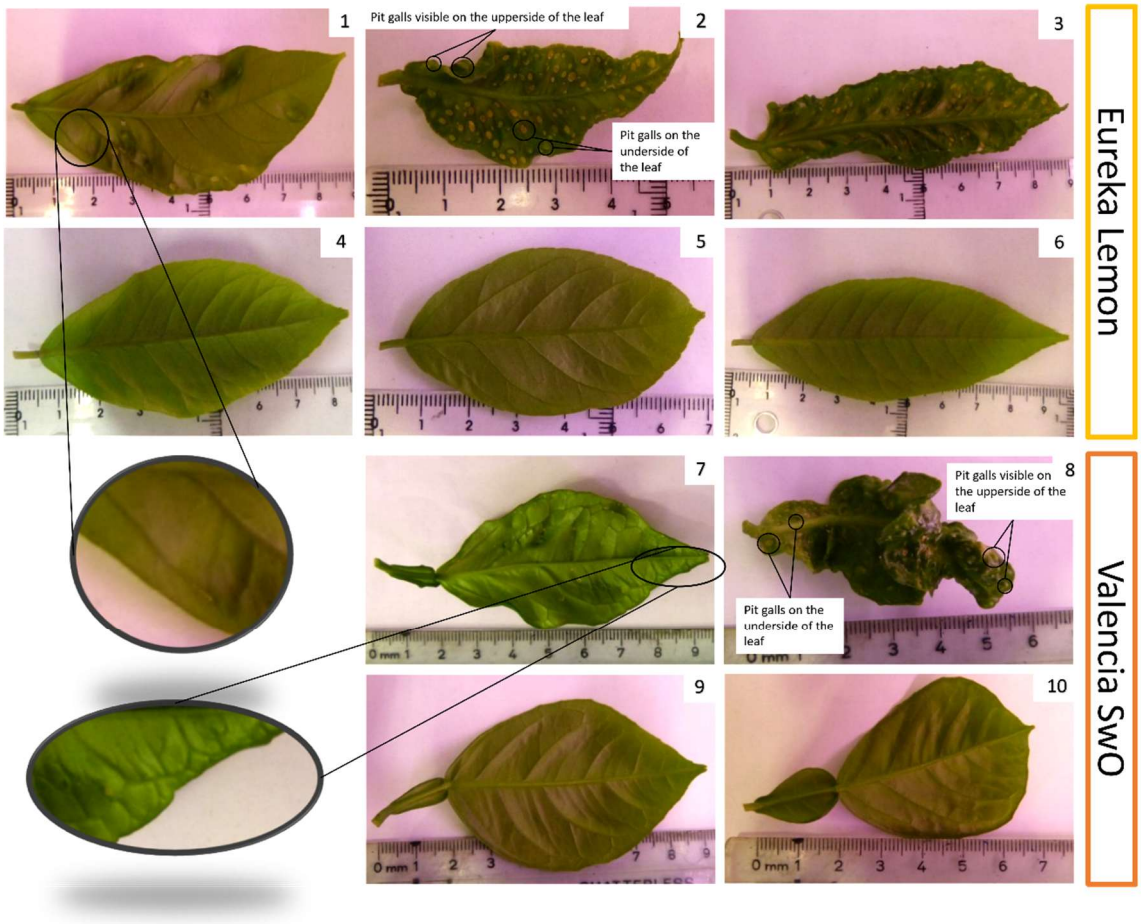

Figure 3.2. The images show the appearance of 'Eureka' lemon and 'Valencia' sweet orange (SwO) leaves 23 to 25 days after *Trioza erytreae* infestation. 1, 2 and 3 are infested 'Eureka' lemon leaves; 4, 5 and 6 are control 'Eureka' lemon leaves; 7 and 8 are infested 'Valencia' SwO leaves; 9 and 10 are control 'Valencia' SwO leaves. 1- an Infested 'Eureka' lemon leaf where 9 nymphs developed, is shown in greater detail in the zoom-in image, which highlights the more pronounced and darker secondary veins; 2- an infested 'Eureka' lemon leaf where 51 nymphs developed, is shown in greater detail in the zoom-in image; 3- an Infested 'Eureka' lemon leaf where 127 nymphs developed, is shown in greater detail in the zoom-in image (Maximum number of nymphs identified on a 'Eureka' lemon leaf); 4, 5 and 6- 'Eureka' lemon control leaves of a similar size to 1, 2 and 3, respectively; 7- Infested 'Valencia' SwO leaf where 9 nymphs developed, see the the zoom-in. This image highlights the more pronounced and darker secondary veins; 8- This image shows an infested 'Valencia' SwO leaf where 58 nymphs developed (the maximum number of nymphs identified on an 'Valencia' SwO leaf); 9 and 10- These images show 'Valencia' SwO control leaves with similar size to 7 and 8, respectively.

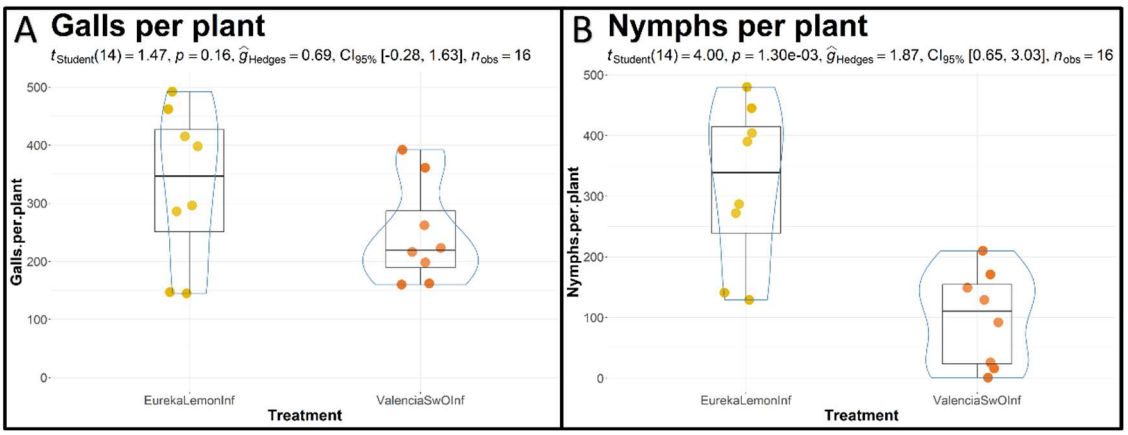

Figure 3.3. Violin/boxplots illustrate the distribution of pit galls (A) and nymphs (B) per infested plant of 'Eureka' lemon (EurekaLemonInf) and 'Valencia' sweet orange (ValenciaSwOInf). The statistical analysis applied was a Student's *t*-test ($t_{Student}$) with 14 degrees of freedom, the *p*-value (*p*), the effect size was assessed by Hedge's *g* ($\hat{g}_{Hedges}$), the confidence interval was set at 95% ($CI_{95\%}$), and the number of observations was 16 (n_{obs}).

3.5.2. Nymph infestation induces greater proteome changes in 'Valencia' sweet orange than in 'Eureka' lemon

The protein extracts from the enriched vascular sap were analysed by SDS-PAGE, which revealed distinct band profiles between infested and control plants (Fig. S3.1 - Appendix). The proteins identified in the enriched vascular sap by nanoLC-MS/MS were profiled and compared between the infested plants and their respective controls with the objective of assessing the molecular response at the proteome level of 'Eureka' lemon and 'Valencia' SwO plants infested with *T. erytreae* nymphs. A total of 5,050 proteins were identified in the enriched vascular sap. Of these, 33 were classified as contaminants and 1,471 had less than two unique peptides and were therefore excluded (Table S3.1 - Appendix). A library comprising 3,141 and 3,370 proteins was generated for 'Eureka' lemon and 'Valencia' SwO plants, respectively (FDR *q*-value <0.01) and used for the following comparisons: a) EurekaLemonInf *vs*. EurekaLemonCon; and b) ValenciaSwOInf *vs*. ValenciaSwOCon. A total of 48 DAPs were identified between infested and control 'Eureka' lemon plants (EurekaLemonInf *vs*. EurekaLemonCon). Of these, 22 were upregulated and 26 were downregulated (Fig. 3.4 and Table S3.2 - Appendix). In contrast, the comparison of the infested and control 'Valencia' SwO plants (ValenciaSwOInf *vs*. ValenciaSwOCon) revealed that 964 of the 1,265 DAPs were upregulated, while 301 were downregulated (Fig. 3.4 and Table S3.2 - Appendix).

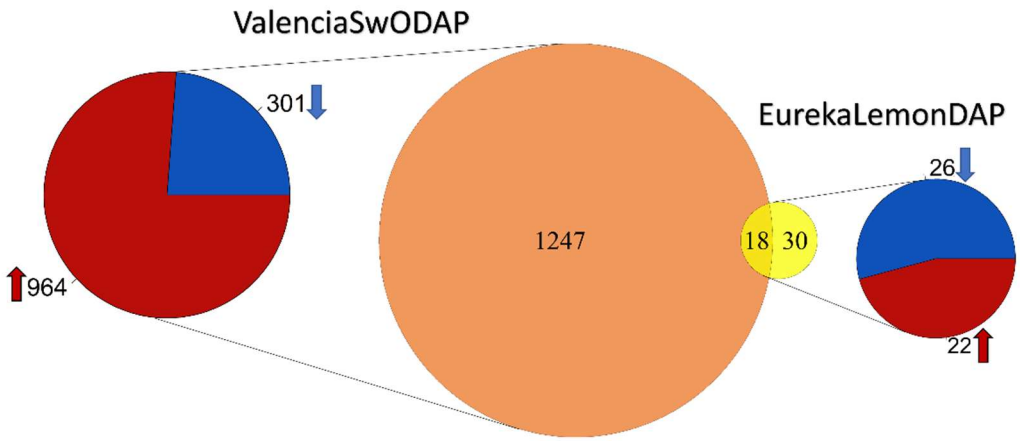

Figure 3.4. Overview of the differentially abundant proteins (DAPs) in 'Eureka' lemon and 'Valencia' sweet orange (SwO) plants infested with *T. erytreae* compared to the control plants (ValenciaSwOInf *vs*. ValenciaSwOCon and EurekaLemonInf *vs*. EurekaLemonCon, respectively). The orange circle in the centre of the figure shows the 1,265 DAPs found in the comparison between infested and control 'Valencia' SwO plants (ValenciaSwOInf *vs.* ValenciaSwOCon). Of these, 18 DAPs were common to the comparison between 'Eureka' lemon and ValenciaSwO plants, as represented in the intersection with the yellow circle. The smaller yellow circle shows the 48 DAPs found in the comparison between infested and control 'Eureka' lemon plants (EurekaLemonInf *vs*. EurekaLemonCon). The pie charts display the distribution of upregulated (red slice) and downregulated (blue slice) proteins in each DAP group. The pie chart on the right-hand side of the figure represents the 'Eureka' lemon group (EurekaLemonDAP), and the one on the left-hand side represents the 'Valencia' SwO group (ValenciaSwODAP).

In response to *T. erytreae*, a total of 18 DAPs were common to both citrus host species (Fig. 3.4). Of these, nine DAPs were upregulated and four were downregulated in both hosts, while the remaining five were downregulated in 'Eureka' lemon plants and upregulated in 'Valencia' SwO plants (Table 3.1). Among the common proteins that were upregulated in infested 'Eureka' lemon and 'Valencia' SwO plants were methyl esterase 10 (MES10), 3-oxo-2(2'-[Z]-pentenyl) cyclopentane-1-octanoic acid OPC-8:0 CoA ligase (OPLC1), cinnamyl alcohol dehydrogenase 8 (ELI3-2), chlorophyllase 1 (CHL1), raffinose synthase 6 (DIN10 or RS6) and catalase 2 (CAT2) (Table 3.1).

Table 3.1. Differentially abundant proteins (DAPs) in response to infestation by *Trioza erytreae* nymphs identified in both the 'Eureka' lemon and the 'Valencia' sweet orange (SwO). The *Arabidopsis thaliana* protein accession codes and protein descriptions were retrieved from the STRING (https://string-db.org/) and TAIR (https://www.arabidopsis.org/) databases.

***C.* ×*sinensis* protein accession number (www.uniprot.org/)**	***A. thaliana* protein accession number (www.arabidopsis.org/)**	**EurekaLemonInf Fold Change**	**ValenciaSwOInf Fold Change**	**Protein description**
A0A067GVT6	CPB (AT1G71790)	0.72	1.33	Subunits of heterodimeric actin filament capping protein Capz superfamily (CPB)
A0A067EE76	MES10 (AT3G50440)	0.65	0.86	Methylesterase 10 (MES10)
A0A067F307	ELI3-2 (AT4G37990)	0.50	0.20	cinnamyl alcohol dehydrogenase 8 (ELI3-2)
A0A067DRF8	AT2G43770	0.46	0.47	Transducin/WD40 repeat-like superfamily protein (AT2G43770)
A0A067G2K8	MTO1 (AT3G01120)	0.39	0.31	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein (MTO1)
A0A067DGV8	CLH1 (AT1G19670)	0.29	0.41	chlorophyllase 1 (CLH1)
A0A067GDP3	DIN10 (AT5G20250)	0.20	0.58	Raffinose synthase family protein (DIN10)
A0A067H294	OPLC1 (AT1G20510)	0.15	0.92	OPC-8:0 CoA ligase1 (OPCL1)
A0A067H2F2	CAT2 (AT4G35090)	0.12	0.65	catalase 2 (CAT2)
A0A067GDZ0	AT5G45910	**(-)** 0.15	0.23	GDSL-like Lipase/Acylhydrolase superfamily protein (AT5G45910)
A0A067FZU2	AT3G11210	**(-)** 0.19	0.33	SGNH hydrolase-type esterase superfamily protein (AT3G11210)
A0A067ESX7	AT3G51680	**(-)** 0.20	0.64	NAD(P)-binding Rossmann-fold superfamily protein (AT3G51680)
A0A067DPT0	AT3G05170	**(-)** 0.34	0.26	Phosphoglycerate mutase family protein (AT3G05170)
A0A067CZJ4	AT1G47480	**(-)** 0.38	0.42	alpha/beta-Hydrolases superfamily protein (AT1G47480)
A0A067GVX6	AT2G18570	**(-)** 0.18	**(-)** 0.37	UDP-Glycosyltransferase superfamily protein (AT2G18570)
A0A067D420	CHIA (AT5G24090)	**(-)** 0.351	**(-)** 0.75	chitinase A (CHIA)
A0A067EVR3	APK3 (AT3G03900)	**(-)** 0.46	**(-)** 0.27	adenosine-5'-phosphosulfate (APS) kinase 3 (APK3)
A0A067EPV6	AT5G09880	**(-)** 0.53	**(-)** 0.39	Splicing factor, CC1-like protein (AT5G09880)

A PCA was performed using all identified proteins from the enriched vascular sap samples of infested and control plants of both citrus host species. The results revealed a clear separation between the two groups along the first principal component axis. The infested plants clustered independently of the control plants in both species, namely in 'Eureka' lemon and 'Valencia' SwO (Fig. 3.5A and 3.5B). The first two principal components captured 48.9% and 62.9% of the total data variability for the 'Eureka' lemon comparison (Fig. 3.5A) and 'Valencia' SwO comparison (Fig. 3.5B), respectively. It may be inferred that the enriched vascular sap proteome was affected by *T. erytreae*.

An additional PCA analysis was conducted using the DAPs from the EurekaLemonInf *vs*. EurekaLemonCon and ValenciaSwOInf *vs*. ValenciaSwOCon comparisons. The results revealed that the first component accounted for 87.6% (Fig. 3.5C) and 76.1% (Fig. 3.5D) of the data variability, respectively. The scatter plot of the DAPs showed a clear separation along the first component axis. The top five proteins with the highest loading values in the two PCA (Table S3.3 - Appendix) are displayed in the score scatter plots (Fig. 3.5C and 3.5D). Two of these proteins, CLH1 and AT3G11210, were classified as "common DAPs" and are highlighted in the 'Eureka' lemon comparison (Table 3.1 and Fig. 3.5C).

The PCA constructed with the DAPs revealed two proteins that were of particular relevance among the five proteins displaying the highest loading values in the EurekaLemonInf *vs*. EurekaLemonCon comparison (Fig. 3.5C). The two proteins are: i) uridine diphosphate glucosyl transferase 76E1 (UGT76E1), which is linked to the wounding response in plants as well as to the formation of JA (Haroth et al., 2019); and ii) adenylylsulfate reductase 1 (APR1), which is related to sulfur metabolism (Bekturova et al., 2021). The proteins with the top five highest loading values in the ValenciaSwOInf *vs*. ValenciaSwOCon comparison (Fig. 3.5D) were linked to biological processes that will be discussed in more detail later. The following proteins were identified: sucrose phosphate synthase 1F (SPS1F), which is related to photosynthesis (Kaiser et al., 2016); pyridoxine/pyridoxamine 5'-phosphate oxidase 1 (PDXH), which is linked to the metabolism of vitamin B6 (González et al., 2007); a protein associated with the membrane trafficking

coatomer (alpha subunit, AT1G62020) (Xia et al., 2020); the protein alpha-galactosidase 1 (AGAL1) and biotin synthetases superfamily (AT3G02760) (Berg et al., 2005).

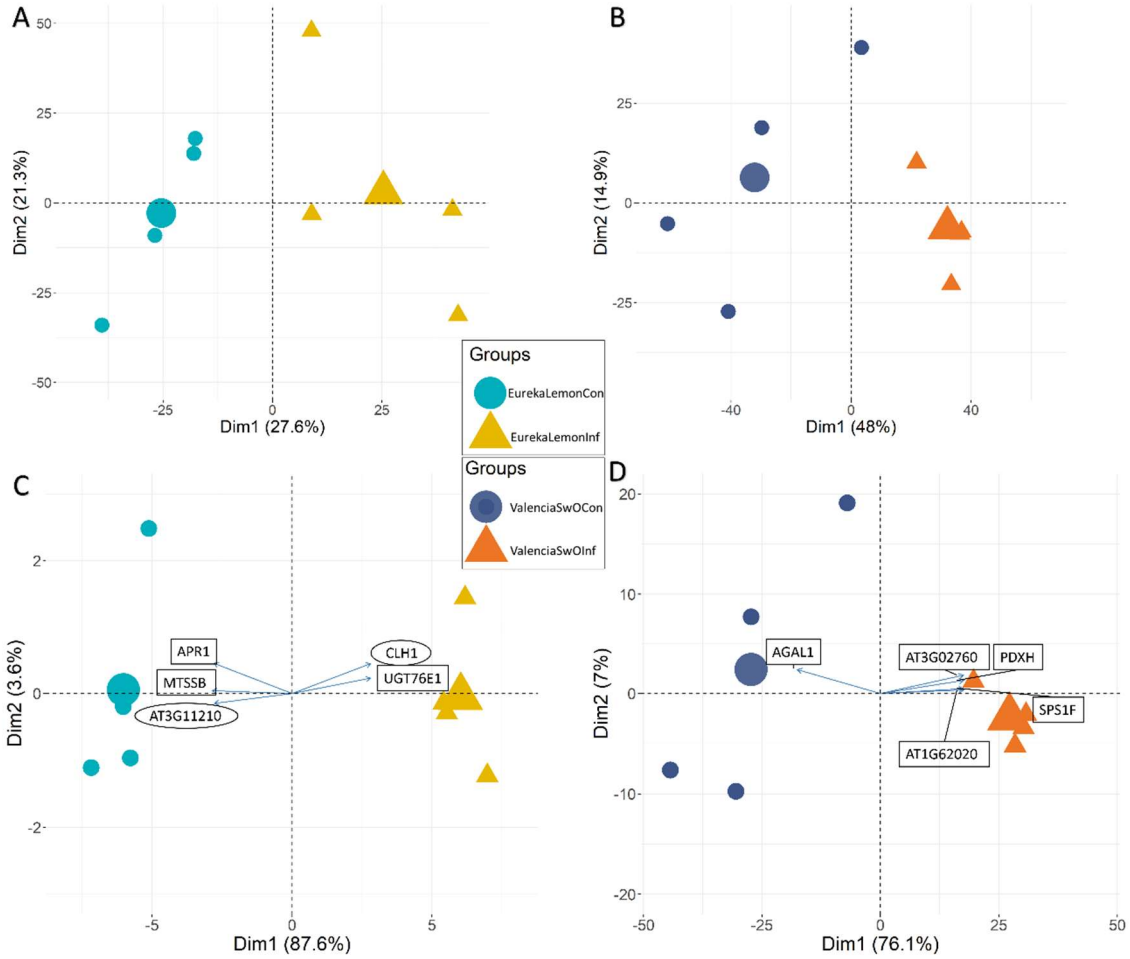

Figure 3.5. Principal component analysis (PCA) was used to analyse the enriched vascular sap proteomics data of 'Eureka' lemon (EurekaLemonInf *vs.* EurekaLemonCon) and 'Valencia' sweet orange (SwO) plants (ValenciaSwOInf *vs*. ValenciaSwOCon). The data is presented in the form of a two-dimensional plot, with biological replicates represented by circles (control samples) and triangles (infested samples). The mean value of each replicate group is represented by the largest circle or triangle. The orange colour represents the infested 'Valencia' sweet orange plants (ValenciaSwOInf), while the dark blue represents the control 'Valencia' sweet orange (ValenciaSwOCon). The yellow colour represents the infested 'Eureka' lemon plants (EurekaLemonInf) while the turquoise represents the 'Eureka' lemon control (EurekaLemonCon). A - PCA of 'Eureka' lemon plants using all identified proteins; B - PCA of 'Valencia' sweet orange plants using all identified proteins; C - PCA of 'Eureka' lemon plants using differentially abundant proteins (DAPs); D – PCA of 'Valencia' sweet orange plants using DAPs. The arrows indicate the top five greatest weighted variables. The percentages on the axes show their contribution to explain variance.

3.5.3. Functional analysis

Three clusters from the KEGG pathways were identified as significantly enriched (FDR < 0.05) in DAPs based on comparisons between the EurekaLemonInf *vs*. EurekaLemonCon group and the ValenciaSwOInf *vs*. ValenciaSwOCon group, using *A. thaliana* orthologues (Table S3.4 - Appendix). These clusters were classified into three distinct categories: 1) common responses in both groups; 2) opposite regulation of common pathways; and 3) species-specific responses. The common responses included all of the significantly enriched pathways with a common regulation in the 'Eureka' lemon and 'Valencia' SwO plants. The term "opposite regulation of common pathways" refers to all the significantly enriched pathways with opposite regulation between the two citrus host species. The species-specific responses comprised the KEGG pathways that were significantly and exclusively enriched in one of the citrus host species under study.

3.5.3.1. *Trioza erytreae´*s nymphs induce common responses from both citrus hosts

The KEGG pathways that were common to both citrus hosts may indicate the general response of these two species to psyllid nymph infestation. The four pathways that were commonly and significantly enriched (FDR < 0.05) were "Galactose metabolism", "Vitamin B6 metabolism" and "Selenocompound metabolism", which were upregulated, and "Amino sugar and nucleotide sugar metabolism", which were downregulated (Fig. 3.6).

3.5.3.2. Protein biosynthesis related pathways with opposite regulation in 'Eureka' lemon and 'Valencia' sweet orange in response to *Trioza erytreae*

The analysis of the enriched vascular sap of both citrus host species showed that there were four common enriched KEGG pathways that displayed opposing regulation. These were identified as being downregulated (FDR < 0.05) in 'Eureka' lemon and upregulated in 'Valencia' SwO plants. The pathways included the "spliceosome", "mRNA surveillance pathway", "protein processing in the

endoplasmic reticulum", which are related to protein biosynthesis, and "pantothenate and coenzyme-A (CoA) biosynthesis" (Fig. 3.6).

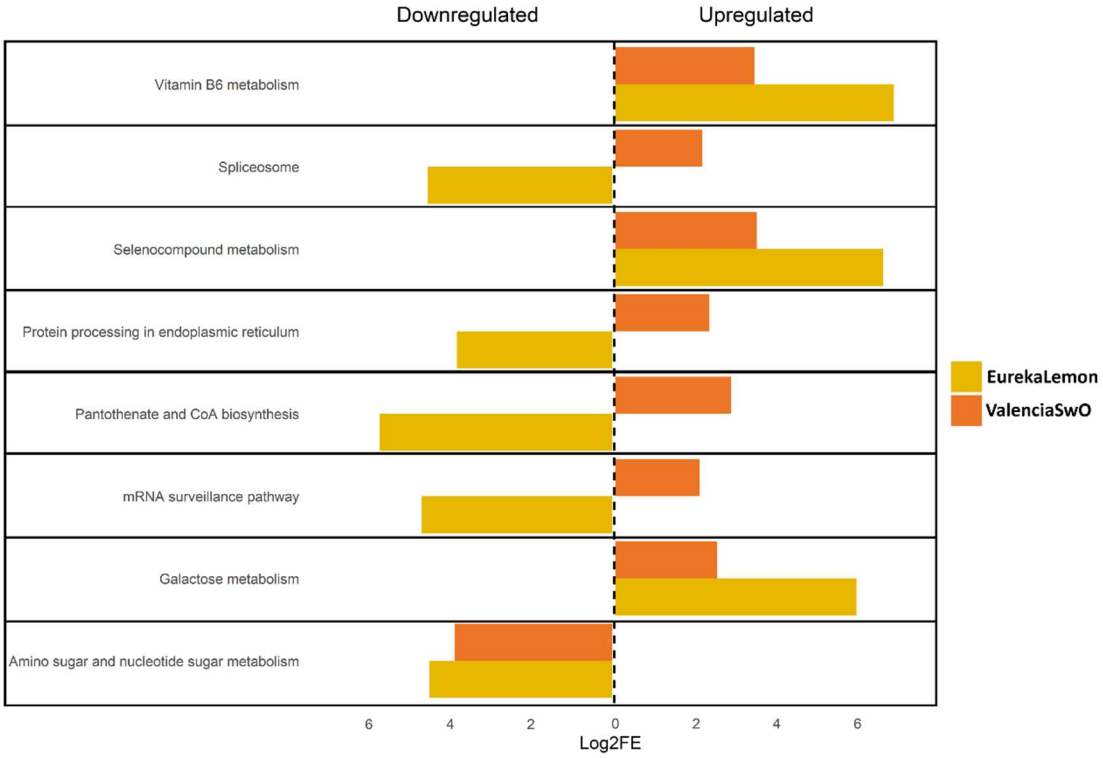

Figure 3.6. Common KEGG pathways significantly enriched (FDR < 0.05) in the response of 'Eureka' lemon and 'Valencia' sweet orange (SwO) to *Trioza erytreae* infestation. The enrichment was performed using differentially abundant proteins (DAPs). The bars to the left side of the vertical dashed line represent the downregulated pathways, while those to the right side represent the upregulated pathways. The yellow bars represent the fold enrichment of the EurekaLemonInf *vs*. EurekaLemonCon comparison, while the orange bars represent the fold enrichment of the ValenciaSwOInf *vs*. ValenciaSwOCon comparison. KEGG pathways were considered to be enriched when the false discovery rate (FDR) adjusted *p*-value was below the 0.05 threshold. Fold enrichment was calculated using the *Arabidopsis thaliana* proteome as a reference and $\log_2$ data transformation.

3.5.3.3. Two specific 'Eureka' lemon pathways in response to *Trioza erytreae*

In the EurekaLemonInf group only two KEGG pathways were identified as uniquely enriched: "Sulfur metabolism" and "Sesquiterpenoid and triterpenoid biosynthesis". These pathways were downregulated (Table S3.4 - Appendix and Fig. 3.7A).

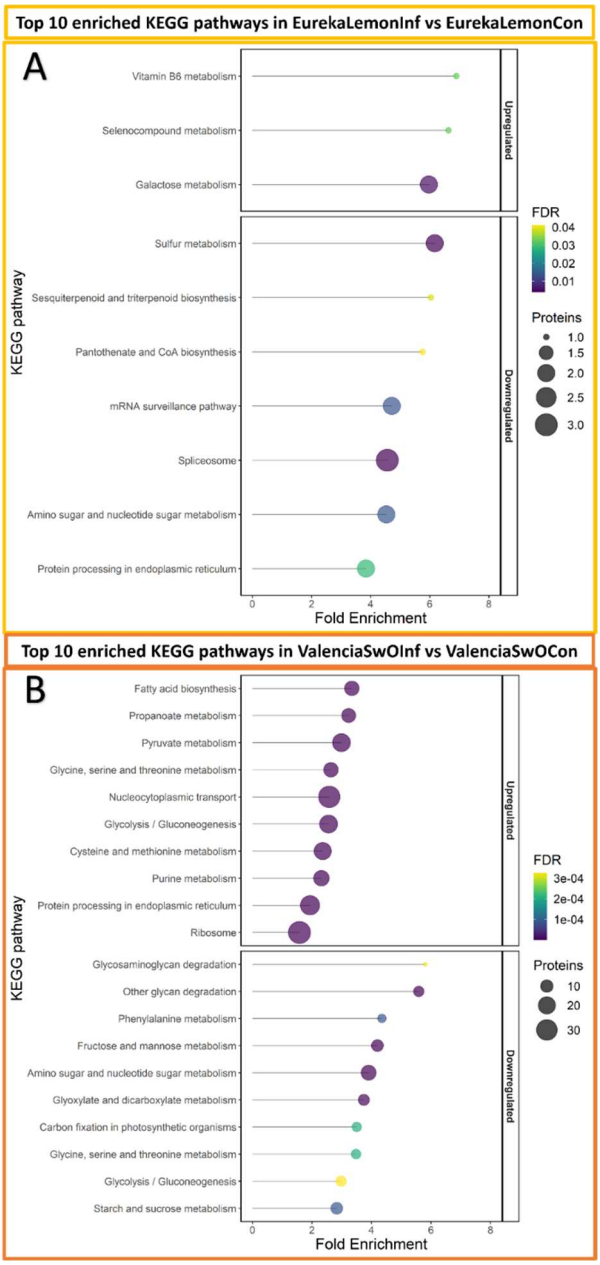

Figure 3.7. Bubble plot of the top 10 enriched KEGG pathways (based on an FDR-adjusted *p*-value threshold of 0.05) of up and downregulated proteins in infested plants. A - Enriched pathways in the EurekaLemonInf *vs*. EurekaLemonCon comparison. B - Enriched pathways in the ValenciaSwOInf *vs*. ValenciaSwOCon comparison. The size of the bubbles is indicative of the number of proteins present in each pathway, while the colour of the bubble represents the FDR-corrected *p*-value and fold enrichment, which were calculated using the *Arabidopsis thaliana* proteome as a reference.

3.5.3.4. Bigger and broader adjustment response of 'Valencia' sweet orange plants towards *Trioza erytreae* infestation

The ValenciaSwOInf group exhibited a considerably higher number of significantly enriched pathways (86), in comparison to the 10 that were enriched in the EurekaLemonInf group. Of the 86 pathways, 58 were upregulated, and 28 were

downregulated (Table S3.4 - Appendix). Fifty-one of the upregulated and 27 of the downregulated pathways were uniquely enriched in ValenciaSwOInf. In the ValenciaSwOInf group, the top 10 KEGG pathways enriched with the 964 upregulated proteins were related to biosynthesis, metabolism, and protein synthesis and processing (Fig. 3.7B), while the top 10 KEGG pathways associated with the 301 downregulated proteins were related to metabolism, degradation, and carbon fixation (Fig. 3.7B). The proteomics results suggest that there was a significant general metabolic adjustment in the 'Valencia' SwO plants, which was characterised by the upregulation of pathways related to respiration, including "Glycolysis and gluconeogenesis" (Fig. 3.7B and 3.8B), "Pyruvate metabolism", the "Citrate cycle", and the "Propanoate metabolism" (Table S3.4 - Appendix and Fig. 3.8B), as well as "Fatty acid biosynthesis". Pathways that were negatively affected, included "Fructose and mannose metabolism", "Glyoxylate and dicarboxylate metabolism", "Starch and sucrose metabolism" and "Carbon fixation in photosynthetic organisms" (Fig. 3.7B and 3.8B). *Trioza erytreae* infestation caused changes in amino acid metabolism, as evidenced by the upregulation of the "Cysteine and methionine metabolism" pathway and the downregulation of the "Phenylalanine metabolism" and "Glycine, serine, and threonine metabolism" pathways (Fig. 3.7B).

It is also noteworthy that two plant defence pathways were found to be upregulated, namely "α-Linolenic acid metabolism" (Table S3.4 - Appendix and Fig. 3.8A) and "Plant-pathogen interaction". However, these pathways were not among the top 10 pathways identified in Table S3.4 - Appendix and Fig. S3.2 - Appendix.

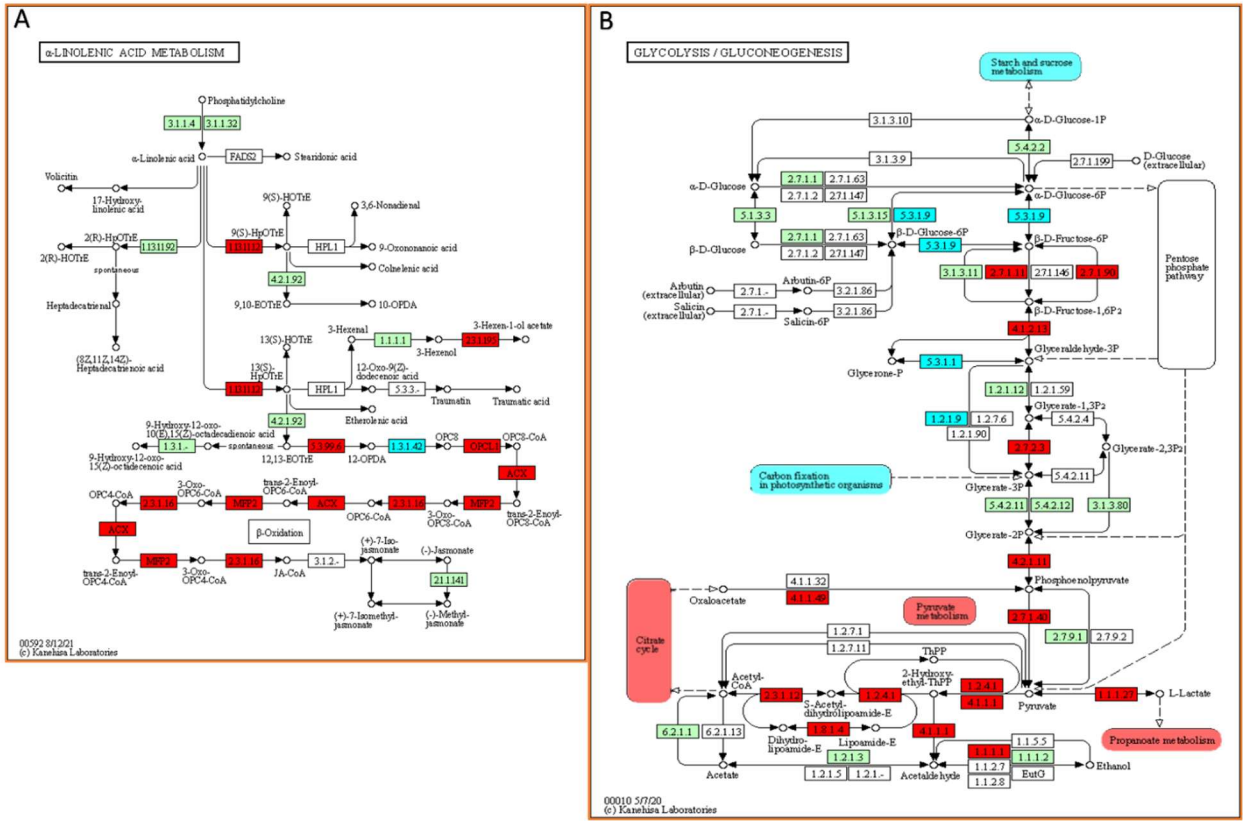

Figure 3.8. Representative scheme of the enriched KEGG pathways in infested 'Valencia' sweet orange plants. KEGG pathway representations are adaptations of the pathway maps available at "https://www.genome.jp/kegg/pathway.html". The red filled rectangles represent the upregulated proteins and pathways, the blue filled rectangles represent the downregulated proteins and pathways, and the green filled rectangles represent the proteins described for *Arabidopsis thaliana* in this pathway (and that were not differential in this study). A. α-Linolenic acid metabolism pathway; B. Glycolysis/Gluconeogenesis pathway

3.6. Discussion

The high protein levels observed in the samples derived from the leaf and petiole midribs may be attributed to the vascular sap and apoplastic fluid, in addition to the cellular contents of various cell types, which may have been collected during the centrifugation process. Such contents include those of parenchyma cells, phloem companion cells, and mesophyll cells, which were adjacent to the midrib vein of the leaf. The sieve elements are expected to facilitate the transport a considerable quantity of protein. In fact, studies have described protein synthesis and turnover in the sieve elements, along with the presence of high levels of small RNAs (sRNA) and proteins that have nuclear functions (Ham and Lucas, 2017; Lin et al., 2009; Ostendorp et al., 2017; Saplaoura and Kragler, 2016). The process of centrifugation to enrich our samples with vascular sap was previously described by Franco et al. (2020) as a method that can also extract proteins from nuclei.

3.6.1. *Trioza erytreae* nymphs developed better in 'Eureka' lemon in comparison to 'Valencia' sweet orange

The sexually mature adult *T. erytreae* individuals used in this experiment were randomly reared on sour orange and lemon plants. The use of these plants for psyllid rearing may induce a preference bias for the lemon tree as a host. A similar bias was observed in *D. citri* (Stockton et al., 2017). However, Moran (1968a) reported that the plant species used for rearing *T. erytreae* (even after two generations) had no effect on host preference. Furthermore, it has been reported that the intensity of flushing affects the development of *T. erytreae* (Catling, 1969). However, the present study revealed no statistically significant differences in the number of new flushes between the two citrus host species assayed, thereby suggesting that this trait is unlikely to serve as a differentiating factor.

It was anticipated that the number of nymphs per citrus host would be comparable, given that each citrus host was exposed to the same number of female and male adult insects. Additionally, a single *T. erytreae* nymph is responsible for the formation of a single pit gall, which typically occurs within the initial days

following oviposition (early instars) (Van den Berg et al., 1991d). The number of pit galls formed on the 'Eureka' lemon and the 'Valencia' SwO hosts was comparable. However, the observation of a reduced number of nymphs developing in 'Valencia' SwO plants, in conjunction with the presence of numerous leaves exhibiting a darker colour in the secondary and tertiary leaf veins, suggests that the 'Valencia' SwO host plant response may have had a detrimental impact on nymph development. The response of the two citrus host species to psyllid infestation was expected to be revealed in the composition of the enriched vascular sap, which transports nutrients and metabolites in response to biotic stress, including molecules of the immune response (Will et al., 2013).

3.6.2. Nymph infestation induces greater proteome changes in 'Valencia' sweet orange than in 'Eureka' lemon

Of the nine common proteins that were upregulated in both citrus hosts (Table 3.1), of particular note was MES10 and OPLC1, which are both related to JA metabolism and the "JA signalling pathway". This pathway represents one of the plant responses to insect infestation (Kienow et al., 2008; Koo et al., 2013; Ruan et al., 2019). Additionally, other upregulated proteins were associated with the response to herbivory and bacteria, respectively ELI3-2, encoded by the gene *AT4G37990*, and CHL1 (Kariola et al., 2005; Klauser et al., 2015). The proteins DIN10 and CAT2 are involved in the response of plants to oxidative stress (Lelarge-Trouverie et al., 2023; Molassiotis and Fotopoulos, 2011). The upregulation of CAT2 and phosphatase 2A (PP2A) in the citrus plants may indicate that these proteins play a pivotal role in the response to the psyllid infestation. Indeed, wild-type *A. thaliana* exhibited a reduction in the population of phloem-feeding insects in comparison to mutant plants lacking one of the aforementioned proteins (Rasool et al., 2020). Among the set of downregulated proteins, chitinase A (CHIA), was identified as one of the four proteins that were downregulated in both citrus hosts (Table 3.1). This protein has previously been described to be a common constituent of phloem and xylem sap (Rodríguez-Celma et al., 2016). Furthermore, diverse chitinases were modified in response to insect infestation in tea plants [*Camellia sinensis* (L.) O. Kuntze] (Bordoloi et al., 2021).

The higher number of DAPs in the ‘Valencia’ SwO in comparison with the ‘Eureka’ lemon plants (Fig. 3.4) points to a higher adjustment of the former´s proteome to *T. erytreae* nymph infestation. It is noteworthy that exposure to the bacteria that causes HLB disease revealed that the ‘Lisbon’ lemon exhibited a lower number of DAPs than the ‘Washington Navel’ SwO (Chin et al., 2021). The reduced reprogramming of the defence response may explain why lemon plants are the preferred host of *T. erytreae* and nymphs develop more successfully. Indeed, it has been demonstrated that nymphs that developed in lemon plants exhibited a larger size (Aidoo et al., 2019c; Aubert, 1987), and that the psyllid had a higher intensity of settlement, probing, and feeding (Benhadi-Marín et al., 2021). Conversely, the interaction between the psyllid and the lemon plant may result in the activation of additional responses that are not covered in a proteomic approach. One potential avenue for further investigation would be to study the transcription of regulatory genes that impact host vulnerability. Another mechanism to be studied may be the induction of the salicylic acid (SA) pathway, which is commonly induced by aphids and sap-sucking insects. This pathway prevents the plant from fully activating the JA and ethylene plant defence pathways (Åhman et al., 2019). Nevertheless, this pathway was not identified in ‘Eureka’ lemon plants in the present study.

3.6.3. Functional analysis

3.6.3.1. *Trioza erytreae*´s nymphs induce common responses in both citrus host species

In response to the infestation of *T. erytreae*, both citrus host species exhibited an upregulation of proteins involved in "Galactose metabolism" and "Vitamin B6 metabolism" pathways (Fig. 3.6). The only protein from the “Galactose metabolism” pathway that was upregulated in both species was raffinose synthase 6 (DIN10 or RS6) (Tables S3.2 and S3.4 - Appendix). In general, the response of plants to stress involves raffinose synthases (Yan et al., 2022) and the “Galactose metabolism” pathway has been related to ascorbate biosynthesis (Bulley and Laing, 2016), which scavenges reactive oxygen species (ROS) and so protects plant cells (Lisko et al., 2014). Additionally, both citrus host species exhibited an upregulation

of proteins related to vitamin B6 synthesis (Fig. 3.6). In particular, aldolase-type triosephosphate isomerase (TIM) barrel family protein (RSR4) was upregulated in the 'Eureka' lemon, whereas in 'Valencia' SwO, bifunctional pyridoxine (pyridoxamine) 5'-phosphate oxidase (PDXH) and pyridoxamine 5-phosphate oxidase (AT2G46580) were found to be upregulated (Tables S3.2 and S3.4 - Appendix). Vitamin B6 has been described as having antioxidant properties and to regulate redox balance during the defence response of tobacco (*Nicotiana tabacum* L.) to bacteria (Denslow et al., 2005). A deficiency of vitamin B6 has been shown to render plants more susceptible to biotic stress (Havaux et al., 2009; Shi et al., 2002). Moreover, the regulation of the sink and source dynamics in plants may also explain the enrichments of both pathways. This phenomenon may be attributed to the fact that insects that feed on the phloem can act as additional sinks for the plant while infesting it (Schultz et al., 2013). The phloem-feeding psyllid, *D. citri*, has been described to ingest galactose (Hijaz et al., 2016a; Hijaz and Killiny, 2014b). We hypothesise that *T. erytreae* might act as a galactose sink, which would explain the upregulation of proteins from the "Galactose metabolism" pathway. Additionally, B-complex vitamins are essential for insect nutrition due to their inability to synthesise these compounds de novo (Douglas, 2017). The downregulation of the "Amino sugar and nucleotide sugar metabolism" pathway in both citrus host species (Fig. 3.6), is consistent with the description of an adjustment in amino acid balance in plants affected by phloem feeders (Douglas, 2006). Indeed, a distinct amino acid profile was observed in mandarin plants in response to infestation by *D. citri* (Malik et al., 2014).

3.6.3.2. Protein biosynthesis related pathways with opposite regulation in 'Eureka' lemon and 'Valencia' sweet orange in response to *Trioza erytreae*

The upregulation of proteins from the "spliceosome", "mRNA surveillance pathway", "protein processing in endoplasmic reticulum", "nucleocytoplasmic transport", "ribosome" and "pantothenate and coenzyme-A (CoA)" pathways in 'Valencia' SwO plants is indicative of an increased flux of proteins and possible enhanced protein synthesis rate (Fig. 3.6 and 3.7B). Pathways related to protein

biosynthesis were identified as being enriched in maize (*Zea mays* L.), and lima bean (*Phaseolus lunatus* L.) in response to herbivory and other biotic stressors (Arimura et al., 2000; Asters et al., 2014). Notably none of these pathways were upregulated in 'Eureka' lemon plants. Furthermore, the "mRNA surveillance pathway" and the endoplasmic reticulum (ER) associated degradation system (ERAD) were significantly different between the two citrus host species. The proteins in question were downregulated in EurekaLemonInf and upregulated in ValenciaSwOInf (Fig. 3.6 and Fig. S3.3 - Appendix). As the ERAD addresses misfolded and unfolded polypeptides (Liu and Howell, 2010), an increase in protein synthesis is typically associated with the plant´s response to biotic stress and the synthesis of stress-related proteins (Sharma and Dubey, 2016).

In summary, the different proteomic fingerprints of the common functional pathways with opposing regulation identified in the enriched vascular sap proteome appear to be related with the suitability of the two citrus host species to *T. erytreae*. It is noteworthy that the 'Eureka' lemon, which is the most suitable host, and 'Valencia' SwO plants seem to exhibit dissimilar responses to the psyllid infestation.

3.6.3.3. Two specific downregulated 'Eureka' lemon pathways in response to *Trioza erytreae*

The proteins that were uniquely enriched and downregulated in EurekaLemonInf belong to the "Sulfur metabolism" and the "Sesquiterpenoid and triterpenoid biosynthesis" pathways (Table S3.4 - Appendix D and Fig. 3.7A). Interestingly, the "Sulfur metabolism" pathway was enriched in SwO and two additional Rutaceae hosts infested with *D. citri*, namely *Murraya paniculata* (L.) Jack and *C. japonica* Thunb.. However, this pathway was not enriched in the more attractive mandarin host (Zhong et al., 2019). The downregulation of proteins from the "Sulfur metabolism" pathway may provide an explanation for the preference of *T. erytreae* nymphs for lemon hosts. Sulfur metabolism plays a pivotal role in plant growth, survival, and defence (Capaldi et al., 2015). In addition, the downregulation of proteins from the "Sulfur metabolism" pathway may be related with the upregulation of proteins from the "Selenocompound (*Se*) metabolism" pathway (Fig. 3.7A), as *Se* and sulfur isolog compounds compete in biochemical processes

(Abdalla et al., 2020; Trippe and Pilon-Smits, 2021). Terpene synthase 21 (TPS21) from the "Sesquiterpenoid and triterpenoid biosynthesis" pathway was downregulated in EurekaLemonInf and upregulated in ValenciaSwOInf (Fig. 3.7A and Table S3.2 - Appendix). This enzyme is involved in the formation of sesquiterpenes (Tholl and Lee, 2011), and in mandarin plants infested by the psyllid *D. citri*, sesquiterpene concentrations as leaf volatiles increases (Jones and Killiny, 2021). These findings also suggest that the detected compounds were not derived solely from the vascular sap.

In conclusion, the limited number of significantly and uniquely enriched pathways in infested 'Eureka' lemon seems to indicate that their defence mechanism is very distinct from that of 'Valencia' SwO. This may also explain the higher survival rate of *T. erytreae* nymphs in 'Eureka' lemon compared to 'Valencia' SwO (Fig. 3.3). It is plausible that additional defensive mechanisms may have been triggered in the 'Eureka' lemon that were not identified in enriched vascular sap using proteomics.

3.6.3.4. Bigger and broader adjustment of 'Valencia' sweet orange plants towards *Trioza erytreae* infestation

Of particular interest among the pathways that were uniquely enriched and upregulated in ValenciaSwOInf was the "fatty acid biosynthesis" pathway (Fig. 3.7B). This pathway is associated with the synthesis of structural barriers to the external environment and the adjustment of cell membrane fluidity, which is a useful stress response (Upchurch, 2008). Furthermore, the "fatty acid biosynthesis" and "α-Linolenic acid metabolism" pathways (Table S3.4 - Appendix and Fig. 3.8A), were upregulated. Proteins involved in the "α-Linolenic acid metabolism" pathway are associated with the synthesis of JA (Fig. 3.8A), a general pattern activated in plants exposed to insect herbivory (Ruan et al., 2019; Upchurch, 2008). For example, 'Valencia' SwO variety exposed to *D. citri* exhibited increased jasmonic acid (JA) levels (Nehela et al., 2018). The same insect–host system also showed upregulated lipoxygenase (LOX), allene oxide cyclase (AOC) and oxophytodienoate-reductase 3 (OPR3) gene transcripts, which are affected to the JA signalling pathway (Erb and Reymond, 2019; Sun et al., 2022). Both LOX2 and AOC3 were upregulated

in *T. erytreae* infested 'Valencia' SwO plants (Table S3.2 - Appendix) and these proteins were previously reported as common constituents of the phloem sap defence response (Kehr, 2006). The current findings suggest that the JA signalling pathway is triggered in SwO in response to the two psyllids.

The upregulation of respiration metabolism-related pathways, including "Pyruvate metabolism", "Citrate cycle", "Glycolysis/Gluconeogenesis", the "Propanoate metabolism", and the concomitant downregulation of photosynthesis-related pathways, such as the "Carbon fixation in photosynthetic organisms" (Table S3.4 - Appendix, Fig. 3.7B and 3.8B) represents a general pattern of plant response to stress (Kerchev et al., 2012).

With regard to the downregulation of the "Carbon fixation in photosynthetic organisms" pathway, it is represented by the alanine aminotransferase 2 (ALAAT2), aspartate aminotransferase 3 (ASP3), triosephosphate isomerase (TPI), and two fructose-bisphosphate aldolases (FBA2 and FBA5) (Tables S3.2 and S3.4 – Appendix). Similarly, SwO 'Madam Vinous' and tomato (*Solanum lycopersicum* L.) plants showed a downregulation of photosynthesis-related pathways and TPI proteins, respectively, in response to sap-feeding insects (Coppola et al., 2013; Mozoruk et al., 2006). The observed phenotypic alterations in the secondary and tertiary veins of 'Valencia' SwO leaves may be attributed to alterations in protein pathways, the disassembly of chloroplasts, and the reallocation of nutrients.

A considerable number of proteins related to the "Photorespiration" pathway module, such as the alanine:glyoxylate aminotransferase (AGT) and serine hydroxymethyltransferase 1 and 3 (SHM1 and SHM3), were negatively regulated (Tables S3.2 and S3.4 - Appendix, and Fig. S3.2 - Appendix). Given that photorespiration affects several primary metabolic pathways, it is plausible that these proteins exert an influence on the growth and development of the 'Valencia' SwO host. On the opposite, the exposure to phloem-feeding insects led to the high expression of photorespiration-linked pepper (*Capsicum annuum* L.) proteins (Florencio-Ortiz et al., 2021), whereas proteins SHM1 and SHM3 of olive (*Olea europaea* L.) were downregulated (Corrado et al., 2012). Therefore, it may be

concluded that the type of stress experienced and the specific host are determining factors in the regulation of this pathway.

The present study identified a wide range of proteins from the "plant–pathogen interaction" pathway (outside of the top 10 KEGGs) that were upregulated in 'Valencia' SwO plants (Tables S3.2 and S3.4 - Appendix and Fig. S3.2 - Appendix). To the best of our knowledge, this pathway has only been observed to be upregulated in citrus plants in response to fungi (Zhang et al., 2021) and bacteria (Peng et al., 2021; Sharma et al., 2021), but not when exposed to a psyllid. In particular, the hypersensitive response seems to be activated in 'Valencia' SwO plants by the upregulation of the protein kinases 3 and 4 (MPK3 and MPK4), cysteine- and histidine-rich domain-containing protein RAR1 (PBS2 or PPHB susceptible 2) and calcium-dependent protein kinases 6 and 9 (CPK6 and CPK9) (Tables S3.2 and S3.4 - Appendix and Fig. S3.2 - Appendix)Although bacteria and fungi are commonly identified as the primary agents responsible for the hypersensitive response, recent research suggest that insects may also elicit this response (Balint-Kurti, 2019). Calcium-related protein kinases (CPK6 and CPK9), which serve as sensors of wounding and feeding (Erb and Reymond, 2019) have been identified in 'Valencia' SwO in response to *D. citri*, being described as phloem sap defence proteins (Kehr, 2006; Wei et al., 2021). According to Sun et al. (2022), the 'Succari' SwO response to *D. citri* resulted in the downregulation of CPK9 transcripts, a response that was not observed in 'Valencia' SwO. Proteins of the "Salicylic acid (SA) pathway", which are frequently upregulated during a plant defensive response, were not identified in the responses of the two citrus host species to the psyllid. In contrast, the 'Succari' SwO responded to *D. citri* by upregulating gene transcripts associated with the SA pathway (Sun et al., 2022).

Overall, the fact that more enriched pathways were found in the infested 'Valencia' SwO plants than in the infested 'Eureka' lemon plants indicates that the former exhibited a more pronounced response to the *T. erytreae* infestation. The response was characterised by metabolic, protein synthesis, and defence adjustments. Furthermore, the present results seem to indicate that the 'Valencia'

SwO triggers the hypersensitive response. The collective effect of these proteomic modifications may hamper the development of *T. erytreae* nymphs.

3.7. Conclusion

This study provides an in-depth analysis of the enriched vascular sap proteome of 'Eureka' lemon and 'Valencia' SwO when infested with *T. erytreae* nymphs. The results reveal that nymphs caused a significant modification of the proteome of the enriched vascular sap of young leaves. Upon exposure to *T. erytreae*, the 'Valencia' SwO showed the most noticeable alterations, which included upregulation of proteins related to respiration, protein biosynthesis, and stress-specific responses, such as the hypersensitive response, antioxidant proteins, and JA signalling pathways. The downregulation of proteins associated with photosynthesis, photorespiration, and carbohydrate synthesis in the enriched vascular sap of 'Valencia' SwO suggests that these proteins are part of a defensive response, as such responses are commonly observed in plants infested by sap-feeding insects. Furthermore, proteins that form structural barriers and alter the fluidity of cell membranes were found to be upregulated. These modifications are typical responses to biotic stress and may be indicative of a defensive mechanism that renders this host less suitable for the growth of *T. erytreae*. The proteomic response of 'Eureka' lemon to *T. erytreae* infestation was less pronounced than that observed in the 'Valencia' SwO response. Of particular note was the downregulation of sulfur metabolism, which plays a pivotal role in plant defence. In conclusion, the proteomic analysis of the enriched vascular sap of the two citrus host species revealed distinct responses to psyllid infestation. The present results prompt further investigation into alternative defence responses that may have been activated and not captured in the proteomic study. The current findings identify promising new areas for research and emphasise the need for further studies into the cross-talk between *T. erytreae* and citrus species, with the aim of defining the factors that affect the choice of host plant by the psyllids.

Chapter 4. A comparative metabolomic and proteomic study of sweet orange and lemon trees infested by *Trioza erytreae* (Del Guercio, 1918)

In preparation:

Magalhães, T., Bento-Silva, A., Anjos, L., Pereira, J. A., Duarte, A., Dandlen, S. A., Bronze, M. R., Power, D. M., Duarte, N., Marques, N. T. A comparative metabolomic and proteomic study of sweet orange and lemon trees infested by *Trioza erytreae* (Del Guercio, 1918)

4.1. Abstract

The psyllid *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae) is the vector of Huanglongbing (HLB), a highly harmful bacterial disease caused by *Candidatus* Liberibacter spp.. *Trioza erytreae* has recently incurred in the Iberian Peninsula, albeit not carrying the bacteria. In this study sweet orange [*Citrus* ×*sinensis* (L.) Osbeck] (SwO), a common host, and lemon [*Citrus* ×*limon* (L.) Osbeck], the preferred host were infested by *T. erytreae*. The number of nymphs that developed on lemon trees was found to be three times higher than on sweet orange. A multi-omics comparative approach was employed for analysis of infested and uninfested samples of both citrus hosts. Enriched vascular sap was analysed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) for the metabolomic profile, and by nanoscale liquid chromatography coupled to MS/MS (nanoLC-MS/MS) for the proteomic profile. Although the number of differentially abundant metabolites (DAMs) was comparable, i.e., 10 DAM in SwO and 12 DAM in lemon, differences were evident in the number of differentially abundant proteins (DAPs). The 1,265 DAPs in SwO yielded 53 enriched pathways, which overlapped with the three DAM enriched pathways, all related to primary metabolism. Furthermore, pipecolic acid and jasmonic acid were upregulated in SwO. In lemon, the 48 DAPs enriched three pathways, which did not overlap with the pathways enriched by DAMs. In lemon, the phenylpropanoid biosynthesis pathway was upregulated with metabolites, such as the phenylalanine and *p*-coumaric acid. Our findings indicate the potential for a resistance-based response towards *T. erytreae* in SwO, while the lemon host adopted a tolerance-based response.

4.2. Abbreviations

CoA (coenzyme A); DAMs (differentially abundant metabolites); DAPs (differentially abundant proteins); FDR (false discovery rate); HLB (huanglongbing); HPLC (high-performance liquid chromatography); HPLC-DAD-MS/MS (high-performance liquid chromatography coupled with a diode array detector and tandem mass spectrometry); HPLC-MS/MS (high-performance liquid chromatography coupled with tandem mass spectrometry); KEGG (Kyoto Encyclopedia of Genes and Genomes); LemonCon ('Eureka' Lemon control); LemonInf (infested 'Eureka' lemon); MRM (multiple reaction monitoring); MS (mass spectrometry); nanoLC-MS/MS (nanoscale liquid chromatography coupled to tandem mass spectrometry); SwO (sweet orange, *C.* ×*sinensis*); SwOCon ('Valencia' sweet orange control); SwOInf (infested 'Valencia' sweet orange). TCA (tricarboxylic acid).

4.3. Introduction

Huanglongbing (HLB) is a bacterial citrus disease associated with the phloem-limited bacteria *Candidatus* Liberibacter (da Graça et al., 2016). The disease has resulted in a 74% reduction in citrus production in Florida (Singerman and Rogers, 2020) and has prompted a transformation in the citriculture management practices in Brazil (Bassanezi et al., 2020). At present, there is no known cure for this disease, and its management is based on the elimination of the inoculum and on vector control (Alquézar et al., 2022). The vectors of HLB have been identified as *Trioza erytreae* (Del Guercio, 1918) (Hemiptera: Triozidae) and *Diaphorina citri* (Kuwayama, 1908) (Hemiptera: Psyllidae) (Aubert, 1987). These two vectors have recently been introduced to the Mediterranean region, *T. erytreae* from the western side (EPPO, 2021) and *D. citri* from the eastern side (EPPO, 2023, 2022), despite not currently harbouring the bacterium. *Trioza erytreae* is expanding within the citrus-producing regions of Spain and Portugal, which are among the top four citrus producers in the European Union (EU) (Duarte et al., 2024; FAO, 2021).

The psyllid *Trioza erytreae* exhibits a marked preference for trees and shrubs of the Rutaceae family, with lemon tree [*Citrus ×limon* (L.) Osbeck] representing the preferred host. Common hosts include sweet orange (SwO) trees [*Citrus ×sinensis* (L.) Osbeck] and occasional hosts include *Citrus trifoliata* L. (Aubert, 1987; Magalhães et al., 2025). The feeding and oviposition rates of *T. erytreae*, as well as its development and morphology, are influenced by the host (Aidoo et al., 2019c; Benhadi-Marín et al., 2021). *Trioza erytreae* life cycle includes five nymphal instars. These nymphal stages of psyllids are mainly sedentary, and their activity is focused on feeding and developing (Van den Berg et al., 1991d). This behaviour makes the fourth and fifth nymphal instars a desirable study object for a high feeding interaction with its host. Until now there is a paucity of studies about the molecular mechanisms underlying host preference of the psyllid, as well as the impact of the host on the psyllid. Although there is evidence that terpenes influence the behaviour of *T. erytreae* (Antwi-Agyakwa et al., 2019), it is anticipated that there are numerous molecular-level interactions in the plant that may contribute to host choice, and which remain to be elucidated. HLB vectors exhibit a preference for feeding on

phloem sap, although the xylem sap is also ingested at a reduced rate (Benhadi-Marín et al., 2021; Ebert et al., 2018).

The response of host plants to biotic stressors, such as insect feeding, can vary considerably, which is attributed to two distinct yet interrelated possible reactions: plant resistance and tolerance to stress. The concept of plant stress resistance entails the allocation of plant resources to deter the stressor, which may result in reduced plant fitness. In contrast, stress tolerance can be defined as the ability to endure stress with low expenditure of resources to cope with it, prioritising the preservation of plant fitness (Leimu and Koricheva, 2006; Simms and Triplett, 1994). The distinct plant defence systems are heritable dynamic mechanisms and vary between citrus species and between cultivars of the same species (Yu et al., 2022). The vascular system is the tissue that facilitates the transmission of molecular signals that modulate tolerance and resistance mechanisms (Dinant and Lucas, 2012). A plant´s response to stress is indicated by a broader molecular adjustment, and an increase in defence-related compounds, which is suggestive of an active/resistant plant response to stress (Kosová et al., 2011). We anticipated that the degree of molecular adjustment in the vascular system in infested plants may serve as an indicator of the plant's type of response.

In the vascular system, the xylem sap plays a role in the plant's defence responses against biotic stress (Yang et al., 2020b). However, the primary contributors are the phloem, the phloem companion cells and the phloem parenchyma cells, which provide metabolites to the sieve elements (Hunt et al., 2023). The diversity of metabolites in the phloem and xylem sap includes amino acids, carbohydrates, organic acids, nucleosides, phytohormones and secondary metabolites (Broussard et al., 2023; Surano et al., 2024). Some of these compounds play important roles in regulating the stress response of plants to phytophagous insects (Gabryś et al., 2015; Hunt et al., 2023). Carbohydrates that circulate in phloem sap, including glucose and fructose, and nucleosides which are involved in both primary and secondary metabolic pathways in plants (Broussard et al., 2023). Amino acids, such as proline, serve as intermediates for bioactive compounds implicated in plant stress responses (Banothu and Uma, 2022; Moormann et al.,

2022). Other compounds that contribute to plant defence mechanisms are the organic acids, such as citric acid, fumaric acid, and quinic acid, alkaloids like pipecolic acid, and the phenylpropanoids like p-coumaric acid, and caffeic acid (Riaz et al., 2018; Vranova et al., 2013; Walsh et al., 1987). The phytohormones jasmonic acid and salicylic acid circulate in the phloem sap, interact with each other and orchestrate plant immune responses, including the regulation of plant defences against insects (Vaishnav and Chowdhury, 2023; Zhu et al., 2014).

An in-depth knowledge of the intricate interactions between psyllids and their host plants is imperative, and the integration of omics studies has proved pivotal in advancing the research in this area. In the present study an untargeted multi-omics (metabolomics and proteomics) approach was used to understand the molecular adjustments occurring in the enriched vascular sap of 'Valencia' SwO and 'Eureka' lemon trees in response to *T. erytreae* infestation. Enriched vascular sap, containing phloem sap, xylem sap and adjacent cell debris, was extracted from the midribs of leaves and petioles from both infested and non-infested trees. Metabolites were analysed by high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) and proteins by nanoscale liquid chromatography coupled to MS/MS (nanoLC-MS/MS). The modifications that occurred in the metabolites and proteins of the enriched vascular sap of 'Valencia' SwO and 'Eureka' lemon trees were analysed and discussed as to their involvement in the response to *T. erytreae*.

4.4. Materials and methods

4.4.1. Citrus host species and psyllid rearing

Trioza erytreae adults were captured with a handheld aspirator into conical centrifuge tubes (50 mL), from lemon orchards with a pesticide-free management, in the Porto district in 2021 (Portugal, 41°18´46.4´´N 8°38'09.7''W). The adult psyllids were used to establish colonies on lemon and sour orange (*Citrus aurantium* L.) plants, within insect-proof covered acrylic cages (40 x 30 x 43 cm). The cages were maintained in a climate chamber at 21 ± 1 °C, with a relative humidity (RH) of 50 ± 5%, and a photoperiod of 16:8 h light:dark (L:D). Mature adults from these colonies were used for infestation.

Sixteen two-year old 'Valencia Midknight Seedless' sweet orange [*C.* ×*sinensis* (L.) Osbeck] (SwO) and 16 two-year old 'Eureka' lemon [*C.* ×*limon* (L.) Osbeck] trees, grafted onto 'Carrizo' citrange (*C. trifoliata* L. × *C.* ×*sinensis*) rootstocks, were used. All trees were purchased from a certified nursery with a phytosanitary passport, with 0.8 and 1 m in height and potted in 5 L pots (19 cm in diameter and 25 cm in height). The potting mix used for all potted plants was a mix of pine bark and coconut fibre (50:50). New shoots were induced by pruning all trees three weeks before the infestation. The number of new flushes was counted per tree, and trees with a similar number of new flushes after pruning were selected for this experiment. For the experiments in this study, all trees and insects were kept in a climate chamber at 23.5 ± 1 °C, 79 ± 5% RH and a photoperiod of 14:10 h (L:D).

4.4.2. Infestation of citrus host trees

All trees were isolated by covering the canopy with insect-proof nets. Out of a total of 16 trees per species, eight were used as controls (SwOCon and LemonCon) and the other eight trees were infested with *T. erytreae* (SwOInf and LemonInf) (Fig. 4.1A). For infestation, a total of 10 sexually mature adults of *T. erytreae* were used per tree, comprising five females and five males. The mature adults were captured from the rearing cages using a handheld aspirator and transferred into conical centrifuge tubes (50 mL). A single tube containing the 10

adult psyllids was left open inside the insect proof net cover of each infested tree. For the remainder of the experiment, which lasted approximately 25 days, the adults were kept inside the net. Twenty-five days following the initial infestation, the number of fourth and fifth-instar nymphs was quantified.

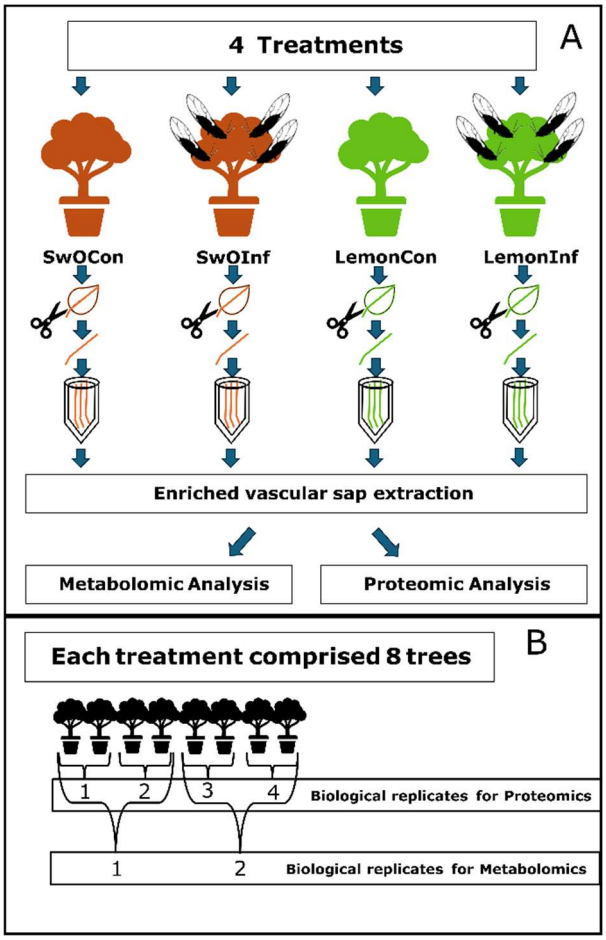

Figure 4.1. A: Experimental design with 4 Treatments: SwOCon represent 'Valencia' sweet orange control trees; SwOInf represent 'Valencia' sweet orange trees infested with *Trioza erytreae*; LemonCon represent 'Eureka' Lemon control trees; SwOInf represent 'Eureka' Lemon trees infested with *Trioza erytreae*; Midrib of the leaf and petiole was cut from 10 leaves in each tree form each treatment and used for enriched vascular sap extraction. B: Scheme for biological replicates for proteomic and metabolomic analysis used for each treatment.

4.4.3. Enriched vascular sap extraction from leaves

All leaves used for enriched vascular sap extraction were excised from the flush growth induced by pruning. Leaves from *T. erytreae* infested and control trees were of similar size and development time. For each species, a total of 8 trees were used, with 10 leaves (from randomly selected new flushes) sampled per tree and per treatment (n=8 trees/treatment, n=10 leaves/tree). Leaves were excised 25 days

after *T. erytreae* infestation and were maintained at 4 °C until the extraction of the enriched vascular sap, performed within 1 h of leaf excision.

The enriched vascular sap extraction protocol was based on Hijaz and Killiny (2014a), with minor adaptations as follows: the midrib vein of the leaves and petiole were excised with a sterilised scalpel, diced, and transferred into a perforated 0.5 mL microtube. Subsequently, the perforated tube was inserted into a 1.5 mL microtube (Fig. 4.1A) and centrifuged at 4 °C and 13,800 × g (12,000 rpm) for 20 minutes. The enriched vascular sap was collected at the bottom of the 1.5 mL microtube and stored at -80 °C. The centrifugation method has been demonstrated to be an effective means of collecting vascular sap, containing phloem sap, xylem sap and apoplastic fluid. However, the process of centrifugation can disrupt the structure of younger cells, so that the extracted fluid is likely to also contain the contents of phloem companion cells, parenchyma cells and mesophyll cells that are in close proximity to the midrib vein. The extracted fluid is henceforth referred to as enriched vascular sap. The same trees were used for both the metabolomic and the proteomic analysis.

4.4.4. Metabolomic analysis

The analyses were conducted using a high-performance liquid chromatography coupled with a diode array detector and tandem mass spectrometry (HPLC-DAD-MS/MS). A preliminary assay was conducted using four mixtures, consisting of a mix of enriched vascular sap from infested and control samples from both citrus trees. This assay consisted of an untargeted approach, whereby observed peaks from the MS full scan were analysed. The peaks that exhibited different intensities in control and infested samples were selected for further quantitation assays. For these assays, eight samples were analysed, consisting of enriched vascular sap pools from four trees, which were used as biological replicates (n = 2) for each treatment (Fig. 4.1B).

4.4.4.1. HPLC conditions

The HPLC analyses were performed on a Waters Alliance 2695 (Waters®, Ireland) equipped with a quaternary pump, solvent degasser, auto sampler and

column oven, coupled to a photodiode array detector Waters 996 PDA (Waters®, Ireland). The separation was performed on a reversed-phase column (Mediterranea Sea 18 5 µm 15x0.21 cm, Teknokroma®) at 35 °C using an injection volume of 20 µL. The mobile phase consisted of Milli-Q water containing 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.30 mL/min. The following gradient of eluents was used: 0% B over 5 min, 0 to 50% B for 30 minutes, 50 to 90% B for 5 minutes, 90% B for 4 minutes, followed by an equilibration step of 15 min in order to establish initial conditions of analysis. A photodiode array detector was used to scan wavelength absorption from 210 to 780 nm.

4.4.4.2. MS conditions

Tandem mass spectrometry (MS/MS) detection was performed on a Micromass® Quattro Micro triple quadrupole (Waters®, Ireland) using an electrospray ionisation (ESI) source operating at 120 °C and applying a capillary voltage of 3.0 kV. High purity nitrogen (N_2) was used both as the drying gas and as the nebulizing gas. Ultra-high purity argon (Ar) was used as the collision gas. For the preliminary analysis, the compounds were ionised in both positive and negative ion modes by applying a source voltage of 30 V. The spectra were recorded in the range *m/z* 60-1,200 and compounds were identified based on their MS/MS spectral profile, applying a collision energy of 20 eV. Whenever possible, the compounds were identified based on commercially available standards (n = 16).

A total of 36 compounds were selected for the quantitation assays. Their MS/MS conditions were optimised based on commercially available standards (n = 16), when available, or based on fragmentation assays in the mixtures from the preliminary assays (n = 20) (Table S4.1 - Appendix). Samples were analysed in multiple reaction monitoring (MRM) mode in order to achieve a higher selectivity and sensitivity. Whenever possible, two transitions were used to identify and quantitate the compounds in samples, with a maximum deviation of 15% between MRM1/MRM2 ratio. The MassLynx software (version 4.1) was used to control the system, for data acquisition and processing.

4.4.5. Proteomics analysis

4.4.5.1. Protein extraction from enriched vascular sap and trypsin digestion

The total protein extraction protocol was based on Zadražnik et al. (2013) with modifications. Each treatment consisted of eight trees. For each treatment, four biological replicates were used (n=4) (Fig. 4.1B). Each biological replicate consisted of a pool of two trees, with 10 leaves per tree, resulting in a total of 20 leaves per biological replicate. A total of 100 µL of enriched vascular sap obtained from biological replicates was solubilised in 1.25 mL of extraction buffer [10% trichloroacetic acid (TClA), 60 mM dithiothreitol (DTT) in acetone], vortexed and stored overnight at - 80 °C. Samples were centrifuged at 14,000 × g for 30 min at 4 °C, and the pellet washed 3 times in 1 mL of wash buffer (60 mM DTT in acetone), followed by centrifugation at 14,000 × g for 5 min at 4 °C. The pellet was left to dry at room temperature and resuspended in 40 µL of denaturing buffer [7 M urea, 2 M thiourea, 30 mM tris-HCl pH 8.5]. Total protein was quantified in a Genesys 1Q-S spectrophotometer (Thermo Electron Corporation, Bremen, Germany) using a Quick Start™ Bradford Protein Assay Kit (Bio-Rad, Hercules, USA) according to the manufacturer's instructions. Fifty micrograms of protein from each sample were submitted to a solid-phase-enhanced sample-preparation (SP3) protocol as described in Hughes et al. (2018) and enzymatic digestion was performed with trypsin/LysC (2 µg) overnight at 37 °C and 1,000 rpm as described in Osório et al. (2021). The concentration of the resulting peptides was measured by fluorescence.

4.4.5.2. NanoLC-MS/MS analysis

The digested peptides from each experimental group (SwOCon, SwOInf, LemonCon and LemonInf) were analysed using nanoLC-MS/MS in an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) for protein identification and quantitation as described in Osório et al. (2021), by an external service provider (Proteomics Scientific Platform of i3S, Ipatimup, Porto, Portugal).

4.4.5.3. Protein quantification and identification

The acquired MS raw data were processed using Proteome Discoverer 2.5.0.400 software (Thermo Scientific). Protein identification searches were performed against the UniProt protein sequence database for *C.* ×*sinensis* (taxon ID 2711, 44,601 entries) and a common contaminant database from MaxQuant (version1.6.2.6, Max Planck Institute of Biochemistry, Munich, Germany). The Sequest HT tandem mass spectrometry peptide database search program was used to identify tryptic peptides using the parameters reported in Osório et al. (2021). Briefly, the ion mass tolerance was of 10 ppm for precursors, 0.02 Da for fragmented ions, and missing cleavage sites were set as 2. Methionine oxidation, asparagine, and glutamine deamidation, peptide N-terminus Gln->pyro-Glut, protein N-terminus acetylation, and loss of methionine and Met-loss+Acetyl were defined as variable modifications. Cysteine carbamidomethylation was defined as a constant modification. The Percolator processing node was enabled with maximum Delta Correlation (deltaCn) 0.05, and validation based on *q*-value. Protein-label-free quantification was performed as in Osório et al. (2021), with the Minora feature detector node at the processing step. For precursor ion quantification the following parameters were used for peptides: unique plus razor; precursor abundance was based on intensity; normalisation mode was based on the total peptide amount; the minimum number of replicate files was set to 50% in each sample group; the pairwise protein ratio calculation and hypothesis test were based on a *t*-test (background based). The Feature Mapper node from the Proteome Discoverer software was used to generate features from unique peptide-specific peaks within a narrow retention time and mass range (a maximum shift of 10 min and 10 ppm of mass tolerance of mapping features between different sample files was permitted). Both peptides and protein identification were considered for a false discovery rate (FDR) of ≤ 0.01. The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2022) partner repository with the dataset identifier PXD043528 and 10.6019/PXD043528.

4.4.6. Metabolite and protein bioinformatic analysis

The fold-change in metabolite abundance was determined by calculating the difference in median peak height (log2-transformed) between the metabolite abundances in the experimental groups. The differentially abundant metabolites (DAMs) were considered for functional analysis, using the MetaboAnalyst web-based tool (https://www.metaboanalyst.ca/MetaboAnalyst/) (Pang et al., 2024) and a hypergeometric Test (p <0.01), in order to gain insight into their biological roles.

The fold-change in protein abundance was calculated using the peptide medians (log2-transformed) between relative protein abundance in the experimental groups. The differentially abundant proteins (DAPs) were considered for functional analysis, which was performed using *Arabidopsis thaliana* (L.) Heynh orthologues of the identified proteins, obtained with the STRING webtool version 11 (https://string-db.org/) (Szklarczyk et al., 2021), with protein names and definitions obtained from the *Arabidopsis* information resource (Berardini et al., 2015). Enrichment analysis was performed using the Plant GeneSet Annotation Database (PlantGSAD) web-based tool (http://systemsbiology.cau.edu.cn/PlantGSEAv2/index.php) (Ma et al., 2022). A hypergeometric test/Fisher's exact test with a Benjamin and Hochberg (Benjamini and Hochberg, 1995) FDR correction method was applied (adjusted p-value at <0.05).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/) (Kanehisa et al., 2022) was used for the functional analysis of metabolites and proteins, with the *A. thaliana* data set serving as the background.

4.4.7. Statistics

The mean number of nymphs per tree was compared between infested trees (SwOInf *vs* LemonInf), using a Student's *t*-test. The mean number of new flushes per tree was compared between both tree species, using a Welch's *t*-test. Both tests were performed in Rstudio software (R Core Team, 2020).

Preprocessing and univariate hypothesis testing for metabolites and proteins were performed in Perseus software version 2.0.7.0. (MaxQuant, Germany) (Tyanova et al., 2016). To determine the DAMs between infested and control treatments (SwOInf *vs* SwOCon and LemonInf *vs* LemonCon) a Student's *t*-test was carried out on the metabolite abundance with the *p*-value adjusted using a permutation-based correction (with 250 randomisations) (FDR <0.05). To determine the DAPs between infested and control treatments (SwOInf *vs* SwOCon and LemonInf *vs* LemonCon), the following filters were considered: (1) the minimum number of biological samples in which a protein was detected in an experimental group was set to 75% (e.g. 3 out of 4); (2) the presence of at least two unique peptides for protein assignment; (3) Protein FDR set to a high q-value < 0.01. A Student's *t*-test was conducted on log10 transformed protein abundance with the *p*-value adjusted using a permutation-based correction (with 250 randomisations) (FDR < 0.05).

4.5. Results

4.5.1. *Trioza erytreae* development on the two citrus host species

The mean number of new flushes per tree was 4.63 for lemon and 6.38 for the SwO, with no significant difference between the host trees (Welch's *t*-test, p =0.15). Twenty-five days following the infestation, the mean number of fourth and fifth instar nymphs in SwO and lemon hosts differed significantly ($p< 0.05$, Student's *t*-test). The mean number of nymphs per SwO tree was 99.3 [± 27.6 standard error of the mean (SEM)] while the mean number per lemon tree was 318.5 (± 47 SEM).

4.5.2. Metabolomic analysis of the enriched vascular sap of citrus in response to *T. erytreae*

4.5.2.1. Compound identification

A total of 36 compounds were selected based on the preliminary HPLC-DAD-MS/MS analysis (Table 4.1). These compounds showed different peak intensities in both infested and control samples, suggesting that they may be involved in the response of citrus to *T. erytreae*. The identity of 16 compounds was confirmed using commercial standards, which were selected based on a review of the literature on citrus vascular sap (Hijaz and Killiny, 2014a) and citrus defence metabolites (Chin et al., 2021; Ibanez et al., 2019). For putative identification of the remaining 20 peaks, their MS/MS spectra were compared with those described in the literature (Table 4.1) (Horai et al., 2010; Kim et al., 2023; Wishart et al., 2007). This process assigned a putative identity to 13 compounds, and the remaining seven were unidentified. The maximum UV absorption and MS/MS fragmentation patterns of these compounds are presented in Table 4.1 for reference. Of the total 36 compounds, 11 were amino acids, 12 were organic acids, two were nucleosides, one was a carbohydrate (glucose), and 10 were classified as other compounds (Table 4.1).

Two of the identified amino acids are imino acids, namely L-pyroglutamic acid and stachydrine (peaks 4 and 6). Two of the identified organic acids were

alkaloids, namely L-pipecolic acid and trigonelline (peaks 16 and 17). In addition, *p*-coumaric acid and ferulic acids, members of the phenylpropanoid family, were identified (peaks 21 and 22). Additionally, demethylnobiletin, a flavone (peak 31), 6-methylcoumarin, a coumarin (peak 33), and O-phenylenediamine (OPDA), a phenylenediamine (peak 35), were within “other compounds” (Table 4.1). Three of the unidentified compounds, namely peaks 29, 30 and 36, exhibited a maximum wavelength at 314 and 270 nm, characteristic of hydroxycinnamic acids and their derivatives (Bengoechea et al., 1995; Wulansari et al., 2023) (Table 4.1).

Table 4.1. Compounds identified by HPLC-DAD-MS/MS (high-performance liquid chromatography coupled with diode array detector and tandem mass spectrometry) in the enriched vascular sap mixture of infested and control lemon and orange trees. #Peak number; λmax (nm): wavelength of maximum height peak in UV spectra (nm); (+) compounds detected in positive ion mode; (-) compounds detected in negative ion mode, ¥ compounds identified by commercial standards, NQ not selected for quantitation due to lack of different intensities in control and infested samples in preliminary studies; RT: retention time (minutes); n/d: not determined due to the peak's low intensity and/or poor peak resolution; **Bold**: characteristic fragment ions described in mass spectrometry databases; Underlined: characteristic fragment ions described by other authors; main MS/MS ions are ordered according to their decreasing intensities; iws: identified with commercial standard.

#	λmax (nm)	RT (min)	Putative Identification	*m/z*	MS/MS ions	References
Amino acids						
1	n/d	1.81	Arginine (+) ¥	175	70, 116	iws
2	n/d	1.85	L-Serine (+) ¥	106	60, 88	iws
3	265	2.31	Aspartic acid (+)	134	**74**, **88**, **46**	https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-IPB_Halle-PB000453&dsn=IPB_Halle; (Thiele et al., 2008)
4	271	2.38	L-Pyroglutamic acid (+)	130	**84**, 82, 42, **56**	https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-Keio_Univ-KO003649&dsn=Keio_Univ
5	271	2.54	Leucine (+) ¥	132	86, 30	iws
6	266	2.64	Stachydrine (+)	144	**58**, **84**	https://pubchem.ncbi.nlm.nih.gov/compound/115244#section=LC-MS
7	264	2.83	L-Methionine (+) ¥	150	56, 61, 74, 104, 87, 102, 133	iws
8	265	3.46	L-Tyrosine (+)	182	**123**, **91**, **119**, **136**, **95**, **147**	https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-BGC_Munich-RP000302&dsn=BGC_Munich; (Taamalli et al., 2015; Thiele et al., 2008)
9	264	5.78	L-Proline (-) ¥	114	68, 45	iws
10	264	5.9	L-Phenylalanine (+) ¥	166	120, 103	iws
11	279/ 312	13.19	Tryptophan (+) ¥	205	188, 146	iws
Organic acids						
12	n/d	1.68	Quinic acid (+) ¥	195	111, 129	iws
13	264	2.08	Malic acid (-) ¥	133	71, 43, 73	iws
14	264	2.11	Citric acid (-) ¥	191	111, 87	iws
15	264	2.16	γ-Aminobutyric acid (+)	104	60, **45**, **58**	https://massbank.eu/MassBank/RecordDisplay?id=KO002141&dsn=Keio_Univ
16	271	2.35	L-Pipecolic acid (+)	130	**84**, 128	https://pubchem.ncbi.nlm.nih.gov/compound/643474#section=LC-MS; (Kite and Hughes, 1997)

#	λmax (nm)	RT (min)	Putative Identification	*m/z*	MS/MS ions	References
17	271	2.38	Trigonelline (+)	138	**92**, **94**, **78**, 67, **110**	https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-Keio_Univ-KO004203&dsn=Keio_Univ; (Szczesny et al., 2018)
18	268	3.12	Succinic acid (-) ¥	117	73, 99	iws
19	278/ 312	13.28	Citramalic acid (-)	147	**85**, **146**, 59, 57, 69, **129**	https://massbank.eu/MassBank/RecordDisplay?id=PR100770&dsn=RIKEN
20	323/ 295	17.26	Caffeic acid (-) ¥	179	135, 134	iws
21	310	19.66	p-coumaric acid (-) ¥	163	119, 93	iws
22	324	20.90	Ferulic acid (-) ¥	193	177, 145	iws
23	285	27.61	Jasmonic acid (-) ¥	209	59, 60	iws
Carbohydrates and nucleosides						
24	n/d	1.82	Glucose (-) ¥	179	89, 119	iws
25	171	2.46	Cytidine (+)	244	**112**, 127, 187, 129, 125, 209	https://massbank.eu/MassBank/RecordDisplay?id=MSBNK-NAIST-KNA00227&dsn=NAIST; (Guo et al., 2013)
26	264	5.46	Adenosine (+)	268	136	https://massbank.eu/MassBank/RecordDisplay?id=KO008860&dsn=Keio_Univ
Other compounds						
27	321	29.47	Unknown 1 (+)	359	326, 344, 298	
28	314	30.13	Unknown 2 (+)	704	686, 668, 591	
29	314/ 270	30.28	Unknown 3 (+)	372	270, 336, 194, 314, 120, 298	
30	314/ 270	30.36	Unknown 4 (+)	380	284	
31	314/ 286	30.65	Demethylnobiletin (+)	389	**359**, **374**, **341**	https://hmdb.ca/spectra/ms_ms/450139; (Liu et al., 2012)
32	299	31.43	Unknown 5 (+)	373	343, 358	
33	287	32.1	6-Methylcoumarin (+)	161	**105**, **103**, **79**, **77**	https://hmdb.ca/spectra/ms_ms/444987; (Tine et al., 2017)
34	287	32.1	Unknown 6 (+)	425	95	
35	330	33.95	OPDA (+)	109	**67**, 94, 55, **81**	https://pubchem.ncbi.nlm.nih.gov/compound/7243#section=MS-MS
36	331/ 270	34.28	Unknown 7 (+)	403	373, 388	

4.5.2.2. Compound quantitation

A total of 16 compounds were quantitated in at least one treatment, using commercial standards. Caffeic acid was only detected in the SwOCon treatment, in all replicates of the other treatments this compound presented levels below the detection threshold of 0.02 µg/mL (Table 4.2). Two of the 16 compounds were not detected in lemon trees (LemonCon and LemonInf), namely Unknown 3 and Unknown 4. Furthermore, demethylnobiletin and Unknown 2 were not detected in LemonInf (Table 4.3). The SwOInf *vs* SwOCon comparison yielded 10 DAMs, eight of which were upregulated, while caffeic acid and proline were downregulated (Tables 4.2 and 4.3). The LemonInf *vs* LemonCon comparison yielded 12 DAMs, of which five were upregulated and seven were downregulated (Tables 4.2 and 4.3). The two comparisons yielded proline and aspartate as common DAMs. Proline was downregulated in the SwO comparison and upregulated in the lemon comparison, while aspartate was upregulated in both comparisons (Tables 4.2 and 4.3).

Table 4.2. Metabolites identified with a commercial standard; MM: Molecular mass (g/mol); RT: Retention time; SwOCon: Control sweet orange trees; SwOInf: Infested sweet orange trees; SwO comparison: SwOInf vs SwOCon; LemonCon: Control lemon trees; LemonInf: Infested lemon trees; Lemon comparison: LemonInf vs LemonCon; SD: standard deviation; Log2FC: Fold change transfrormed by log base 2; Sig: Significance of test (+ means significant); Differentially abundant metabolites are highlighted in bold (in the respective comparisons).

Compound	MM (g/mol)	RT (min)	SwOCon	SwOInf	SwO Comparison		LemonCon	LemonInf	Lemon Comparison	
			Mean ± SD (µg/mL)	Mean ± SD (µg/mL)	Log2FC	Sig	Mean ± SD (µg/mL)	Mean ± SD (µg/mL)	Log2FC	Sig
Arginine	174.2	1.81	30.32 ± 4.10	40.75 ± 4.02	0.43		42.66 ± 0.20	43.18 ± 26.05	-0.13	
Caffeic acid	**180.16**	**17.26**	**0.06 ± 0.01**	**< 0.02**	**-2.47**	**+**	< 0.02	< 0.02	0.00	
Citric acid	**192.12**	**2.21**	206.40 ± 17.84	298.33 ± 26.36	0.53		**257.37 ± 1.79**	**228.70 ± 13.70**	**-0.17**	**+**
Ferulic acid	**194.18**	**20.9**	15.80 ± 2.82	17.54 ± 1.92	0.16		**4.26 ± 0.32**	**3.23 ± 0.14**	**-0.40**	**+**
Glucose	180.16	1.82	883.61 ± 317.96	1603.36 ± 770.00	0.82		1512.27 ± 639.60	1906.99 ± 469.17	0.38	
Jasmonic acid	**210.27**	**27.61**	**0.06 ± 0.01**	**0.18 ± 0.00**	**1.57**	**+**	0.47 ± 0.01	0.45 ± 0.00	-0.07	
Leucine	131.17	2.54	35.51 ± 7.14	54.29 ± 10.84	0.61		51.42 ± 4.05	52.98 ± 17.57	0.00	
Malic acid	**134.09**	**2.08**	**464.35 ± 47.40**	**2882.98 ± 281.53**	**2.63**	**+**	2319.09 ± 56.80	1626.75 ± 722.51	-0.59	
Methionine	149.21	2.23	4.23 ± 0.91	5.89 ± 0.75	0.49		6.23 ± 0.23	4.63 ± 2.31	-0.52	
***p*- coumaric acid**	**164.16**	**20.78**	5.82 ± 0.90	3.01 ± 0.29	-0.83		**3.91 ± 0.66**	**5.85 ± 0.32**	**0.52**	**+**
Phenylalanine	**165.19**	**5.9**	76.31 ± 15.42	29.23 ± 6.38	-1.39		**26.31 ± 2.52**	**42.71 ± 7.17**	**0.69**	**+**
Proline	**115.13**	**5.78**	**78.78 ± 4.11**	**40.83 ± 0.36**	**-0.95**	**+**	**30.03 ± 1.40**	**48.93 ± 3.06**	**0.70**	**+**
Quinic acid	**192.17**	**1.97**	4887.35 ± 1768.42	5239.75 ± 1255.55	0.13		**1266.74 ± 412.02**	**4705.46 ± 623.24**	**1.93**	**+**
Serine	105.09	1.85	73.88 ± 4.16	66.61 ± 0.05	-0.15		84.13 ± 0.19	82.80 ± 46.91	-0.15	
Succinic acid	**118.09**	**3.12**	**3.06 ± 0.67**	**11.14 ± 0.64**	**1.88**	**+**	8.48 ± 0.65	7.82 ± 0.06	-0.11	
Tryptophan	**204.22**	**13.19**	8.65 ± 1.78	14.04 ±2.32	0.70		**25.98 ± 0.48**	**46.31 ± 9.24**	**0.82**	**+**

Table 4.3. Putatively identified metabolites; MM: Molecular mass (g/mol); RT: Retention time; SwOCon: Control sweet orange trees; SwOInf: Infested sweet orange trees; SwO comparison: SwOInf vs SwOCon; LemonCon: Control lemon trees; LemonInf: Infested lemon trees; Lemon comparison: LemonInf vs LemonCon; SD: standard deviation, n/d means not detected (minimum peak area was used for statistical tests); Log2FC: Fold change transfrormed by log base 2; Sig: Significance of test (+ means significant); Differentially abundant metabolites are highlighted in bold (in the respective comparisons).

Compound	MM (g/mol)	RT (min)	SwOCon	SwOInf	SwO Comparison		LemonCon	LemonInf	Lemon Comparison	
			Mean ± SD (area)	Mean ± SD (area)	Log2FC	Sig	Mean ± SD (area)	Mean ± SD (area)	Log2FC	Sig
6-Methylcoumarin	160.00	32.10	47058 ± 43711	39646 ± 32909	-0.14		9390 ± 2300	8633 ± 1805	-0.12	
Adenosine	**267.10**	**5.46**	238846 ± 23426	98225 ± 14649	-1.29		**1821703 ± 297394**	**1339 ± 243**	**-10.41**	**+**
Aspartic acid	**133.04**	**2.31**	**394 ± 106**	**4211 ± 432**	**3.44**	**+**	**2215 ± 365**	**7350 ± 1189**	**1.73**	**+**
Citramalic acid	148.04	13.28	13047 ± 7542	10281 ± 5848	-0.34		2816 ± 681	2751 ± 180	-0.01	
Cytidine	**243.09**	**2.46**	**1023 ± 193**	**53147 ± 5554**	**5.71**	**+**	76578 ± 5133	89650 ± 10711	0.22	
Demethylnobiletin	**388.00**	**30.65**	172409 ± 43094	71716 ± 23034	-1.28		**810 ± 21**	**n/d**	**-16.31**	**+**
L-Pyroglutamic acid	**129.04**	**2.38**	**84941 ± 25297**	**580227 ± 26771**	**2.80**	**+**	382921 ± 54479	421776 ± 41303	0.14	
L-Pipecolic acid	**129.08**	**2.35**	**88272 ± 23961**	**581625 ± 23492**	**2.75**	**+**	386382 ± 56640	435030 ± 59015	0.17	
OPDA	108.07	33.95	1111 ± 551	5062 ± 1969	2.23		3399 ± 144	1519 ± 257	-1.17	
Stachydrine	143.09	2.64	465367 ± 210082	7301729 ± 2105878	4.02		6064976 ± 1447053	5130823 ± 742080	-0.23	
Trigonelline	137.00	2.38	358069 ± 76906	138840 ± 28507	-1.37		244133 ± 57923	182361 ± 18650	-0.40	
Tyrosine	**181.00**	**3.46**	**107037 ± 14862**	**556695 ± 17519**	**2.39**	**+**	498949 ± 38895	620350 ± 136926	0.30	
γ-Aminobutyric acid	103.00	2.16	1130 ± 623	439950 ± 67603	8.72		416407 ± 85985	371441 ± 28906	-0.15	
Unknown 1 (+)	**358.00**	**29.47**	648037 ± 157119	408123 ± 133943	-0.69		**6647 ± 1109**	**1168 ± 169**	**-2.51**	**+**
Unknown 2 (+)	703.00	30.13	578739 ± 153545	360013 ± 119519	-0.70		5326 ± 1514	n/d	-5.90	
Unknown 3 (+)	371.00	30.28	4361 ± 1744	2802 ± 750	-0.60		n/d	n/d	0.00	
Unknown 4 (+)	379.00	30.36	311 ± 30	160 ± 53	-1.00		n/d	n/d	0.00	
Unknown 5 (+)	**372.00**	**31.43**	825043 ± 224770	469887 ± 157969	-0.83		**5458 ±939**	**1477 ± 29**	**-1.88**	**+**
Unknown 6 (+)	424.00	32.10	38308 ± 24737	34628 ± 20284	-0.11		12612 ± 1697	11645 ± 520	-0.11	
Unknown 7 (+)	**402.00**	**34.28**	4071835 ± 1180473	2358652 ± 799187	-0.80		**40482 ± 1839**	**8479 ± 533**	**-2.26**	**+**

Functional analysis of identified DAMs yielded three enriched pathways (p < 0.01) in the SwOInf *vs* SwOCon comparison. These were the "citrate cycle (TCA cycle)", the "carbon fixation in photosynthetic organisms", and the "alanine, aspartate and glutamate metabolism" pathways (Table S4.2 - Appendix). Functional analysis of the DAMs in the LemonInf *vs* LemonCon comparison identified three enriched pathways (p < 0.01), namely the "phenylpropanoid biosynthesis", the "phenylalanine, tyrosine and tryptophan biosynthesis" (Fig. 4.2) and the "cyanoamino acid metabolism" pathways, by the DAMs phenylalanine and aspartic acid (Table S4.2 - Appendix).

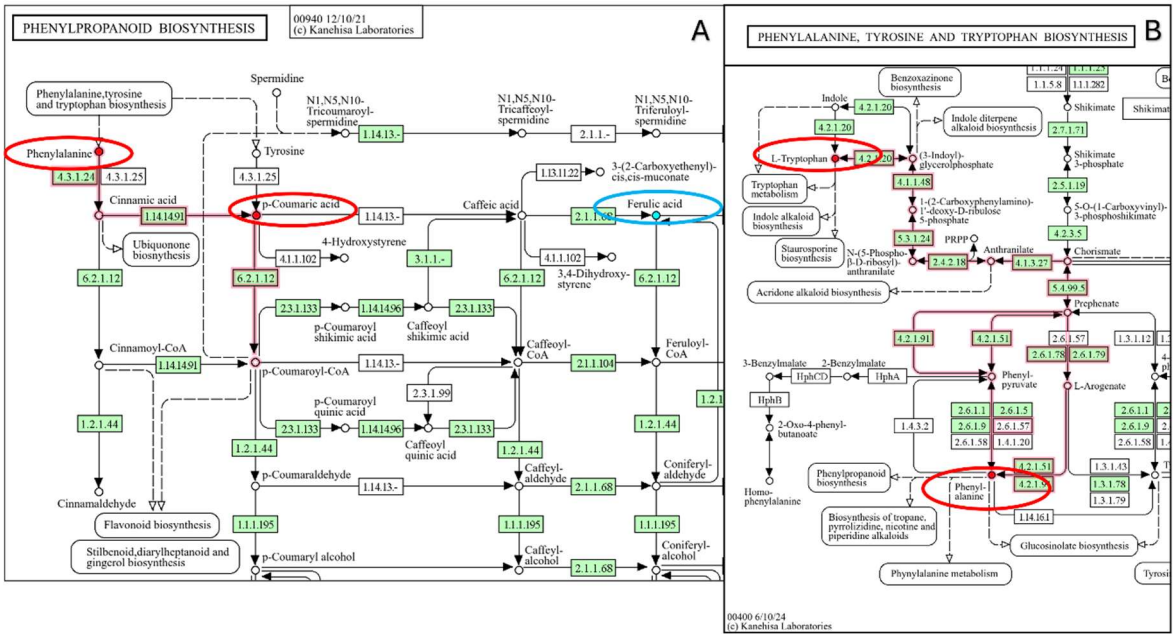

Figure 4.2. KEGG pathways identified based on identified DAMs in lemon infested with *Trioza erytreae*. The red colour indicates molecules with an increased concentration; the cyan blue colour indicates molecules with a reduced concentration; and the green colour represents the molecules described for *Arabidopsis thaliana* in this pathway (and that were not DAMs in this study). Metabolites (DAMs) are highlighted in the circular nodes and by an external rig of the same colour. A: "phenylpropanoid biosynthesis" pathway, the "flavanone biosynthesis" module is highlighted by the addition of pink outlines to the arrows and forms. B: "phenylalanine, tyrosine and tryptophan biosynthesis" pathway, the "tryptophan biosynthesis" and the "phenylalanine biosynthesis" are highlighted with pink outlines on arrows, boxes and circular nodes.

4.5.3. Proteomic analysis of the enriched vascular sap of citrus in response to *T. erytreae*

A total of 5,050 proteins were identified in the enriched vascular sap (Table S4.3 - Appendix). Of these, 1,471 were excluded since less than two unique peptides were identified, and an additional 33 classified as contaminants were excluded. Based on the identification of at least three of the four replicates of each treatment, a library of 3,370 and 3,141 proteins was generated for SwO and lemon trees, respectively.

The SwOInf *vs* SwOCon proteome comparison yielded 1,265 DAPs, of which 964 were upregulated and 301 were downregulated (Table S4.4 - Appendix). The LemonInf *vs* LemonCon comparison, yielding 48 DAPs, of which 22 were upregulated and 26 were downregulated (Table S4.4 - Appendix).

Functional analysis based on the DAPs obtained in the SwOInf *vs* SwOCon comparison identified 53 enriched KEGG pathways (FDR < 0.05) (Table S4.5 - Appendix). The results of the metabolic and proteomic analysis indicated that the 'Valencia' SwO underwent a significant reallocation of molecular resources related to carbohydrate metabolism and lipid metabolism, in response to infestation by *T. erytreae*. The proteomic findings corroborated the metabolomics results since 10 of the 53 identified DAP-enriched pathways in 'Valencia' SwO trees were related to the carbohydrate metabolism. These included "glycolysis/ gluconeogenesis", "galactose metabolism" "pyruvate metabolism" and the "pentose phosphate pathway" pathways, among others. The seven enriched pathways associated with lipid and energy metabolism included, "fatty acid biosynthesis", "fatty acid degradation" and the "methane metabolism" (Table S4.5 - Appendix).

4.5.3.1. The metabolomic and proteomic analysis revealed an overlap in SwO response to *Trioza erytreae*

Three of the 53 enriched KEGG pathways in SwO were enriched in both the metabolomic and proteomic analysis. These pathways were the "citrate cycle (TCA cycle)" (Fig. 4.3A), the "carbon fixation in photosynthetic organisms" pathway and

the “alanine, aspartate and glutamate metabolism” pathways. Proteins associated with aspartic acid biosynthesis, namely aspartate aminotransferases (ASP3 and ASP5), as well as proteins related to aspartic acid catabolism/metabolism, such as asparagine synthetases (ASN1 and ASN3) and L-aspartase (AT5G10920), were downregulated in proteomic analysis (Tables S4.5 and S4.4 - Appendix). Additionally, the “alpha-linolenic acid metabolism” pathway was found to be enriched. This pathway is related with the jasmonic acid biosynthesis, and therefore with the plant´s response to insect infestation (Fig. 4.3B), and the metabolite jasmonic acid was upregulated in ‘Valencia’ SwO trees.

Differentially abundant proteins related with amino acid metabolism, such as the proline biosynthesis-related protein delta1-pyrroline-5-carboxylate synthase 1 (P5CS1) were upregulated while the aldehyde dehydrogenase 12A1 (ALDH12A1) was downregulated (Tables S4.5 and S4.4 - Appendix). Both are related to the metabolite proline which was downregulated in SwO (Table 4.2). In the context of organic acids synthesis, the upregulation of isopropylmalate dehydrogenase (AT5G14590) and dihydrolipoamide succinyltransferase (AT4G26910) was observed. These enzymes are linked to the steps preceding succinic and malic acid biosynthesis (Tables S4.5 and S4.4 - Appendix). Both the succinic and malic acid metabolites were upregulated in SwO trees (Table 4.2).

Proteins related to the polyphenol caffeic acid catabolism/metabolism were upregulated, namely 4-coumarate:CoA ligase (AT4G05160) and the Caffeoyl-CoA O-methyltransferase 1 (CCoAOMT1) (Tables S4.5 and S4.4 - Appendix). And the metabolite caffeic acid was downregulated in SwO trees (Table 4.2). The defence related sulfur containing glutathione synthetase 2 (GSH2, AT5G27380), methionine sulfoxide reductase B2 (MSRB2, AT4G21860) were found to be upregulated in ‘Valencia’ SwO trees (Table S4.5 - Appendix).

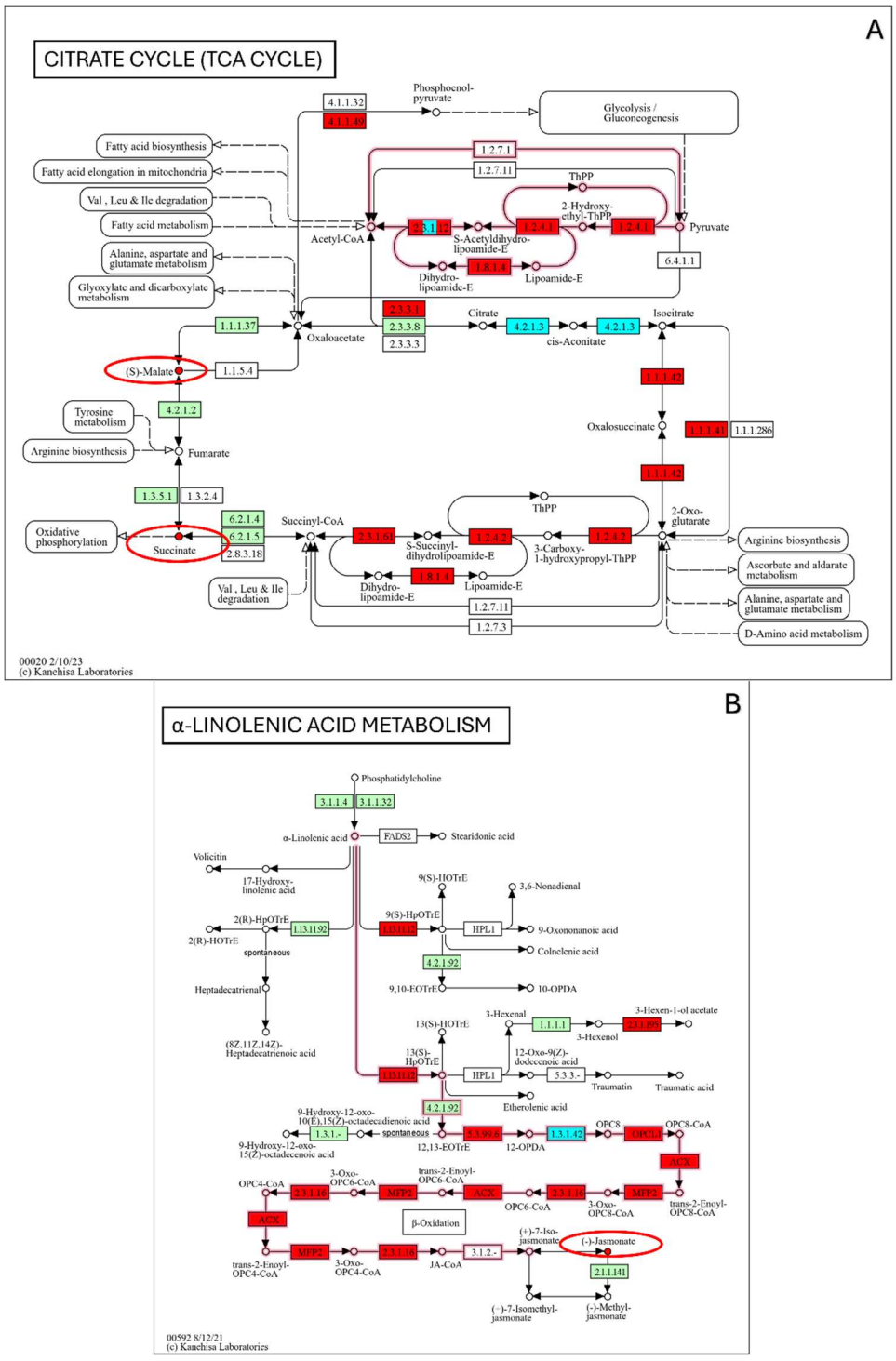

Figure 4.3. Selected KEGG pathways highlighted in SwO: the red colour indicates molecules that are upregulated; the cyan blue colour indicates molecules that are downregulated; and the green colour represent the molecules described for *Arabidopsis thaliana* in this pathway (and that were not differential in this study). Metabolites (DAM) are highlighted by the circular nodes and by an external ring of the same colour; proteins (DAP) are highlighted by rectangular boxes. **A**: "citrate cycle (TCA cycle)" pathway, the "pyruvate oxidation" module is highlighted with pink outlines on arrows and forms. **B**: "alpha-linolenic acid metabolism pathway", the "jasmonate biosynthesis" module is highlighted with pink outlines on arrows, boxes and circular nodes.

4.5.3.2. Minimal overlap between the proteomic and metabolomic analysis with respect to lemon hosts

Only three KEGG enriched pathways (FDR < 0.05) were obtained with the DAPs identified in the LemonInf *vs* LemonCon comparison (Table S4.5 - Appendix). The identified pathways were “sulfur metabolism”, “selenocompound metabolism” and the “amino sugar and nucleotide sugar metabolism”. Interestingly, the same three pathways were also enriched in the SwOInf *vs* SwOCon comparison. Moreover, the “assimilatory sulfate reduction” module of the “sulfur metabolism” pathway, was predominantly enriched by downregulated proteins in lemon hosts and by upregulated proteins in SwO hosts (Fig. 4.4). It is noteworthy that the three pathways were not enriched by the metabolomic analysis. The only pathway enriched by both DAMs and DAPs was the phenylpropanoid biosynthesis pathway, and only one protein, the upregulated cinnamyl alcohol dehydrogenase 8 (ELI3-2), was related to this pathway.

Although no DAPs were found to be related to the “phenylalanine, tyrosine, and tryptophan biosynthesis” pathway (enriched by DAMs), one DAP was found to be related to the biosynthesis of the amino acid methionine, namely the methionine overaccumulation 1 (MTO1), which was upregulated.

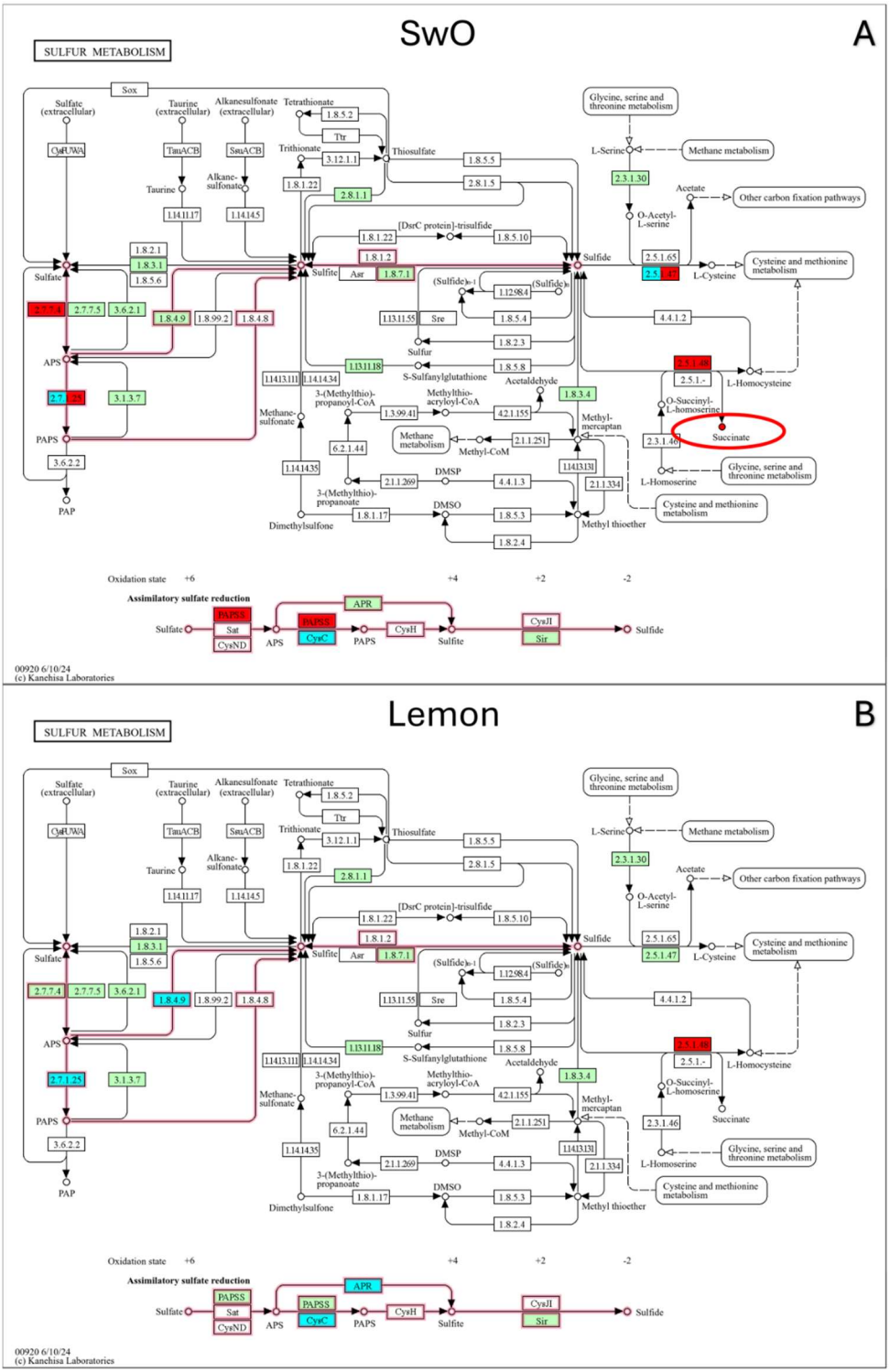

Figure 4.4. Regulation of the "Sulfur metabolism" KEGG pathway in SwO (A) and lemon trees (B): the red colour boxes indicate molecules that are upregulated; the cyan blue colour boxes indicate the molecules that are downregulated; and the green colour boxes indicates the molecules described for *Arabidopsis thaliana* in this pathway (and that were not differential in this study). Metabolites (DAM) are highlighted by in the circular nodes and by an external ring of the same colour; proteins (DAP) are highlighted by rectangular boxes. The "assimilatory sulfate reduction" module is highlighted by pink outlines on arrows, boxes and circular nodes.

4.6. Discussion

Moran (1968a) showed that the future host preference of *T. erytreae* was not influenced by the plant host species used for rearing. The sour orange and lemon plants used for rearing *T. erytreae* in cages should therefore not introduce any bias in the current study. The lack of a significant difference in the mean number of new flushes when comparing both hosts, indicates that plant growth should not be a major factor affecting the differences observed in *T. erytreae* development. The lower number of nymphs that successfully developed in 'Valencia' SwO trees relative to 'Eureka' lemon trees corroborate the results of Tamesse (2000), who observed a higher number of nymphs in lemon trees (cultivars 'Lisbon' and 'Eureka') relative to SwO trees (cultivars 'Hamlin', 'Pineapple', and 'Valencia' cultivars). Furthermore, this outcome is consistent with the evidence indicating that *T. erytreae* has a preference for lemon trees as a host (Aidoo et al., 2019c; Aubert, 1987). The molecular response to *T. erytreae* in 'Valencia' SwO and 'Eureka' lemon trees exhibited significant differences, as identified by the metabolomic and proteomic analysis. In addition, numerous points of convergence between both omics analyses were identified. The following discussion will focus on the metabolic response, and the metabolite families that were affected, integrating and highlighting the convergence and divergence with the proteomics results. The high nymphal infestation on lemon trees would suggest a high metabolic adjustment, and defence-related response from this host. However, as will be discussed in the following sections, it was the SwO trees with the lower nymphal infestation that showed a severe response.

4.6.1. Citrus hosts adapt their primary metabolism in response to *T. erytreae* infestation

Primary metabolites play an integral role in the processes of normal growth, development and reproduction. In the present study, both citrus host species adapted their primary metabolism, in distinct ways, in response to *T. erytreae* infestation.

In ‘Valencia’ SwO trees, the upregulation of malic acid and succinic acid, and the downregulation of citric acid was observed (Table 4.2). Organic acids are a common class of metabolites found in phloem sap (Broussard et al., 2023). The high production of malic acid and succinic acid as “citrate cycle (TCA cycle)” intermediates seem to be a response necessary for growth and survival under stress (Panchal et al., 2021). These metabolites enriched also the “glycolysis/gluconeogenesis” pathway, and the “carbon fixation in photosynthetic organisms” pathway (Table S4.2 and S4.5 - Appendix). In addition, the TCA cycle is part of a strategy used by plants to combat the oxidative stress related with biotic stress (MacLean et al., 2023). Succinic acid may act as a signalling molecule related to detoxification through reactive oxygen species (ROS), and in this way the TCA-cycle is engaged to fortify defence mechanisms targeted in an oxidative environment (MacLean et al., 2023) (Table S4.2 and S4.5 - Appendix) (Fig. 4.2A).

The increase in TCA-cycle intermediates was previously described to be related to the catabolism of proteinogenic amino acids in citrus infested by the bacterium *C*. Liberibacter (Killiny and Nehela, 2017). Therefore, the upregulated TCA cycle pathway in ‘Valencia’ SwO, and the “glycolysis/gluconeogenesis” pathway, suggest an important activity in the cells towards the synthesis of amino acids. In fact, aspartic acid and tyrosine were upregulated during the adaptation of this host to the biotic stress caused by *T. erytreae* (Table 4.3), while proline was downregulated (Table 4.2). Another compound produced by the TCA cycle, proline was found to be dowregulated in ‘Valencia’ SwO, which may reflect the high demand in the stressed host (MacLean et al., 2023). In an oxidative environment, proline as well as tyrosine, are also precursors for the synthesis of secondary metabolites (Banothu and Uma, 2022; Yang et al., 2020c).

The metabolic and proteomic results related to the metabolism of malic and succinic acid in ‘Valencia’ SwO trees show convergence. DAPs like isopropylmalate dehydrogenase (AT5G14590) and dihydrolipoamide succinyltransferase (AT4G26910) that are in the pathways of succinic and malic acid biosynthesis, were upregulated (Klodmann et al., 2011; Lee et al., 2008a). Conversely, divergence is suggested in the metabolism/catabolism of aspartate, as the proteins ASN1 and

ASN3 and L-aspartase AT5G10920 were upregulated, while proteins linked to aspartate biosynthesis were downregulated (ASP3 and ASP5) (Viola, 2000; de la Torre et al., 2014). In addition, divergence was also found in DAPs related to proline with upregulation of delta1-pyrroline-5-carboxylate synthase 1 (P5CS1), which is in the initial stages of proline biosynthesis, and downregulation of aldehyde dehydrogenase 12A1 (ALDH12A1), which is involved in the catabolism of proline to glutamate (Rizzi et al., 2015). The upregulation of the "TCA cycle", "glycolysis/gluconeogenesis" and "carbon fixation in photosynthetic organisms" pathways contribute to mitochondrial metabolism, ATP production and carbohydrate synthesis. These results provide an insight into the physiological response of 'Valencia' SwO to *T. erytreae* infestation stress: the plant strives to sustain its metabolic activity and adapt to the imposed challenge.

The response of 'Eureka' lemon trees to the infestation by *T. erytreae*, resulted in the upregulation of the amino acid phenylalanine and tryptophan (Table 4.2), thereby enriching the "phenylalanine, tyrosine and tryptophan biosynthesis" pathway (Fig. 4.1). A rise in tryptophan levels has previously been reported in SwO 'Valencia' in response to *D. citri* (Killiny and Nehela, 2017), while 'Owari' mandarin trees had reduced phenylalanine in response to *D. citri* (Malik et al., 2014). In HLB-tolerant citrus species, both tryptophan and phenylalanine were more prevalent (Rao et al., 2018b) and it is tempting to speculate that the upregulation of both amino acids in 'Eureka' lemon trees in the present study indicate high levels of protein synthesis, and specialised compounds involved in plant defence (Yoo et al., 2013). The lemon tree proteomics results did not converge with the metabolomics results, as the pathways enriched by DAMs were different from those enriched by DAPs, and proteins related to the biosynthesis or catabolism of the identified amino acids were not differentially abundant.

4.6.2. *Trioza erytreae* infestation induces defence responses in both citrus host species

The plant defence hormone jasmonic acid was upregulated in 'Valencia' SwO in response to *T. erytreae*. Jasmonic acid plays a role in several plant signalling pathways and is involved in regulating the defence against herbivorous insects

(Vaishnav and Chowdhury, 2023). Interestingly, jasmonic acid was identified in 'Valencia' SwO in response to *D. citri* (Patt et al., 2018). In the present study DAMs and DAPs in plants infested with *T. erytreae* converged and the "jasmonate biosynthesis" module was represented in the DAPs (Fig. 4.3B and Table 4.2). In addition, the upregulation of the sulfur-containing defence proteins (GSH2, AT5G27380 and MSRB2, AT4G21860) (Table S4.5 - Appendix) and the upregulation of the pathway module "assimilatory sulfate reduction" (Fig. 4.4) were also observed. The enrichment of these pathways is probably related to the activity of jasmonic acid, since it induces the assimilation of sulfur and the production of sulfur-containing defence compounds like glutathione and methionine related compounds (Capaldi et al., 2015).

The infested 'Valencia' SwO trees showed increased levels of the antioxidants pyroglutamic acid, tyrosine, and L-pipecolic acid (Table 4.3). These compounds have previously been identified in the phloem sap and increase in trees exposed to biotic stresses (Fiehn, 2003; Killiny, 2017; Teixeira et al., 2023). In contrast to our findings with *T. erytreae* infestation, in 'Valencia' SwO trees infested with *D. citri*, pyroglutamic acid was downregulated (Nehela and Killiny, 2019).

The amino acid tyrosine is the precursor for the biosynthesis of flavonoids, alkaloids, and phytoalexins, secondary metabolites with antioxidant properties and involved in plant defence against herbivorous insects (War et al., 2012; Xu and Fu, 2022). Tyrosine was found to increase in *C. aurantium* (sour orange) in response to the mite *Tetranychus urticae* Koch (Acari: Prostigmata) (Agut et al., 2014). Pipecolic acid is the precursor of the immune regulatory metabolite N-hydroxypipecolic acid (NHP) and induces systemic acquired resistance (SAR) in distant leaf tissue (Bernsdorff et al., 2016; Yildiz et al., 2021). Vranova et al. (2013) found that pipecolic acid negatively impacted growth and development of insects that feed on plants. The upregulation of pipecolic acid detected in 'Valencia' SwO may explain the significantly lower number of fourth and fifth instar nymphs of *T. erytreae* compared to the 'Eureka' lemon trees.

The secondary metabolite caffeic acid was downregulated in the 'Valencia' SwO in response to *T. erytreae* (Table 4.2). Similarly, caffeic acid was downregulated

in the mandarin 'Owari' infested with *D. citri*. The psyllids seem to have the ability to reduce caffeic acid levels in plants to make them more vulnerable to feeding (Malik et al., 2015). The proteomic analysis in the present study was aligned with the metabolomic findings, with the upregulation of proteins that catabolise caffeic acid through esterification with CoA (AT4G05160) and methylation (CCoAOMT1) (Cao et al., 2016; Do et al., 2007).

The stress responses of 'Eureka' lemon trees against *T. erytreae* differed significantly from those of 'Valencia' SwO trees. As described in section 4.6.1, the upregulated amino acids phenylalanine and tryptophan point to a high level of synthesis of plant defence compounds (Yoo et al., 2013). Both amino acids are formed by the shikimic acid pathway and phenylalanine is a precursor for phenylpropanoid pathway-derived compounds such as hydroxycinnamic acids, previously identified in the phloem sap, which include ferulic and p-coumaric acids, which have functions in plant defence (Banothu and Uma, 2022; Killiny, 2016; Kumar et al., 2023). Furthermore, the "flavanone biosynthesis" module of the "phenylpropanoid biosynthesis" pathway was also upregulated in 'Eureka' lemon trees (Fig. 4.2A). The secondary metabolites identified in 'Eureka' lemon trees were mainly phenolic compounds, which is indicative of a specific host response to *T. erytreae* infestation.

Tryptophan plays a direct role in regulating the defence response to insect herbivory in plants (Radwanski and Last, 1995). Additionally, other compounds such as quinic acid and coumaric acid, which were both upregulated, also play a role in protecting against biotic stress caused by insect herbivory (Islam et al., 2024). In agreement with our findings in 'Eureka' lemon trees, an increase in coumaric phenylpropanoids levels was observed in cotton plants (*Gossypium hirsutum* L.) in response to insect feeding (Li et al., 2016).

In infested 'Eureka' lemon trees, ferulic acid was found to be downregulated which is consistent with the results of 'Valencia' SwO trees exposed to *D. citri* (Killiny and Nehela, 2017). The observed decrease in citric acid levels in 'Eureka' lemon trees may be linked to an increased activity within the shikimate pathway potentially influencing the synthesis of phenylalanine and tryptophan, which were both

upregulated in enriched vascular sap (Fig. 4.2B) (Walsh et al., 1987). In accordance with our results, quinic acid levels increased in 'Valencia' SwO infested with *D. citri*, although contrary to our results citric acid levels also increased (Killiny and Nehela, 2017).

The three unidentified metabolic compounds (peaks 29, 30 and 36) exhibited a λmax of 314/ 270 (Table 4.1), which is characteristic of hydroxycinnamic acids and their derivatives (Bengoechea et al., 1995; Wulansari et al., 2023). The compounds of peaks 29 and 30 showed very low intensity in 'Eureka' lemon trees, and the compound of peak 36 was downregulated in infested 'Eureka' lemon trees (Table 4.3). Considering the putative identity of peaks 29, 30 and 36 and potential role in plant defence mechanisms, a follow-up study will be important to identify these compounds and elucidate their role. Overall, a minimal overlap existed between the metabolomic and proteomic results in 'Eureka' lemon trees infested with *T. erytreae*. However, the upregulated protein cinnamyl-alcohol dehydrogenase 8 (ELI3-2) suggests that lignin biosynthesis may have been stimulated instead of flavanol biosynthesis (Sibout et al., 2005).

Only aspartic acid and proline were identified as common metabolites in both citrus host species infested with *T. erytreae*. In both cases, aspartic acid was upregulated, while proline was upregulated only in the lemon host (Table 4.2 and 4.3). Proline is an amino acid that contributes to stress tolerance and serves as a link between primary and secondary metabolism. It facilitates the oxidative pentose phosphate pathway and thereby increases phenolic metabolism through the phenylpropanoid biosynthesis pathway (Caretto et al., 2015). This suggests that the upregulation of proline contributed to the vascular sap enrichment of the aforementioned phenylpropanoid compounds. Our findings in 'Eureka' lemon are in accordance with those of Banothu and Uma (2022), who described proline as a metabolite produced in response to sap-sucking insects.

Overall, it is evident that there are significant differences in the response of the two citrus host species to infestation by *T. erytreae*, with alterations occurring in both primary and secondary metabolism. Primary metabolic processes were identified as the most altered in 'Valencia' SwO, followed by the modification of

specific secondary metabolites. The upregulation of TCA-cycle intermediates and jasmonic acid-related responses was identified at both the metabolomic and the proteomic level. In contrast, the metabolomic adjustments in 'Eureka' lemon trees pointed to a targeted response, involving the synthesis of amino acids towards the synthesis of secondary metabolites, mainly phenylpropanoids. In agreement with our results, compounds of primary metabolism were unaltered or decreased in infested 'Valencia' SwO in response to *D. citri* (Killiny and Nehela, 2017). The observed increase in levels of phenylalanine and tryptophan in 'Eureka' lemon trees is consistent with the increased tolerance of certain citrus cultivars to HLB (Rao et al., 2018b).

4.7. Conclusion

Distinct stress response mechanisms were identified in the enriched vascular sap of two infested citrus host species, 'Valencia' SwO and 'Eureka' lemon trees. 'Valencia' SwO trees showed a broader functional enrichment of metabolic pathways related with primary metabolism, along with activation of the jasmonic acid signalling pathway, even at lower nymphal infestation intensities. This suggests that the 'Valencia' SwO trees employed a resistance-based response towards *T. erytreae*. It is proposed that this type of response may have a negative effect on the development of the psyllid and may explain the low number of developed psyllids in 'Valencia' SwO. In contrast, specific resistance responses were discernible in 'Eureka' lemon trees, accompanied by a lower molecular adjustment and tolerance-related responses. This suggests that the citrus host primarily adopted a tolerance-based response towards *T. erytreae*.

Chapter 5. Comparative proteomic analysis of *Trioza erytreae* nymphs developed on *Citrus* ×*limon* and *Citrus* ×*sinensis* plants

This chapter has been adapted from the submitted article:

Magalhães, T., Anjos, L., Power, D. M., Pereira, J. A., Duarte, A., Marques, N. T. Comparative proteomic analysis of *Trioza erytreae* nymphs developed on *Citrus* ×*limon* and *Citrus* ×*sinensis* plants

5.1. Abstract

Trioza erytreae is a vector of the highly damaging citrus disease Huanglongbing (HLB). The citrus hosts have a significant influence on the development of the psyllid, with lemon plants (*Citrus ×limon*) being the preferred host. The present study analysed *T. erytreae* oviposition, and nymphal development on 'Eureka' lemon and 'Valencia Midknight Seedless' sweet orange (SwO) (*C. ×sinensis*) hosts. A comparative proteomic analysis was performed on the fourth and fifth instar nymphs developing on each citrus host. In parallel a 24 h sucrose feeding study was undertaken on nymphs reared on lemon and SwO, along with a comparative proteome analysis, aiming to evaluate the impact of feeding in each citrus host on nymphal development and its biology. The proteomes were examined by nanoscale liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS). *Trioza erytreae* exhibited significant variations in oviposition and infestation patterns depending on the host, with a high number of nymphs developing on lemon plants. Nymphs that developed on lemon exhibited a heightened energy metabolism revealed by proteome analysis, and an increase in initiation translation factors. Nymphs that developed on SwO showed a high abundance of proteins associated with "muscle" and "neuronal muscle development". Feeding on sucrose and on SwO induced an enrichment of "semi-sterile" and "abnormal development" phenotype groups. In conclusion, the nutrients obtained through feeding on lemon was conducive to the successful development of *T. erytreae*, whereas feeding on SwO seemed to be suboptimal. Furthermore, a diet comprised solely of sucrose induced a stress response and potential hinderance of psyllid development.

5.2. Abbreviations

AGC (automatic gain control); ATP (adenosine triphosphate); CkIIbeta (casein kinase II beta subunit); DAI (days after infestation); DAP (differentially abundant proteins); FA (formic acid); FDR (false discovery rate); HLB (huanglongbing); "Lemon" (a treatment group formed by *Trioza erytreae* nymphs developed exclusively on lemon plants); LemonSuc (a treatment group comprised by *Trioza erytreae* nymphs developed on lemon plants that underwent a 24 h

sucrose feeding treatment); nanoLC-MS/MS (nanoscale liquid chromatography coupled to tandem mass spectrometry); SDM (standard deviation of the mean); SEM (standard error mean); SwO (sweet orange, *C.* ×*sinensis*); "SwO" (a treatment group formed by *Trioza erytreae* nymphs developed exclusively on sweet orange plants); SwOSuc (a treatment group comprised by *Trioza erytreae* nymphs developed on sweet orange plants that underwent a 24 h sucrose feeding treatment)

5.3. Introduction

Huanglongbing (HLB) is a highly destructive citrus disease transmitted by the psyllids *Trioza erytreae* (Del Guercio, 1918) and *Diaphorina citri* (Kuwayama, 1908) and the causal agent is the phloem-limited bacterium *Candidatus* Liberibacter spp. (Bové, 2006). As there is currently no effective treatment against this disease, vector control and the removal and elimination of infected plants are the only control measures (Alquézar et al., 2022). Since Europe is one of the few continents that remains free of HLB (Alquézar et al., 2022), the implementation of diversified vector control measures is needed to prevent the introduction and spread of HLB disease. Interaction analysis between vector and host plant is a crucial field that may assist in identifying critical points for the management of the psyllid vector as well as for the development of novel management practices (Magalhães et al., 2025). *Trioza erytreae* and *D. citri*, are hemimetabolous hemiptera insects (Mito et al., 2010). Prior to the emergence of the alate adult, the nymphs undergo five nymphal instars that feed on plant sap (Aubert, 1987). The emergence of young flushes and the presence of young leaves is a necessity for oviposition and nymphal development in both psyllid species (Catling, 1969; Cifuentes-Arenas et al., 2018).

Spain and Portugal, the first and fourth largest citrus producing countries in Europe, respectively, have recently reported the spread of *T. erytreae* in their continental territories. This pest was introduced to mainland Portugal and Spain in 2015, specifically in the Porto and Galicia regions, respectively (Duarte et al., 2024; EPPO, 2023, 2022, 2021; FAO, 2021). *Trioza erytreae* exhibits specific developmental symptoms, characterised by the formation of pit galls on the leaves of the infested plants, with one nymph developing in each pit gall (Van den Berg, 1990).

Rutaceae species constitute the functional hosts for this psyllid, with the subfamily Aurantioideae, which includes the genus *Citrus*, containing most of the highly suitable hosts for *T. erytreae* (Aubert, 1987). Lemon plants [*Citrus* ×*limon* (L.) Osbeck] have been identified as a preferred host for *T. erytreae* (Aidoo et al., 2019a; Benhadi-Marín et al., 2021; Magalhães et al., 2025). According to Aubert (1987), the sweet orange (SwO) [*Citrus sinensis* (L.) Osbeck] is a common host. Compared to

other hosts, *T. erytreae* development on lemon hosts yields larger nymphs and adults (Aidoo et al., 2019c). The volatile profile and flushing rhythm of lemon plants have been identified as the primary factors influencing the selection of these hosts by adult *T. erytreae* (Antwi-Agyakwa et al., 2019; Catling, 1969). Nonetheless, the underlying mechanisms driving the psyllid´s development on lemon plants remain to be elucidated.

Proteomics studies have yielded insights into the developmental biology of insects (Shashank and Bollineni, 2014; Si et al., 2020). The fruit fly, *Drosophila melanogaster*, (Meigen, 1830) (Casas-Vila et al., 2017; Roberts, 2006), is the ideal ortholog model for the functional analysis of insect development. Even though the fruit fly undergoes holometabolism, while *T. erytreae* exhibits hemimetabolism (Mito et al., 2010), and although development differs, several metabolic pathways, such as the "hedgehog (Hh) signalling pathway" are conserved and play a crucial role in insect development (Lin and Smagghe, 2019; Villarreal et al., 2015; Yamanaka et al., 2013). The nutritional intake and dietary habits of insects exert a significant influence on their developmental processes (Delisle and Hardy, 1997; Layalle et al., 2008). These dietary factors affect the insect's proteome causing increased energy metabolism, upregulation of initiation translation factors and protein synthesis, as well as affecting its growth potential (Arrese and Soulages, 2010; Nagarajan and Grewal, 2014). Proteomics studies facilitate the identification of essential molecular interactions between the insect and plant hosts. Furthermore, Hunter et al. (2017) patented a molecular approach that promotes the expression of a specific gene in citrus plant hosts that impairs *D. citri* development when it feeds on them. This patent was based on omics studies that showed the importance of the identified gene in insect development and on studies that showed it interfered on normal *D. citri* development (Liu et al., 2020; Shukla et al., 2015; Yang et al., 2020a).

As demonstrated by Franco et al. (2020) and Killiny (2017), the sap content of different citrus species varies. Consequently, the diet of *T. erytreae* differs based on the specific host citrus species. Furthermore, hosts exhibit different responses to sap feeding insects, which likewise exhibit distinct responses to these pests. The

diversity of defence mechanisms and the ability to adjust the sap composition to create a less conducive environment for the insect, underscores the complexity of insect–plant interactions (Kehr, 2006; Thompson and Goggin, 2006). Moreover, sap feeding insects have developed different strategies to circumvent the plant's defence mechanisms. These include the use of salivary effectors that alter plant metabolism, and the avoidance of detection by plants (Walling, 2008; Will et al., 2013). Furthermore, the phloem sap is characterised by a rich sugar content, which has led to the evolution of adaptations that enable these insects to cope with high sugar diets (Dinant et al., 2010; Sharma and Raman, 2017). Omics studies have proven effective in explaining the interactions between sap-feeding insects and their plant hosts (Oates et al., 2016; Zogli et al., 2020). A proteome adjustment in the vascular sap has been observed in sweet orange plant hosts in response to *T. erytreae* infestation, which is less pronounced in lemon hosts (Magalhães et al., 2024). The use of artificial diets has proven instrumental in the identification of dietary components that influence insect development (Catalani et al., 2021; Chen et al., 2017a). However, research employing artificial diets for psyllid vectors of HLB remains limited. To the best of our knowledge, there is a paucity of research studies conducted on *D. citri* (Hall et al., 2010; Russell and Pelz-Stelinski, 2015) and no studies have been undertaken on *T. erytreae*.

The present study employed proteomics to analyse the proteome of fourth and fifth instar nymphs of *T. erytreae* reared on the preferred host, lemon (*C.* ×*limon*), and the common host, SwO (*C. sinensis*), aiming to better understand the host effect on psyllid development. The oviposition, infestation behaviour and development of *T. erytreae* on these two hosts were monitored over time. Furthermore, an artificial sucrose feeding treatment protocol was established to ascertain the impact of a 24 hour dietary transition to a sucrose-only regimen on nymphs that developed and emerged on lemon or SwO plants. The proteome was analysed using nanoscale liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS). This study aimed to better understand the influence of the host plant on *T. erytreae* metabolism and development and the effect of a sucrose only diet on these insects.

5.4. Materials and methods

5.4.1. Insect origin and rearing

Adults of *Trioza erytreae* were captured using a handheld aspirator from pesticide-free lemon orchards in Caracoi, district of Porto, Portugal (41°18'46.4"N 8°38’09.7"W), in 2021. The captured psyllids adults were collected in conical centrifuge tubes (50 mL) and were then released onto the lemon and sour orange (*C. aurantium* L.) plants within acrylic cages (40 x 30 x 43 cm) covered with insect-proof nets. The rearing cages were maintained within a climate chamber at 21°C ± 1 °C with a relative humidity of 50 ± 5% and a photoperiod of 16:8 h of light: dark (L:D).

5.4.2. Infestation and psyllid development

Adult specimens from the rearing cages were used to infest ‘Valencia Midknight Seedless’ sweet orange [*C.* ×*sinensis* (L.) Osbeck] (SwO) and ‘Eureka’ lemon [*C.* ×*limon* (L.) Osbeck] (lemon) plants, both of which were grafted onto ‘Carrizo’ citrange (*C. trifoliata* × *C.* ×*sinensis*) rootstock. A total of 16 plants were used in the study, eight lemon plants and eight SwO plants. The plants used for infestation experiments (two-year-old plants with 0.8 to 1 m in height) were acquired from a certified nursery with a phytosanitary passport. All plants were potted in 5 L tall pots (19 cm diameter and 25 cm high) and were maintained in a climate chamber with controlled conditions of temperature (23.5 ± 1 °C), humidity (79 ± 5%) and photoperiod [14:10 h (L:D)]. Three weeks prior to the infestation, the citrus plants were pruned to stimulate new shoot formation, and the plants selected for the experiment exhibited a comparable number of new shoots. Plants were isolated within a cylindrical insect-proof net, which was fixed above the canopy with a wooden structure and secured to the tree trunk above the pot, enveloping the entire canopy. *Trioza erytreae* mature adults were aspirated from the rearing cages with a handheld aspirator and collected into a conical centrifuge tube (50 mL). The psyllids were then introduced onto the netted citrus hosts using the conical centrifugal tubes. Ten (five males and five females) *T. erytreae* mature adults were used per plant.

The intensity of egg-laying per leaf was categorised using the following scale: AO- no eggs; BO- 1 to 10 eggs; CO- 11 to 20 eggs; DO- 21 to 40 eggs; EO- 41 to 100 eggs; FO- more than 100 eggs. The intensity of nymphs per leaf was categorised using the following scale: A- no nymphs; B- 1 to 10 nymphs; C- 11 to 20 nymphs; D- 21 to 40 nymphs; E- 41 to 100 nymphs; F- more than 100 nymphs. The egg-laying and nymphal intensity was evaluated two days after infestation (DAI) and then at three-day intervals throughout the experiment. Plant hosts were observed daily, and the first appearance of each developmental stage was recorded (from first instar nymphs to new adult emergence).

Upon attaining the fourth and fifth instar developmental stage, nymphs were removed from the leaves and divided into two groups: one group for protein extraction and the other group underwent sucrose feeding followed by protein extraction. The nymphs were counted as they were removed from the leaves, along with the pit galls that had formed on the leaves. Dead nymphs attached to the leaves were not counted and were not used in the aforementioned protocols.

5.4.3. Treatment groups and sucrose feeding

The study comprised four distinct treatment groups: a) two treatment groups consisted of nymphs collected directly from the citrus host plants and were designated as “Lemon” and “SwO”, for nymphs that exclusively developed on lemon and SwO plants, respectively; b) two treatment groups consisted of nymphs that underwent a 24 h sucrose feeding regimen after being detached from the citrus host plant and these groups were designated as “LemonSuc” and “SwOSuc”, for nymphs that developed on lemon and SwO plants, respectively. Each treatment group was represented by four biological replicates (n= 4) and each biological replicate contained 100 nymphs, randomly collected from plants within the same treatment group.

The fourth and fifth instar nymphs from the “Lemon” and “SwO” treatments were removed from the leaves, weighed in 2 mL microcentrifuge tubes and immediately frozen in liquid nitrogen and stored at -80 °C until the protein extraction protocol. The fourth and fifth instar nymphs from the “LemonSuc” and “SwOSuc”

treatments were removed from the leaves and subjected to a 24 h sucrose feeding regimen (Fig. 5.1).

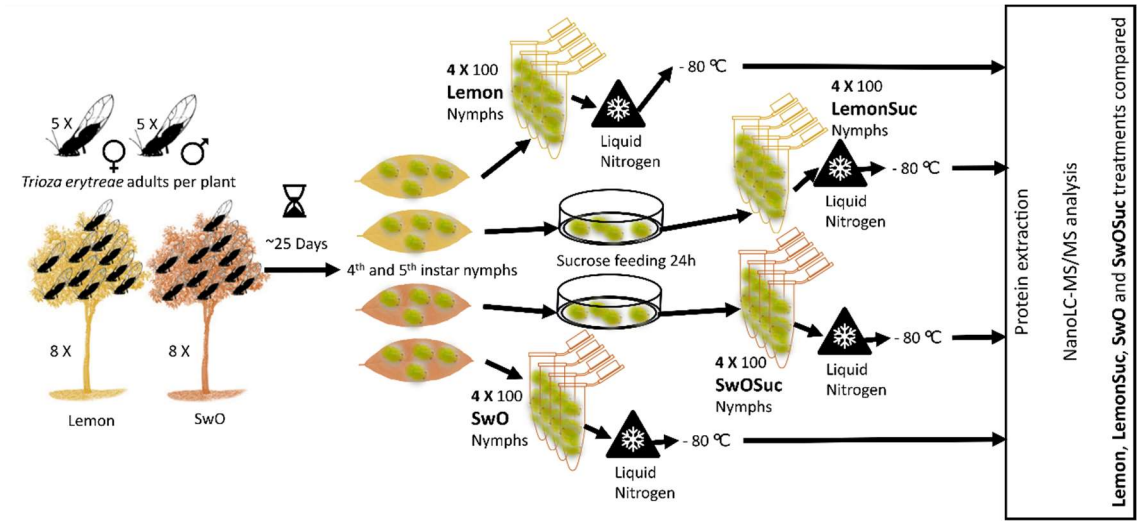

Figure 5.1. An overview of the experimental workflow: infestation of citrus hosts with *T. erytreae*, nymphal development and sucrose feeding. "Lemon": a treatment group formed by nymphs developed exclusively on lemon plants. "LemonSuc": a treatment group composed of nymphs developed on lemon plants and then fed for 24 h on sucrose. "SwO": a treatment group formed by nymphs reared exclusively on sweet orange (SwO). "SwOSuc": a treatment group composed of nymphs developed on SwO plants and then fed for 24 h on sucrose. The following comparisons were made SwO *vs* Lemon; LemonSuc *vs* Lemon; SwOSuc vs SwO and SwOSuc *vs* LemonSuc.

For the 24 h sucrose feeding regimen nymphs were removed from their hosts and placed on a Petri dish with filter paper (Whatman Grade 1), soaked in 1 mL of a 0.5 M sucrose solution. The nymphs were maintained in the Petri dish for a period of 24 h, using the same climatic conditions used for the infestation of host plants and nymphal development. Nymphs were observed to quickly settle and new honeydew excretions were observed, which indicated active feeding by the nymphs (Urbaneja-Bernat et al., 2023; Van den Berg et al., 1991d). The nymphs were removed from the Petri dishes, weighed in 2 mL microcentrifuge tubes, frozen in liquid nitrogen and stored at -80 °C until the protein extraction protocol.

The 24 h feeding regime on the sucrose diet was selected based on preliminary studies, which demonstrated a nymphal survival rate of 94%, while after 48h it decreased to 82%. After a period of sucrose feeding for more than 48h, most of the surviving nymphs emerged as new adults. In fact, after 72h an average of 60% of the nymphs were either dead or morphed into adults.

5.4.4. *Trioza erytreae* nymphs' protein extraction and analysis

Total protein was extracted from the fourth and fifth instar nymphs collected from the four experimental groups: "Lemon", "SwO", "LemonSuc" and "SwOSuc" (Fig. 5.1). The protein extraction method was adapted from Cilia et al. (2009) using the TCA-Acetone method for protein extraction from aphids. Modifications were made to the homogenisation procedure and centrifugation force, based on the *D. citri* protein extraction protocol (Ramsey et al., 2015). Briefly, frozen samples (of 100 nymphs each in 2 mL microcentrifuge tubes) were mechanically homogenised using a 3 mm stainless steel ball in a TissueLyser Mixer Mill 400 (Retsch, Haan, Germany). The first cycle was set to last for 1 min and was executed without the addition of any buffer (dry cycle) to guarantee the destruction of the samples. Subsequently, 500 µL of protein extraction buffer (10% TCA with 2% β-mercaptoethanol in acetone) was added to each sample, and four homogenisation cycles, (of 1 min, except the second cycle, which was 2 min) with 5 min incubation pauses on ice between each cycle were performed. All cycles (wet and dry) were configured at 30 Hz. The steel balls were removed, and the samples were vortexed, stored at -80 °C for 5h and centrifuged at 14,000 × g for 30 min at 4 °C. The resulting pellets were washed with 1 mL of acetone at 4 °C and centrifuged at 14,000 × g for 5 min at 4 °C (this step was repeated two more times). Pellets were dried and resuspended in 200 µL of protein solubilisation buffer [7 M Urea, 2 M Thiourea, 4% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate (CHAPS)] and stored at -20 °C overnight. Samples were centrifuged at 14,000 × g for 20 min at 4 °C and the soluble protein fraction was recovered as the final extraction product (Fig. 5.1) and was stored at -80ºC. The total soluble protein extracted from *Trioza erytreae* nymphs was quantified using a Genesys 1Q-S spectrophotometer (Thermo Electron Corporation, Bremen, Germany) with a Quick Start™ Bradford Protein Assay Kit (Bio-Rad, Hercules, USA), according to the manufacturer's instructions and using bovine serum albumin (BSA) as the standard. The quality of the nymph protein extract was evaluated (30 µg of total soluble protein/ sample) by electrophoresis using 12% sodium dodecyl-sulphate polyacrylamide gels (SDS-PAGE), according to the Laemmli method (Laemmli, 1970), and stained with Coomassie blue. Prior to

nanoLC-MS/MS proteomic analysis, 50 µg of soluble protein from each sample underwent a solid-phase-enhanced sample-preparation (SP3) protocol (Hughes et al., 2018), followed by overnight enzymatic digestion with trypsin/LysC (2 µg) at 37 ºC and 1,000 rpm. The concentration of the resulting peptides was then quantified using fluorescence.

5.4.5. Proteomic analysis of nymphs

5.4.5.1. Proteomics data acquisition

The proteome of the nymphs from the four experimental conditions (Lemon, LemonSuc, SwO and SwOSuc, n = 4 samples of 100 nymphs/ condition) was obtained based on the protocol described by Osório et al. (2021). Protein identification and quantification were carried out using a nanoscale liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS) on an Ultimate 3000 liquid chromatography system coupled to a Q-Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany), by an external service provider (Proteomics Scientific Platform of i3S, Ipatimup, Porto, Portugal).

In summary, 500 ng of trypsin/LysC-digested samples were loaded into a trapping cartridge (Acclaim PepMap C18 100 Å, 5 mm × 300 µm i.d., 160454, Thermo Scientific) in a mobile phase of 2% acetonitrile (ACN), and 0.1% formic acid (FA) at a flow rate of 10 µL/min. Following a 3 min loading interval, the trap column was switched in-line to a 50 cm × 75 µm inner diameter EASY-Spray column (ES803, PepMap RSLC, C18, 2 µm, Thermo Scientific) at a flow rate of 250 nL/min. The separation was achieved by employing a solvent mixture of A: 0.1% FA and B: 80% ACN, 0.1% FA with a gradient elution program: 5 min (2.5% B to 10% B), 120 min (10% B to 30% B), 20 min (30% B to 50% B), 5 min (50% B to 99% B), and 10 min (hold 99% B). Subsequently, the column was subjected to a 17 min equilibration period with 2.5% B. Data acquisition was controlled by Xcalibur 4.0 and Tune 2.9 software (Thermo Scientific).

The mass spectrometer was operated in data-dependent (dd) positive acquisition mode, alternating between a full scan (*m/z* 380-1,580) and subsequent

higher-energy collisional dissociation tandem mass spectrometry (HCD MS/MS). This method was established for the 10 most intense peaks from a full scan (normalised collision energy of 27%). The electrospray ionisation (ESI) spray voltage was set to 1.9 kV, with the global settings configured as follows: optimal lock mass (*m*/*z* 445.12003), lock mass injection, full MS, and chromatographic peak width at a full width half maximum (FWHM) of 15 s. The full scan settings were as follows: 70 k resolution (*m*/*z* 200), automatic gain control (AGC) target 3×10^6, maximum injection time 120 ms; dd settings: minimum AGC target 8×10^3, intensity threshold 7.3×10^4, charge exclusion: unassigned, 1, 8, >8, peptide matches preferred, exclude isotopes on, and dynamic exclusion 45 s. The MS2 settings were as follows: microscans - 1, resolution - 35 k (*m*/*z* 200), AGC target - 2×10^5, maximum injection time - 110 ms, isolation window - 2.0 *m*/*z*, isolation offset - 0.0 *m*/*z*, dynamic first mass and spectrum data type profile.

5.4.5.2. Data processing, protein-label-free quantification and identification

The mass spectrometry (MS) raw data were processed using Proteome Discoverer 2.5.0.400 software (Thermo Scientific). Protein identification searches were conducted using the UniProt protein sequence database for *D. citri* (taxon ID 121845, 21,517 entries) and for *C.* ×*sinensis* (taxon ID 2711, 44,601 entries) and a common contaminant database from MaxQuant (version1.6.2.6, Max Planck Institute of Biochemistry, Munich, Germany). The Sequest HT tandem mass spectrometry peptide database search program was employed to identify tryptic peptides, with an ion mass tolerance of 10 ppm for precursors and 0.02 Da for fragmented ions and missing cleavage sites was set as 2. Cysteine carbamidomethylation was defined as a constant modification. Methionine oxidation, asparagine, and glutamine deamidation, peptide N-terminus Gln->pyro-Glut, protein N-terminus acetylation, and loss of methionine and Met-loss+Acetyl were defined as variable modifications. The peptide confidence was set to high and the Inferys rescoring node was considered for this analysis. The Percolator processing node was enabled with the following settings: maximum Delta

Correlation (deltaCn) 0.05; decoy database search target False Discovery Rate (FDR) 1%; validation based on the *q*-value.

Protein-label-free quantification was conducted using the Minora feature detector node at the processing stage. The following parameters were employed for precursor ion quantification: 1) Unique peptides plus razor; 2) Precursor abundance was determined based on intensity; 3) Normalisation mode was based on total peptide amount; 4) The minimum number of replicate files was set to 50% in each sample group; 5) Pairwise protein ratio calculation and hypothesis testing were based on a *t*-test (background based). The Feature Mapper node in Proteome Discoverer software was employed to generate features from distinctive peptide-specific peaks within a narrow retention-time and mass range (a maximum shift of 10 min and 10 ppm of mass tolerance was permitted for the mapping of features from different sample files). For the purpose of feature linking and mapping, the signal to noise (S/N) threshold was set at 5 for each comparison. Libraries of proteins and peptides confidently identified and quantified for each experimental condition were generated and the mass spectrometry proteomics data were deposited in the ProteomeXchange Consortium via the PRIDE (www.ebi.ac.uk/pride) (Perez-Riverol et al., 2022) partner repository with the dataset identifier PXD059807 and 10.6019/PXD059807. Fold-change in protein abundance was calculated in each established comparison (SwO *vs* Lemon; LemonSuc *vs* Lemon; SwOSuc *vs* SwO and SwOSuc *vs* LemonSuc) using the medians (log2-transformed) between relative protein abundance in the experimental groups and differentially abundant proteins (DAPs) were considered.

5.4.5.3. Bioinformatics analysis

Functional analysis of the proteome was performed using *Drosophila melanogaster* Meigen, with 1,830 orthologues of the identified proteins, obtained with the STRING webtool version 12.0 (https://string-db.org/) (Szklarczyk et al., 2023). Enrichment analysis was performed using the webtool PANGEA, PAthway, Network and Gene-set Enrichment Analysis a multi-species enrichment tool, version 1 beta 1 (https://www.flyrnai.org/tools/pangea/) (Hu et al., 2023). A

hypergeometric test/Fisher's exact test was conducted with a Benjamin and Hochberg (Benjamini and Hochberg, 1995) FDR correction, considering an adjusted *p*-value of <0.05. The following databases were used for all the enrichment analysis: Gene Ontology (Aleksander et al., 2023; Ashburner et al., 2000), FlyBase (Öztürk-Çolak et al., 2024) and Reactome (Milacic et al., 2024). Bubble plots were created using R software (R Core Team, 2020) with the ggplot2 package (Wickham, 2016). Venn diagrams were created using the Venny 2.1 web tool (https://bioinfogp.cnb.csic.es/tools/venny/) (Oliveros, 2015).

5.4.6. Statistical analysis

Statistical tests were performed using Rstudio software (R Core Team, 2020), unless otherwise specified in this section. The normality of all data was assessed using a Shapiro–Wilk test, while homoscedasticity was evaluated with Levene's test for normally distributed data and the Brown–Forsythe test for non-normally distributed data.

A Welch's *t*-test was employed to compare the mean number of flushes per plant between SwO and lemon plants. A Wilcoxon–Mann–Whitney test was employed to compare the average development time of *T. erytreae* on the two hosts. A χ^2 (Chi-squared) test was used to compare leaves containing eggs and those devoid of eggs in both citrus hosts. Each egg intensity class on the oviposited leaves was compared among citrus hosts using a Fisher's exact test. Finally, the mean number of nymphs and pit galls per plant was compared between SwO and lemon plants, using a Student's *t*-test. In proteomic analysis and to determine the DAPs, the following criteria were considered: (1) a minimum number of biological samples in which a protein was detected in an experimental group was set to 75% (e.g. 3 out of 4); (2) the identification of at least two unique peptides for protein assignment; (3) a stringent FDR with the *q*-value set at <0.01. Protein intensities were log2-transformed and were normalised based on the quantile method. Protein-wise linear models for paired samples combined with empirical Bayesian statistics were employed for differential abundance analysis, using the R Bioconductor package limma (Ritchie et al., 2015). FDR correction was achieved using the Benjamini-Hochberg (BH) method, and DAPs were identified with an adjusted p-value of < 0.05.

5.5. Results

5.5.1. *Trioza erytreae* exhibited a citrus host-specific oviposition pattern and nymphs developed better on lemon than on SwO plants

The psyllids and the citrus hosts exhibited signs of growth and development throughout the course of the experiment. Regarding the host plant, at the onset of the experiment, the mean number of new flushes per plant was 4.6 for 'Eureka' lemon and 6.4 for the 'Valencia' SwO and were not significantly different (Welch's *t*-test, $p = 0.15$). The increase in the number of new leaves from 5 DAI and 23 DAI was of 9.4% for lemon and 9.6% for SwO plants (Table 5.1), indicating that the citrus plants had a similar growth pattern.

Table 5.1. Total number of oviposited and infested leaves of citrus plants at 5 DAI and 23 DAI, respectively. Increase in new leaves and new infested leaves of citrus plants from 5 DAI to 23 DAI. "SwO" refers to sweet orange plants. "Available leaves" refers to the quantity of new leaves from the growth induced by pruning, which were the ones available for infestation. "Oviposited leaves" denotes the number of leaves harbouring *Trioza erytreae* eggs, with the percentage of these leaves in relation to the "Available leaves" indicated in brackets. "Infested leaves" denotes the number of leaves containing *T. erytreae* nymphs and/or eggs with the percentage of these leaves in relation to the "Available leaves" indicated in brackets. The emergence of "New leaves" was determined by calculating the difference between the "Available leaves" at 5 DAI and the "Available leaves" at 23 DAI, with the percentage increase in new leaves indicated in brackets. Similarly, the calculation of "New infested leaves" involved the subtraction of "Oviposited leaves" at 5 DAI from "Infested leaves" at 23 DAI, with the percentage increase of new infested leaves indicated in brackets.

Host citrus	5 DAI		23 DAI		Increase from 5 DAI to 23 DAI	
	Available leaves	Oviposited leaves	Available leaves	Infested Leaves	New leaves	New infested leaves
SwO	251	106 (42.2%)	275	158 (57.5%)	24 (9.6%)	52 (49.1%)
Lemon	181	25 (13.8%)	198	128 (64.6%)	17 (9.3%)	103 (412%)

Henceforth the term "available leaves" refers to the new leaves from the growth induced by pruning, as these are the young leaves that the psyllid requires for a successful infestation. The first indications of oviposition were observed at 2.6 and 2.5 DAI on average in lemon and SwO, respectively. The peak oviposition period on the lemon host was recorded at 5 DAI, with 13.8% of the available leaves having eggs. In contrast, over the same 5 DAI, a more dispersed pattern was observed on SwO, with 42.2% of the available leaves having eggs (Table 5.1). The peak oviposition

on SwO was recorded at 8 DAI, and eggs were found on 45.7% of the available leaves. In contrast, 12.5% of the available leaves had eggs at 8 DAI in lemon. The citrus host influenced the proportion of available leaves with eggs (p- value <0.05, Chi squared test) for all DAIs evaluated except at 23 DAI.

The egg intensity, number of eggs per available leaf, categorised as AO (no eggs per leaf), BO (1-10 eggs per leaf) and FO (more than 100 eggs per leaf) was influenced by the citrus host (p <0.05) at both 5 and 8 DAI, although EO (50-100 eggs per leaf) was only influenced by the citrus host at 8 DAI. The present study revealed higher levels of oviposition intensity per leaf on lemon, in comparison to SwO. At 5 DAI, 52% of the leaves with eggs in lemon plants exhibited more than 20 eggs, and 24% had over 100 eggs (Fig. 5.2A). Conversely, at 5 DAI the SwO plants had a low egg intensity, and 27.4% of leaves with eggs had more than 20, and 5.7% had over 100 eggs (Fig. 5.2A). A difference in egg intensity between the citrus hosts was also observed at 8 DAI. In lemon 66.67% of leaves with eggs had more than 20 and this fell to 28.19% in SwO.

The quantity of infested leaves increased notably from 5 DAI to 23 DAI and was more notable for lemon (412%) than SwO (49.1%), although at 23 DAI a similar number of infested leaves was recorded (128 on lemon and 158 on SwO) (Table 5.1). The evaluation of infestation intensity at 23 DAI showed the nymph intensity class distribution was not host dependent (p > 0.05, Chi squared test). Although, a greater proportion of the infested leaves in the lemon (5.5%) had more than 100 nymphs, compared to the infested leaves of SwO (2.5%, Fig. 5.2B). The average number of pit galls per plant which varied between 330.1 [(± 47.5 standard error mean (SEM)] for lemon and 246.8 (± 30.8 SEM) for SwO plants did not differ significantly between plant hosts (p-value= 0.16, Students t-test). A significantly higher number of nymphs (fourth and fifth instar) developed on lemon compared to SwO (p< 0.05, Student t-test). The average number of fourth and fifth instar nymphs per plant was 318.5 (± 47.3 SEM) for lemon plants and 99.3 (± 27.6 SEM) for SwO plants.

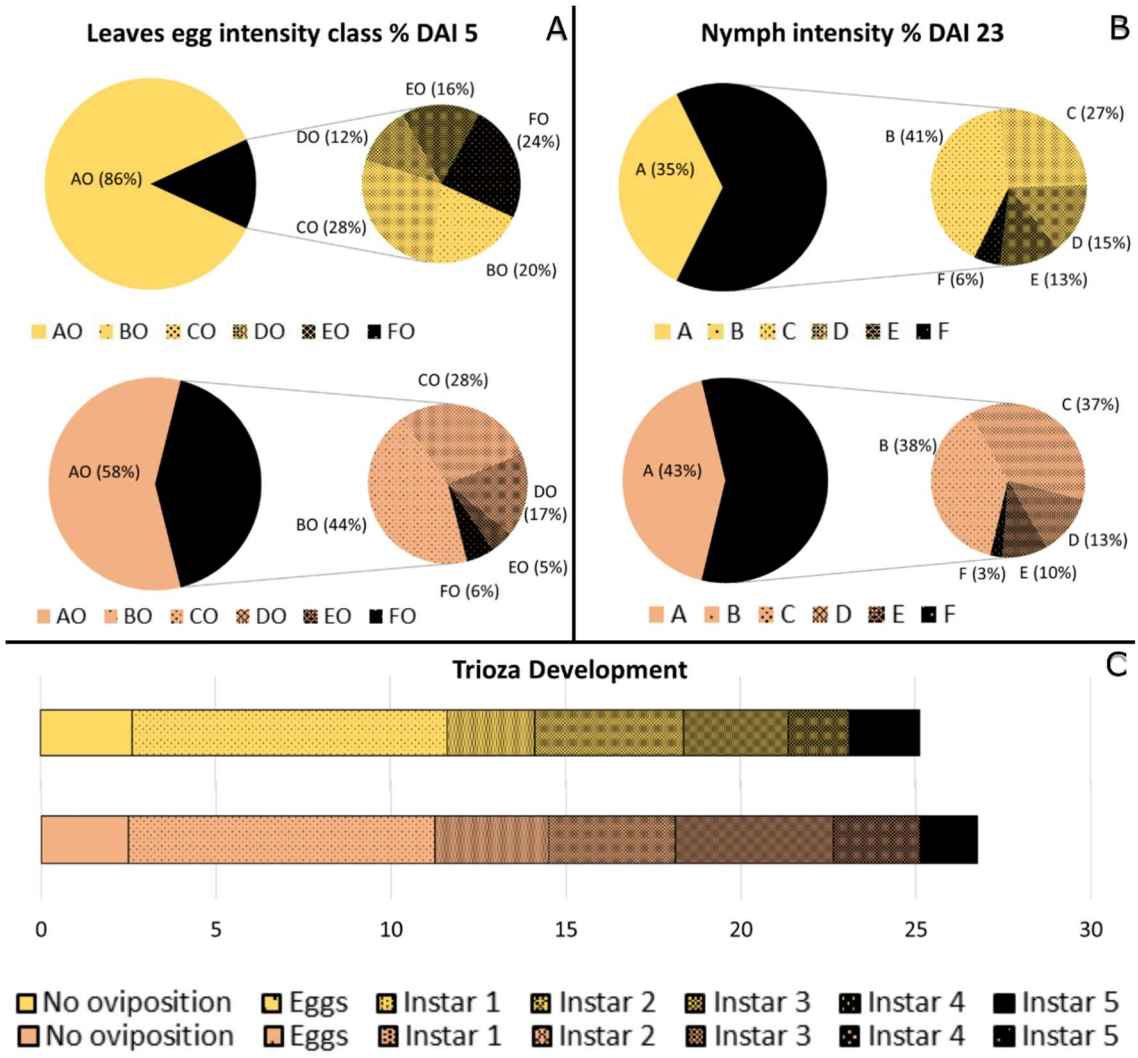

Figure 5.2. A: Pie charts depicting the presence and intensity of eggs deposited per leaf five days after infestation (DAI); B: Pie charts illustrating the presence and intensity of nymphs per leaf at 23 DAI (B); C: First observation of each psyllid developmental stage in each host. The leaf egg-laying intensity scale is as follows: AO- no eggs; BO- 1 to 10 eggs; CO- 11 to 20 eggs; DO- 21 to 40 eggs; EO- 41-100 eggs; FO- more than 100 eggs. The nymph intensity scale is as follows: A- no nymphs; B- 1 to 10 nymphs; C- 11 to 20 nymphs; D- 21 to 40 nymphs; E- 41-100 nymphs; F- more than 100 nymphs. The yellow colour background represents data recorded from lemon plants. The orange colour background represents data recorded from sweet orange plants.

The duration of the psyllid development was similar in both hosts (Fig. 5.2C). Although the time required (DAI) for the initial fifth instar nymph to mature (*p*-value= 0.013) and for the first adult to emerge (*p*-value= 0.018) was significantly higher in SwO (25.1 DAI ± 1.46 standard deviation of the mean SDM) compared to the lemon plants (23.1 DAI ± 0.64 SDM). The time required for the first adult emergence was 25.1 DAI (± 0.64 SDM) on lemon and 26.8 DAI (± 1.28 SDM) on SwO.

5.5.2. Citrus host plant and sucrose feeding affected *Trioza erytreae* protein identification

The soluble protein extracts of fourth- and fifth-instar *T. erytreae* nymphs from the four experimental groups were first resolved by Coomassie blue one-dimensional SDS-PAGE and showed consistent high-quality protein extracts (Fig. S5.1- Appendix). Similar protein band profiles between the four different experimental groups were observed by visual inspection of gels.

Protein libraries that were confidently identified/quantified in the nymphs at the fourth and fifth instar by nanoLC-MS/MS, were profiled and compared between those that developed on lemon and those that developed on SwO, with and without sucrose treatment. A total of 2,777 proteins were identified in the nymph samples, of which 2,617 were identified as belonging to *D. citri,* and 161 were identified as belonging to *C.* ×*sinensis*. Following the exclusion of 25 proteins classified as contaminants, a total of 1,500 proteins confidently identified/quantified were filtered based on the following rigorous criteria's: a) proteins with a high FDR (*q*-value < 0.01); b) more than two unique peptides and c) proteins identified in at least 75% of the samples from each independent treatment group. Of these, 1,477 were identified as *D. citri,* and 23 were identified as *C.* ×*sinensis* (Fig. 5.3A and Table S5.1-Appendix).

The global comparative proteome analysis exhibited substantial overlap among the detected proteins in the four experimental groups. Among the 23 citrus host plant proteins and 1,477 psyllid proteins identified, 15 and 1,410, respectively, were common across all four experimental groups (Fig. 5.3A).

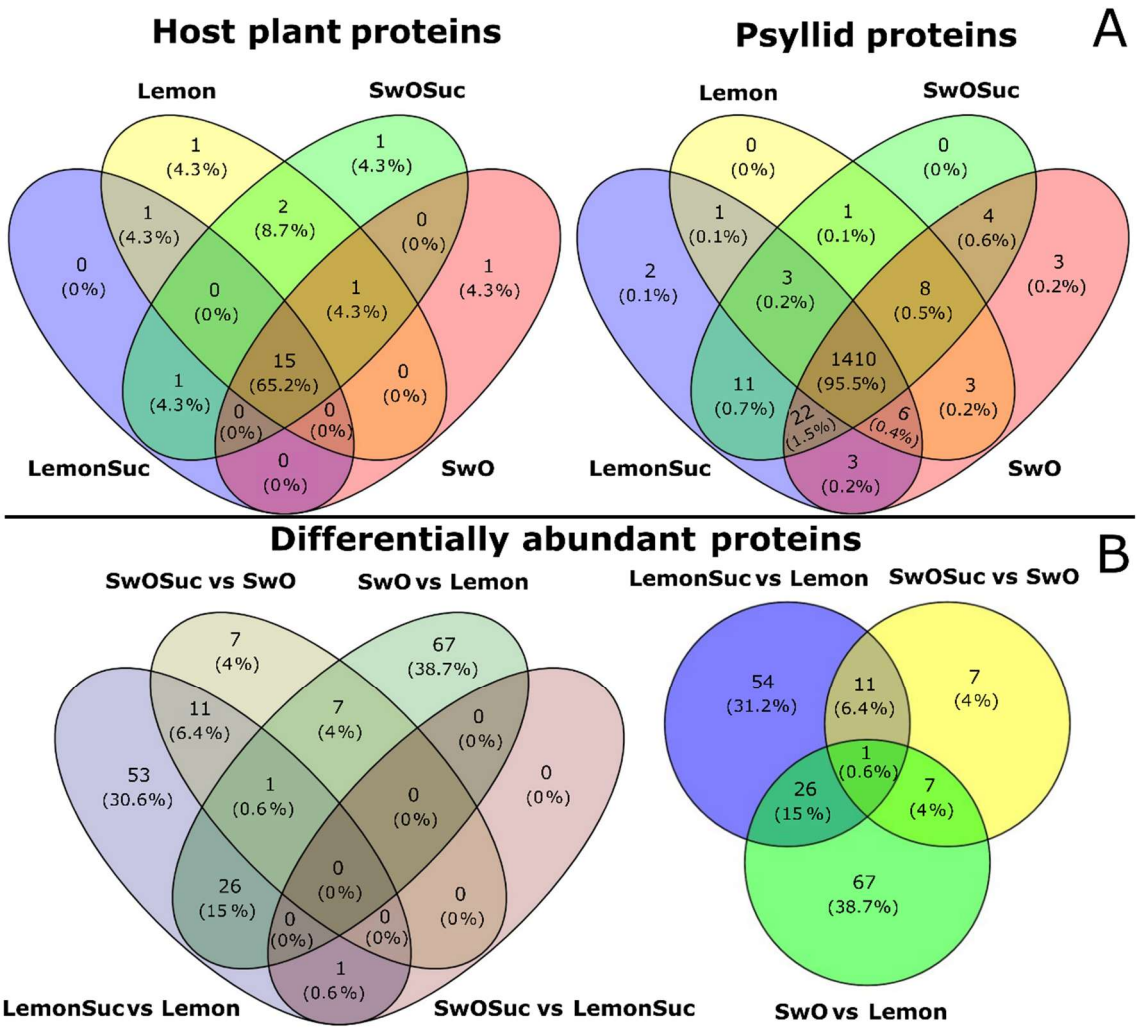

Figure 5.3. Venn diagrams illustrating the comparison of the number of proteins identified between the four experimental groups. **A:** Confidently identified/quantified "host plant proteins" (proteins identified as belonging to the *Citrus ×sinensis*, UniProt protein sequence database) and as "psyllid proteins" (proteins identified as belonging to the *Diaphorina citri* UniProt protein sequence database). **B:** Differentially abundant proteins (DAP´s, $p < 0.05$) between the specified comparisons. "Lemon": refers to the nymphs that developed on lemon plants. "LemonSuc": refers to the nymphs developed on lemon plants followed by 24 h sucrose feeding. "SwO": refers to the nymphs that developed on sweet orange (SwO). "SwOSuc": refers to the nymphs developed on SwO plants followed by 24 h sucrose feeding. The Venn diagrams were generated using the webtool https://bioinfogp.cnb. csic.es/tools/venny/.

5.5.2.1. Citrus hosts proteins identified on *Trioza erytreae* nymphs

Of the 15 common citrus host plant proteins identified in the nymphs from all the treatment groups (Fig. 5.3A). Five of these proteins are related to protein metabolism, A0A067GTS6 (KH type-2 domain-containing protein), A0A067EVG3 (26S protease regulatory subunit 6B), A0A067DE71 (Isoleucine--tRNA ligase), A0A067DP37 (60S ribosomal protein L23), A0A067EFK3 (Peptidyl-prolyl cis-trans isomerase) (Table S5.1- Appendix); and four proteins were associated with the plant

stress response, comprising two heat shock proteins, i.e., A0A067FWR4 (heat shock cognate 70 kDa protein) and H9NHJ9 (Hsp90). In addition, two proteins were identified that are associated with the oxidative stress response: A0A067F9T7 (Peroxidase) and A0A067DDL8 (Superoxide dismutase family protein) (Table S5.1-Appendix). Two additional citrus host proteins associated with antioxidant activity and the plant stress response were identified: one exclusive to nymphs from lemon, (A0A067H2F2, Catalase) and the other exclusive to nymphs from SwO (A0A067H6D4, Peroxidase) (Table S5.1- Appendix).

5.5.2.2. Treatment-specific psyllid proteins

In nymphs fed sucrose irrespective of the plant host (LemonSuc and SwOSuc) 11 common proteins were identified, and included “MRP” (Multidrug-Resistance like protein 1, isoform A), “Mi-2” (Chromodomain-helicase-DNA-binding protein Mi-2 homolog), “SMC1” (Structural maintenance of chromosomes 1), “bt” (Bent, isoform F), “Nedd4” (E3 ubiquitin-protein ligase Nedd-4) and “wrd” (Well-rounded, isoform B) (Table S5.1- Appendix). Four proteins were identified both in SwO and SwOSuc and were not identified in lemon fed nymphs (Fig. 5.3A), namely “bw” (Brown), “CG13185” (Midasin), and “Klp61F” (kinesin-like protein Klp61F) (Table S5.1- Appendix).

5.5.3. Citrus host plant and sucrose feeding affect the *Trioza erytreae* proteome

A total of 173 DAPs were identified across the four comparisons of the four experimental groups (p <0.05, Limma, with Benjamin-Hochberg correction). The SwO *vs* Lemon comparison yielded the highest number of DAPs (101), followed by the LemonSuc *vs* Lemon comparison (92 DAPs), the SwOSuc *vs* SwO comparison (26 DAPs), and finally, the SwOSuc *vs* LemonSuc comparison (1 DAP) (Table S5.2-Appendix, Fig. 5.3B and 5.4).

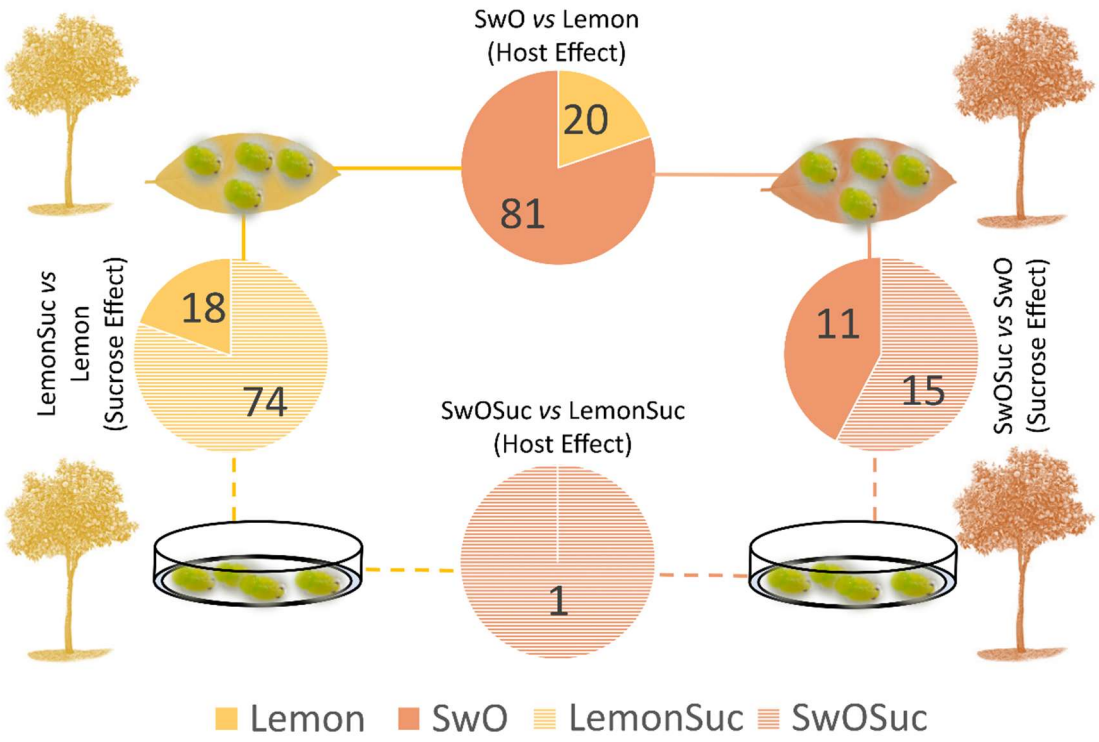

Figure 5.4. Differentially abundant proteins (DAP) identified in nymphs from the four experimental groups. Each pie chart represents one of the 4 comparisons performed and the numbers inside the pie charts represent the DAPs that were most abundant in the different experimental groups. The yellow colour denotes the lemon plant treatment groups. The orange colour denotes the sweet orange (SwO) plant treatment group. Dashed lines and striped colouring in pie charts represent nymphs that were fed sucrose (24 h). Solid lines and solid colours in pie charts represent nymphs that were not exposed to sucrose feeding. "Lemon": nymphs that developed exclusively on lemon plants. "LemonSuc": nymphs that developed on lemon plants and were then fed sucrose for24 h before sampling. "SwO": nymphs exclusively developed on SwO. "SwOSuc": nymphs that developed on SwO plants and were then fed sucrose for 24 h before sampling.

5.5.3.1. The citrus host effect: lemon plants modify nymph energy metabolism while SwO plants hinder nymph fertility and development

Among the 101 DAPs identified in the SwO *vs* Lemon comparison, 81 were found to be more abundant in SwO nymphs, whereas 20 were more abundant in Lemon (Fig. 5.4). The 20 proteins identified were mainly related with energy metabolism, and enriched pathways were "generation of precursor metabolites and energy", "carbohydrate metabolism process", "formation of ATP by chemiosmotic coupling", "mitochondrial fatty acid beta-oxidation", among other energy-related pathways (Fig. 5.5). Two specific ATP synthases were identified, namely "blw" (ATP synthase subunit alpha, mitochondrial) and "ATPsynbeta" (ATP synthase subunit beta, mitochondrial) (Table S5.2- Appendix).

In the SwO *vs* Lemon comparison, nymphs that developed on SwO exhibited an enrichment of the semi-sterile phenotype groups (Fig. 5.5). Two proteins that contributed to this enrichment were: “CkIIbeta” (Casein kinase II beta subunit) and “Abi” (Abelson interacting protein) (Table S5.2- Appendix).

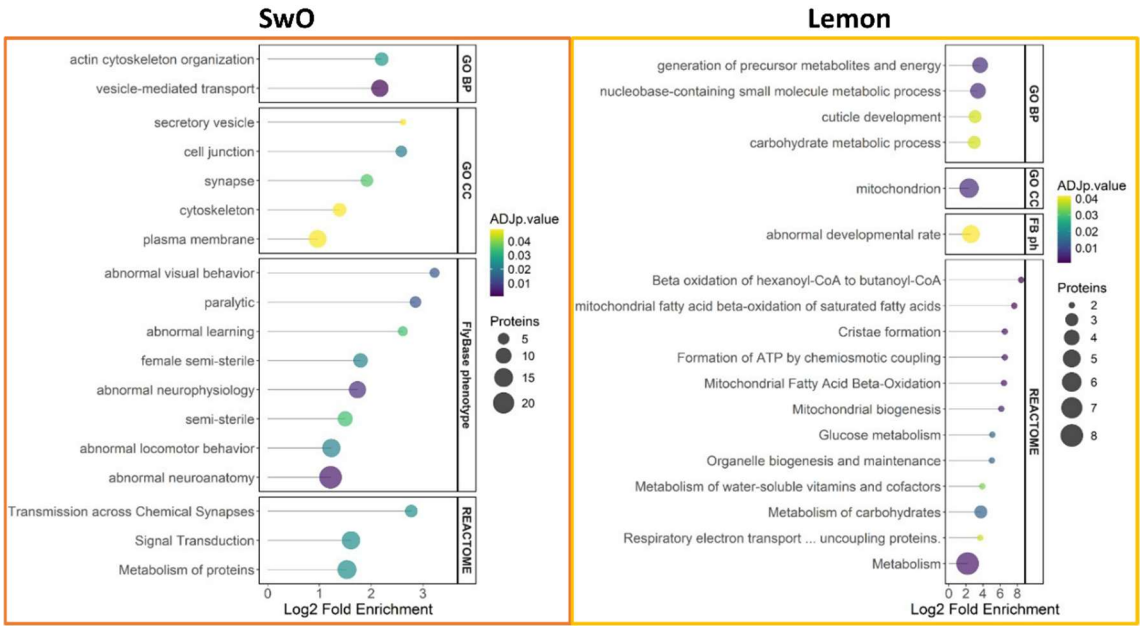

Figure 5.5. Bubble plots of the enriched parameters identified when differentially abundant proteins (DAPs) in nymphs from the SwO and Lemon comparison were used to interrogate different databases (FDR adjusted *p*-value threshold of 0.05). The size of the bubbles indicates the number of proteins in each pathway, whereas the colour denotes the FDR corrected *p*-value and fold enrichment. The *Drosophila melanogaster* proteome was used as the reference. “Lemon”: nymphs that developed exclusively on lemon plants. “SwO”: nymphs exclusively developed on SwO. GO BP: Gene ontology biological process database; GO CC: Gene ontology cellular component database (https://geneontology.org/); FlyBase phenotype or FB ph: Flybase phenotype database (https://flybase.org/); REACTOME: reactome pathway database (https://reactome.org/).

The results of the proteomic profiles of SwO and Lemon revealed that the most abundant proteins identified in SwO were associated with the biological processes “actin cytoskeleton organisation”, and “signal transduction” and “transmission across chemical synapses” pathways (Fig. 5.5). This was coherent with the higher abundance of “Syn” (synapsin-1) and “sls” (titin) observed in SwO (Table S5.2- Appendix). These two proteins also enriched the phenotype groups “paralytic” and “abnormal locomotor behaviour”. Additionally, the “abnormal neuroanatomy” and “abnormal neurophysiology” were enriched by “Dap160” (dynamin associated protein 160) and “CadN” (neural-cadherin) (Table S5.2- Appendix).

A comparison of the SwO and lemon bubble plots (Fig. 5.5) revealed a significant disparity in the enriched pathways between the two infested citrus host plants. The lemon plants exhibited enriched pathways related to growth, development and energy metabolism, including “organelle biogenesis and maintenance”, “mitochondrial biogenesis” and “cuticle development” (Fig. 5.5). In contrast, none of these pathways were enriched in the SwO plants, which had enriched pathways linked to abnormal development (Fig. 5.5).

5.5.3.2. Sucrose feeding had a higher impact on nymphs that developed on lemon plants, with the hedgehog signalling pathway affected

The proteome analysis of nymphs in the SwOSuc *vs* SwO comparison yielded a similar outcome, and 15 proteins were more abundant in SwOSuc nymphs and 11 were more abundant in SwO nymphs. In the LemonSuc *vs* Lemon comparison, 74 proteins were more abundant in LemonSuc nymphs, while 18 were more abundant in Lemon nymphs (Fig. 5.4). Comparison of nymphs exposed to sucrose feeding revealed an enrichment of the “Hh signalling pathway” in nymphs that developed on lemon plants (Fig. 5.6 and 5.7). The three DAPs that enriched the pathways in both sucrose feeding treatments were “Tnpo” (Transportin, isoform A), “wdb” (Widerborst) and “CG5504” (Protein tumorous imaginal discs, mitochondrial) (Table S5.2- Appendix). Three proteins were exclusively enriched in this pathway in LemonSuc, namely “UbcE2M” (Nedd8-conjugating enzyme UbcE2M), “flw” (Serine/threonine-protein phosphatase beta isoform) and “Gbeta76C” (Guanine nucleotide-binding protein subunit beta-2).

The 18 proteins with increased abundance in the Lemon nymphs, in the LemonSuc *vs* Lemon comparison (Fig. 5.4), were predominantly associated with protein translation-related functions, including “translational regulator activity”, “translation initiation complex formation”, and “ribosomal scanning and start codon recognition”, among others (Fig. 5.6). Proteins with high abundances that enriched these pathways included “eIF3b” (Eukaryotic translation initiation factor 3 subunit B) and “RpS28b” (40S ribosomal protein S28) (Table S5.2- Appendix). The semi-sterile phenotype groups were also enriched by the more abundant proteins in the SwOSuc (Fig. 5.7) and LemonSuc (Fig. 5.6) treatment groups.

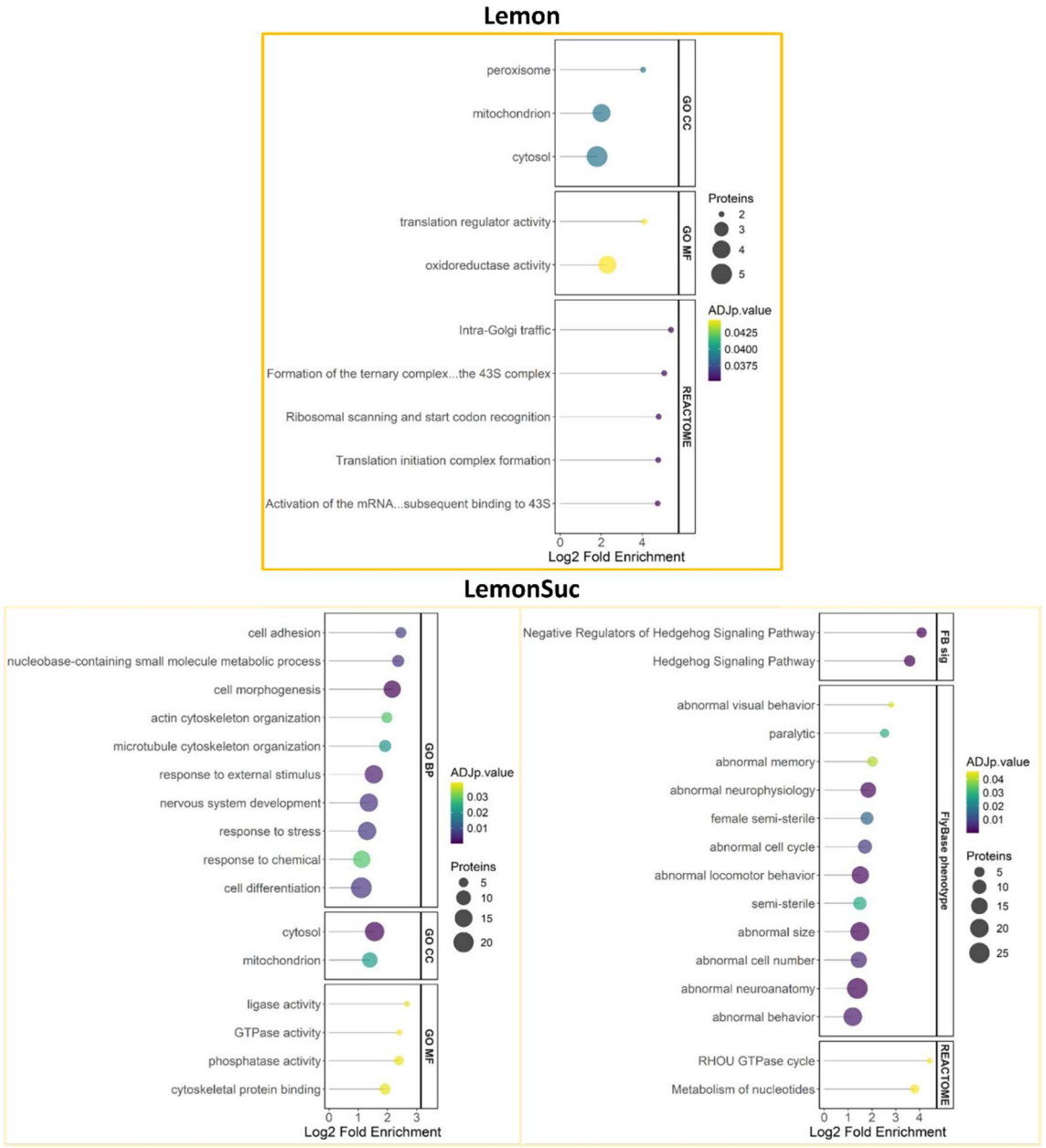

Figure 5.6. Bubble plots of the enriched parameters identified when differentially abundant proteins (DAPs) in nymphs from the SwO and Lemon comparison were used to interrogate different databases (FDR adjusted *p*-value threshold of 0.05). The size of the bubbles indicates the number of proteins in each pathway, whereas the colour denotes the FDR corrected *p*-value and fold enrichment. The *Drosophila melanogaster* proteome was used as the reference. "Lemon": nymphs that developed exclusively on lemon plants. "LemonSuc": nymphs that developed on lemon plants and were then fed sucrose for 24 h before sampling. GO BP: Gene ontology biological process database; GO CC: Gene ontology cellular component database (https://geneontology.org/); FlyBase phenotype or FB ph: Flybase phenotype database (https://flybase.org/); REACTOME: reactome pathway database (https://reactome.org/).

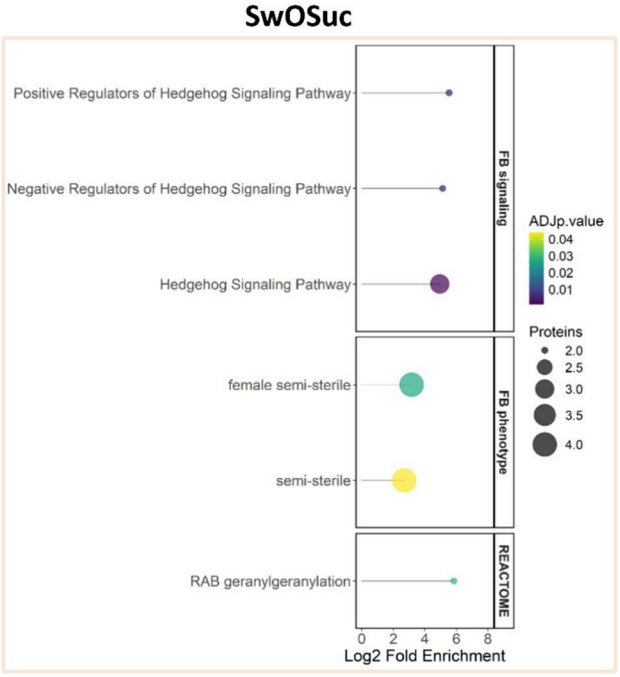

Figure 5.7. Bubble plot of the enriched parameters identified when differentially abundant proteins (DAPs) in nymphs from the SwOSuc and SwO comparison were used to interrogate different databases (FDR adjusted *p*-value threshold of 0.05). The size of the bubbles indicates the number of proteins in each pathway, whereas the colour denotes the FDR corrected *p*-value and fold enrichment. The *Drosophila melanogaster* proteome was used as the reference. "SwO": nymphs exclusively developed on SwO. "SwOSuc": nymphs that developed on SwO plants and were then fed sucrose for 24 h before sampling. FB signalling: Flybase signalling pathways database (https://flybase.org/); FB phenotype: Flybase phenotype database (https://flybase.org/); REACTOME: reactome pathway database (https://reactome.org/).

The biological processes related with the response to external stimuli, chemicals and stress were enriched in LemonSuc nymphs in the LemonSuc *vs* Lemon comparison (Fig. 5.6). Higher abundance was observed in proteins such as "Hrb87F" (heterogeneous nuclear ribonucleoprotein at 87F), "Letm1" (mitochondrial proton/calcium exchanger protein), "coro" (coronin), and "NUCB1" (calcium-binding protein) (Table S5.2- Appendix). Additionally, abnormal phenotype groups were enriched in LemonSuc nymphs including "abnormal behaviour", "abnormal neurophysiology" and "abnormal neuroanatomy" (Fig. 5.6). The proteins that were found to enrich the abnormal and the paralytic phenotype groups included "shot" (short stop), "twz" (Tiwaz, isoform B), "tau" (Microtubule-associated protein), and the previously mentioned "Dap160" and "Syn".

In the transition of *T. erytreae* nymphs from lemon plants to a sucrose diet, pathways that were enriched between LemonSuc vs. Lemon, were suggestive of

reactive adaptation since they included “response to external stimulus”, “response to chemical”, “response to stress”, “actin cytoskeleton organisation” and “microtubule cytoskeleton organisation” (Fig. 5.6).

5.6. Discussion

This section will discuss potential explanations for the observed lower success of nymphal development on SwO plants in comparison to lemon plants. This discussion will centre on the observed disparities of the hosts impact on the nymphs’ proteome and elucidates the potential mechanisms underpinning the variations in nymphal developmental success. Additionally, the greater proteome adjustment observed in the nymphs that developed on lemon plants in response to the dietary shift to sucrose complements our understanding of this plant–insect interaction.

5.6.1. *Trioza erytreae* exhibited a citrus host-specific oviposition pattern and nymphs developed better on lemon than on SwO plants

The presence of young flushes and their young leaves has been demonstrated to be essential for oviposition by *T. erytreae* (Catling, 1969). The consistency observed in the initial number of flushes and new leaf development among the citrus hosts in the current study suggests that flushing should not be used as a differentiating factor among the two citrus hosts.

The oviposition behaviour exhibited by adult females of *T. erytreae* differed markedly when infesting lemon or SwO plants. A more dispersed oviposition pattern characterised by a reduced egg count per leaf, was observed on SwO plants. Conversely, a denser pattern with a high number of eggs per leaf was observed on lemon plants (Table 5.1 and Fig. 5.2A). Previous studies have demonstrated that the number of *T. erytreae* eggs laid per shoot differs according to the host plant. In contrast to the findings of the present study, two other studies reported that hosts with a high number of oviposited flushes in relation to the total available flushes, generally tended to have a greater number of eggs per flush (Aidoo et al., 2019a; Hernández-Suárez et al., 2021). In accordance with the patterns of oviposition

identified in the current study, it seems that *T. erytreae* females appear to initiate oviposition on a leaf of SwO, but subsequently seek alternative leaves since they consider this leaf suboptimal for oviposition. Conversely, the observed behaviour of *T. erytreae* on lemon plants suggests that the initial leaf encountered is perceived as optimal, with the majority of the eggs deposited on the same leaf.

The duration of the life cycle observed in the current study (25 to 27 days) (Fig. 5.2C) is closely aligned with the optimal development time (23,9 days) identified by Pérez-Otero et al. (2024), who tested the effect of climatic conditions on *T. erytreae* development. Moran (1968b) showed that the host plant exerts an influence on the duration of *T. erytreae* development, and that the psyllid has a longer development time in some hosts compared to others, which was also observed in the current study. The markedly greater increase in newly infested leaves in lemon plants from 5 to 23 DAI (Table 5.1) is likely to be closely related to the highly concentrated oviposition pattern observed in this host. *Trioza erytreae* are known to disperse more when encountering already infested flushes (Van den Berg et al., 1991b). Therefore, it seems that the emerging nymphs were compelled to seek new leaves with fewer competing nymphs and sufficient space to complete their full development.

The greater number of empty pit galls and dead fourth and fifth instar nymphs, that remained attached to the leaf (classified as vacant galls) on SwO plants, indicate that a high number of the nymphs were unable to complete their development. In agreement with this observation, Tamesse (2000) observed a comparable number of *T. erytreae* in the initial stages (eggs and first instar nymphs) per flush in 'Eureka' lemon and 'Valencia' SwO, and a significantly lower number of fourth and fifth instar nymphs on 'Valencia' SwO.

5.6.2. Citrus host plant and sucrose feeding affected *Trioza erytreae* protein identification

5.6.2.1. Citrus host plant proteins identified in *Trioza erytreae* nymphs

The present study identified host plant proteins in *T. erytreae* nymphs, suggesting that they are ingested and retained by the nymphs. The relatively low

number of identified host plant proteins (Fig. 5.3A) in *T. erytreae* nymphs was expected, as although insects are known to ingest and utilise plant proteins, they also metabolise them and affect their conformation, leaving only a few highly stable plant proteins intact (Chen et al., 2007; Salvucci et al., 1998). The identified host plant proteins were associated with the heat shock, and oxidative stress response, and with protein metabolism. Heat shock proteins important in the response of SwO to stress (Shafqat et al., 2020). Additionally, plant protein metabolism increases in response to infestation by phloem feeding insects, and specifically in the response of SwO to *T. erytreae* (Du et al., 2015; Magalhães et al., 2024; Wu et al., 2019). Furthermore, oxidative proteins present in plants can reduce the digestibility of plant food by the insects (Wang and Constabel, 2004) and this supports emerging concepts about the host plants response to psyllid feeding. The same plant proteins were found in nymphs growing on both hosts. Except for two enzymes, catalase (A0A067H2F2) and a peroxidase (A0A067H6D4) that were exclusively identified in nymphs developing on lemon and SwO, respectively (Table S5.1- Appendix). In a previous study of tomato (*Solanum lycopersicum* L.) plants catalase and peroxidase were induced in response to phloem-feeding insects, and catalase activity was positively correlated with the hosts resistance to aphids (Zhao et al., 2016). A similar number of total plant proteins was identified between the treatments in our study (Fig. 5.3A), and this may be related to the relatively small number of stable plant proteins that resist digestion by insects (Chen et al., 2007; Salvucci et al., 1998).

5.6.2.2. Treatment-specific psyllid proteins

Three of the four proteins that were exclusively identified in the nymphs that developed on SwO plants (SwO and SwOSuc), namely “bw”, “CG13185” and “Klp61F” (Table S5.1- Appendix), are associated with the molecular function of ATP binding (Garbarino and Gibbons, 2002; Mackenzie et al., 1999; Van den Wildenberg et al., 2008). The nymphs subjected to the sucrose feeding treatment (LemonSuc and SwOSuc) produced four proteins that are also associated with the molecular function of ATP binding, namely “MRP”, “Mi-2”, “SMC1” and “bt” (Bent, isoform F) (Table S5.1- Appendix) (Cole, 2014; Dege and Hagman, 2014; Yi et al., 2017). ATP-

binding proteins have been shown to be essential for development and the ability to cope with xenobiotic stress (Broehan et al., 2013). However, ATP-binding proteins were not detected in nymphs that developed exclusively on lemon plants. This may indicate that lemon host plants do not induce xenobiotic stress in the nymphs, or alternatively, the stress experienced by the nymphs in the lemon plants was of a lower intensity when compared to the other three groups.

The unique proteins detected on the sucrose-fed nymphs, “Nedd4” and “wrd”, are required for proper synaptic growth (Viquez et al., 2006; Zhong et al., 2011), and sucrose diets have been shown to initially increase synaptic currents in *D. melanogaster* embryos (Suzuki et al., 2002), which may explain the presence of these proteins in the sucrose-fed nymphs in the current study.

5.6.3. Citrus host plant and sucrose feeding affect *Trioza erytreae* proteome

The greater number of DAPs in the SwO *vs* Lemon comparison (101 DAPs) in relation to the other comparisons (Fig. 5.4) showed that the citrus host has a higher influence over the *T. erytreae* proteome than the diet shift. A comparable pattern was observed in a study involving *D. citri,* where the proteomes of the psyllids that remained on one diet throughout the experiment were more divergent than psyllids that underwent dietary alterations (Ramsey et al., 2022).

The sucrose feeding treatment had a more pronounced impact on nymphs that developed on lemon plants (92 DAPs), than on nymphs that developed on SwO plants (26 DAPs) (Fig. 5.4). Lemon and SwO plants leaves have different nutritional profiles (Galvez-Sola et al., 2015). Furthermore, in response to *T. erytreae* infestations, a greater proteome adaptation of the leaf-enriched vascular sap was observed in ‘Valencia’ SwO in relation to ‘Eureka’ lemon’ plants (Magalhães et al., 2024). The divergent nutritional content and proteome response of ‘Valencia’ SwO and ‘Eureka’ lemon’ to *T. erytreae* infestation may explain the divergent proteome response of the nymphs when transitioning from the host to the sucrose diet

5.6.3.1. The citrus host effect: lemon plants modify nymph energy metabolism while SwO plants hinder nymph fertility and development

A comparison of the enriched pathways in nymphs from the SwO and lemon plants, suggests that the latter are more conducive to the development of *T. erytreae* than the former. The enriched pathways in nymphs from the SwO plants were related to protein metabolism, whereas in the lemon plants, the enriched pathways are related to growth, development, and energy metabolism (Fig. 5.5). As demonstrated by Chen et al. (2017b), an increase in protein metabolism occurred in insects subjected to nutrition deprivation.

In nymphs that developed on lemon plants, the developmental and energy metabolism-related pathways were enriched (Fig. 5.5). The pathways involved are vital for insect development and for periods of nutrient scarcity (Arrese and Soulages, 2010; Fraga et al., 2013). The knockdown of ATP synthases causes developmental arrest in the early stages of the insect life cycle (Tsang and Lemire, 2003). Furthermore, *D. citri* had higher abundance of ATP synthases when developing on *Murraya paniculata*, a highly suitable plant host compared to when developing on a less suitable host (Ramsey et al., 2022). The higher abundance of the ATP synthases ("blw" and "ATPsynbeta") and the induction of energy-related pathways in nymphs that developed on lemon suggests that psyllid development was facilitated by this host compared to SwO.

In the SwO *vs* Lemon comparison, the nymphs that developed on SwO plants were enriched in semi-sterile phenotype groups (Fig. 5.5). The "CkIIbeta" and "Abi" proteins that enriched this phenotype are known to affect oogenesis (Squarr et al., 2016; Wong et al., 2011), and in the psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae), the plant host affected fertility rates (Mustafa et al., 2015). The diet contents were identified as a potential factor influencing fertility, as observed in other insects, where lower quality diets resulted in lower fertility rates (Chen et al., 2017b; Wang et al., 2013), and insects feeding on low quality diets prioritise survival over reproduction (Parthasarathy and Palli, 2011). The semi-sterile phenotype groups include proteins in *D. melanogaster* phenotypes that have a decreased (by at least 50%) capacity to produce fertilised eggs in comparison to the wild-type. In

the current study nymphs that developed on SwO plants, within the SwO *vs* Lemon comparison, the semi-sterile phenotype groups were enriched, and a high abundance of the "CkIIbeta" and "Abi" proteins (Table S5.2- Appendix), was observed. These proteins are known to affect oogenesis (Squarr et al., 2016; Wong et al., 2011). In *T. erytreae* a lower number of fourth and fifth instar nymphs on the SwO plants, may indicate that SwO has a detrimental effect on fertility. In the psyllid *B. cockerelli* plant host also influenced psyllid fertility, and divergent fertility rates occurred with development on different plant hosts (Mustafa et al., 2015). Further investigation of "CkIIbeta" and "Abi" proteins will contribute to improve understanding of *T. erytreae* fertility and may yield strategies for control.

In SwO-developed nymphs the "signal transduction" and "transmission across chemical synapses" were enriched (Fig. 5.5) by "Syn", a protein known to modify locomotor behaviour (Godenschwege et al., 2004), and "sls", a protein related to muscle development (Burkart et al., 2007) (Table S5.2- Appendix). The latter protein was identified in higher abundance in *D. citri* developed on a host where psyllids present narrower wing shapes (Paris et al., 2016; Ramsey et al., 2022). This finding suggests a potential impact of SwO on the nymph's development and behaviour. Furthermore, the "abnormal neuroanatomy" and "abnormal neurophysiology" phenotype groups were found to be enriched (Fig. 5.5) by "Dap160", which induces endocytosis (Tang et al., 2005), and "CadN", which is crucial for insect development (Hummel and Zipursky, 2004) (Table S5.2- Appendix). In the present study, *T. erytreae* infestation patterns observed in SwO were found to be abnormally dispersed (Table 5.1). Furthermore, the number of nymphs that developed successfully was found to be three times lower than the number of nymphs that developed on lemon plants. The settling and oviposition behaviours of psyllids have been demonstrated to be influenced by host-feeding and probing activities(Horton and Krysan, 1990; Moran and Buchan, 1975). We suggest that the SwO plant host diet may have induced production of proteins linked to abnormal behaviour and development phenotypes in the psyllid.

5.6.3.2. Sucrose feeding effect had a high impact on nymphs that developed on lemon plants, with hedgehog signalling pathway affected

The results of the present study revealed that the proteome of nymphs fed sucrose after developing on lemon was enriched in the "Hh signalling pathway" (Fig. 5.6 and 5.7). Furthermore, negative regulators of the "Hh signalling pathway" were dominant (Fig. 5.6). In contrast, in SwOSuc, the "Hh signalling pathway" was enriched in positive and negative regulators (Fig. 5.7). The three common DAPs enriched in both SwOSuc and LemonSuc have different regulatory roles. "Tnpo", is a positive regulator, "wdb" is a context-dependent regulator and "CG5504" (Table S5.2- Appendix) is a negative regulator (Canamasas et al., 2003; Jia et al., 2009; Shi et al., 2014; Su et al., 2011). The negative regulators of Hh signalling, "UbcE2M" and "Gbeta76C", were more abundant in LemonSuc nymphs (Table S5.2- Appendix) (Du et al., 2011; Li et al., 2018). Both negative and positive regulators of the "Hh signalling pathway" can cause developmental malformations in insects (Villarreal et al., 2015). The knockdown of the hedgehog (*hh*) gene (GenBank accession number NM_001114365), and the subsequent negative regulation of the "Hh signalling pathway" in the red flour beetle *Tribolium castaneum* (Herbst, 1797) resulted in the development of severe limb abnormalities (Villarreal et al., 2015). Increased levels of "Tnpo" protein cause lethality in early stages of fruit fly development, which is accompanied by abnormalities in the eyes and wings of adult flies (Goodman et al., 2021). The proteome results obtained linked to the "Hh signalling pathway" in *T. erytreae* nymphs after 24 h sucrose feeding suggests development may have been modified and also revealed that the plant host can influence the outcome.

The proteome analysis revealed high abundance of the "eIF3b" and "RpS28b" proteins (Table S5.2- Appendix) in lemon-developed nymphs that were not submitted to sucrose feeding, in the LemonSuc *vs* Lemon comparison. Both these translation-related proteins are crucial for cell proliferation and cell division (Dong and Zhang, 2006; Marygold et al., 2007). The results suggests that sucrose feeding hinders cell growth and division in *T. erytreae* nymphs. Similarly, a significant reduction in the number of translation transcripts was observed in *D. melanogaster*

larvae when transitioned from a full food diet to a state of starvation, or to a diet consisting exclusively of sugar, (Nagarajan and Grewal, 2014).

The sucrose feeding treatment appears to have exerted a more pronounced influence on the proteome of nymphs that developed on lemon, as revealed by the enrichment of active responses (stress response, response to chemical, response to external stimulus) (Fig. 6). The protein "Hrb87F", present in higher abundance is associated with active responses and enhanced tolerance of insects to starvation (Singh and Lakhotia, 2012). Additionally, in LemonSuc a higher abundance of proteins linked to biotic and abiotic stress response was observed, namely "Letm1", "coro" (coronin) and "NUCB1" (Table S5.2- Appendix) (Berkey et al., 2009; Jin et al., 2008; Lee et al., 2008b). It has been shown that insects that feed on more restricted diets are more resilient to external pressures, attributable to the priming of stress responses (Chen et al., 2017b, 2017a). Furthermore, a stress response was also observed in *Spodoptera exigua* (Lepidoptera: Noctuidae) when feeding on an artificial diet or a less suitable host (Breeschoten et al., 2019).

The findings of the present study indicate that the sucrose feeding treatment is eliciting stress responses, which could possibly result in developmental deficiencies and behavioural alterations in *T. erytreae*. This hypothesis is consistent with the documented reduction in the size of *D. melanogaster* with leaner bodies that developed on protein-deficient diets (Henry et al., 2020). The impact of high sucrose diets on the development of various organs in insects has been demonstrated to be detrimental (Catalani et al., 2021; Rani et al., 2020). Further functional studies are required to elucidate the impact of the proteins induced by sucrose feeding on *T. erytreae* homeostasis and development.

5.7. Conclusions

The experimental results showed that *T. erytreae* exhibited divergent oviposition and infestation behaviour patterns on the SwO and lemon plants. The developmental time was slightly prolonged and the number of nymphs that reached the fourth and fifth instars was significantly diminished when the psyllids were developing on SwO. The proteome analysis of nymphs indicates that their growth

and development were facilitated by the lemon plants. This was evidenced by the proteome enrichment analysis, which revealed greater enrichment in developmental and energy-metabolism related pathways, such as “generation of precursor metabolites and energy”, “formation of ATP by chemiosmotic coupling”, “mitochondrial biogenesis” and “cuticle development”. Furthermore, an increase in initiation translation factors was observed in nymphs that developed on lemon compared to the nymphs that developed on SwO or that were submitted to sucrose feeding. The sucrose and SwO diets resulted in the enrichment of “semi-sterile” phenotypes, as well as “abnormal development” and “abnormal behaviour” phenotypes. These findings suggest that the diet from lemon is more conducive to successful *T. erytreae* development. The observation that nymphs that developed on lemon exhibited a greater proteome adjustment to the sucrose feeding treatment than the nymphs that developed on SwO lend support to this hypothesis. Some of the proteins highlighted in this study may serve as potential targets for the control of *T. erytreae* infestation.

Chapter 6. Jasmonic acid spray affects *Citrus* ×*limon* (L.) Burm. f. volatiles and hinders *Trioza erytreae* (Del Guercio) development

In preparation:

Magalhães, T., Ruano, D., Rodrigues, N., Duarte, A., Marques, N. T., Pereira, J. A. Jasmonic acid spray affects *Citrus* ×*limon* (L.) Burm. f. volatiles and hinders *Trioza erytreae* (Del Guercio) development

6.1. Abstract

Exogenous jasmonic acid (JA) application triggers plant defences and influences plant volatile organic compounds (VOCs) emission. Determining the JA spray effect on lemon (*Citrus ×limon* ((L.) Burm. f.) plants' VOC profile and on *Trioza erytreae* (Del Guercio), a vector of the citrus Huanglongbing pathogen, could help develop novel control strategies against this vector. Exogenous JA application reduced *T. erytreae* oviposition by 69% in lemon plants. It also affected egg success with 94.9% of the eggs resulting in successfully developed nymphs for non-JA sprayed plants (Psyll) and only 53.6% on JA sprayed plants (JAsp_Psyll). Lemon plant VOCs were identified using headspace solid-phase microextraction (HS-SPME) in combination with gas chromatography–mass spectrometry (GC–MS). Lemon plant VOC emissions and endogenous JA levels were affected by JA spray and *T. erytreae* infestation. The isolated effect of adult psyllid feeding and oviposition had a greater impact on lemon plants five days after spray (DAS). The combined effect of JA spray and continuous nymphal feeding had a higher impact on lemon plants 25 DAS. Monoterpenes, sesquiterpenes, alcohols, esters and aldehydes were involved in lemon plants' response to JA spray and *T. erytreae* infestation. Exogenous application of JA induced VOCs and JA related responses on lemon plants and greatly hindered *T. erytreae* infestation, demonstrating the great potential of JA against the pest. Nevertheless, JA application in field conditions should be further investigated.

6.2. Abbreviations

ANOVA (analysis of variance); "Control" (lemon plants sprayed with control spray mix and without *T. erytreae* infestation); DAS (days after spray); ELISA (enzyme-linked immunosorbent assay); HS-SPME-GC/MS (headspace solid phase microextraction gas chromatography coupled to mass spectrometry); HLB (huanglongbing); JA (jasmonic acid); "JAsp" (lemon plants sprayed with JA spray mix and without *T. erytreae* infestation); ; "JAsp_Psyll" (lemon plants sprayed with JA spray mix and infested with *T. erytreae*); PCA (principal component analysis); "Psyll" (lemon plants sprayed with control spray mix and infested with *T. erytreae*); SA (salicylic acid); TIC (total ion chromatogram); VOC (volatile organic compounds)

6.3. Introduction

Huanglongbing (HLB), caused by the bacterium *Candidatus* Liberibacter africanus, *Candidatus* Liberibacter americanus and *Candidatus* Liberibacter asiaticus is the most devastating citrus disease in the world (Bové, 2006; Gottwald et al., 2007). This disease is spread by two psyllid vectors, *Trioza erytreae* (Del Guercio) and *Diaphorina citri* (Kuwayama), which in the last decade have reached countries of the Mediterranean basin, specifically, *T. erytreae* has reached mainland Portugal and Spain (Benhadi-Marín et al., 2022; EPPO, 2021, 2015), and *D. citri* Israel and Cyprus (EPPO, 2023, 2022). The Mediterranean basin is currently still HLB-free (EPPO, 2025). However, the introduction of the vector usually precedes subsequent outbreaks of HLB, and in recent years, the time between these two events has been progressively reduced (Alquézar et al., 2022; Bové, 2006). This poses a significant threat to European citriculture, and governmental authorities have deemed both the vectors and the causal agents of HLB as priority quarantine pests (EPPO, 2025).

Vector control is a major factor in HLB management, given that there are no available curative measures or resistant cultivars of citrus for this disease (Alquézar et al., 2022; Galvañ et al., 2023). The three-pronged strategy, that consists of (1) planting of certified healthy citrus material; (2) removal of inoculum sources-infected trees; and (3) application of insecticide treatments to control psyllid populations is the main strategy to manage HLB (Galvañ et al., 2023). Successful examples for HLB management have relied on intense pesticide spray applications (Galvañ et al., 2023; Singerman et al., 2017). This can lead to the development of pesticide resistance by the vectors and other pests, with risks for the environment and human health (Chen et al., 2022; García-Méndez et al., 2019). Therefore, alternatives to conventional insecticide sprays for vector control are needed for the sustainable management and prevention of HLB.

Preparing and enhancing the plants' response for future stress, also known as priming, has been shown to be an effective alternative insect control method (Frost et al., 2008; Kim and Felton, 2013). Specific chemical stimuli can successfully prime plants' defences, and an effective elicitor is needed (Mauch-Mani et al., 2017). A holistic approach to discover these elicitors includes the exploration of the plant–insect interaction (Balmer et al., 2015; Enders and Begcy, 2021). Phytohormone signalling

response is a known layer of plants' response to insects, in particular the JA phytohormone (Enders and Begcy, 2021; Schuman and Baldwin, 2016). Previous studies demonstrated that JA levels and JA signalling pathways of citrus plants have been affected by *T. erytreae* and *D. citri* infestation (Gao et al., 2023; Patt et al., 2018). And the psyllids development has been hindered in hosts where the JA signalling pathways were upregulated, and/or JA levels were higher (Gao et al., 2023; Patt et al., 2018). In *D. citri*, JA priming has shown promising results as a potential alternative control method (Rao et al., 2018a).

It is known that, in conditions with low levels of JA, the jasmonate ZIM-domain (JAZ) proteins bind and limit the activity of various transcription factors of JA response genes. While with high levels of JA, a degradation of the JAZ proteins occurs through the 26S proteasome pathway, leading to an activation of JA response genes (Fig. 6.1) (Chini et al., 2007; Ruan et al., 2019).

The JA response genes activate a myriad of responses, including the activation of JA biosynthesis that creates a positive feedback loop of the JA signalling pathway. Additionally, JA response genes activate specific plant defence mechanisms and induce the biosynthesis of many secondary metabolites, including volatile organic compounds (VOCs) (Chini et al., 2007; Luo et al., 2023). VOCs are emitted by plants and serve as a communicating agent within the plant and other living organisms, like other plants, microorganisms and biological agents present in their environment (Dudareva et al., 2004; Paré and Tumlinson, 1999). These compounds have a crucial role in plant–insect interactions, acting as repellents for plant pests, as attractants of pollinators and natural enemies. Insects have the ability to interpret these compounds and identify their preferred plant host (Antwi-Agyakwa et al., 2019; Paré and Tumlinson, 1999). The influence of VOCs on the behaviour of HLB vectors had been demonstrated (Mann et al., 2012; Patt et al., 2018). Lemon plants *Citrus ×limon* (L.) Burm. f. are classified as highly suitable hosts of *T. erytreae*, and the host VOCs emission profile has been shown to influence the psyllid preference (Antwi-Agyakwa et al., 2019; Magalhães et al., 2025).

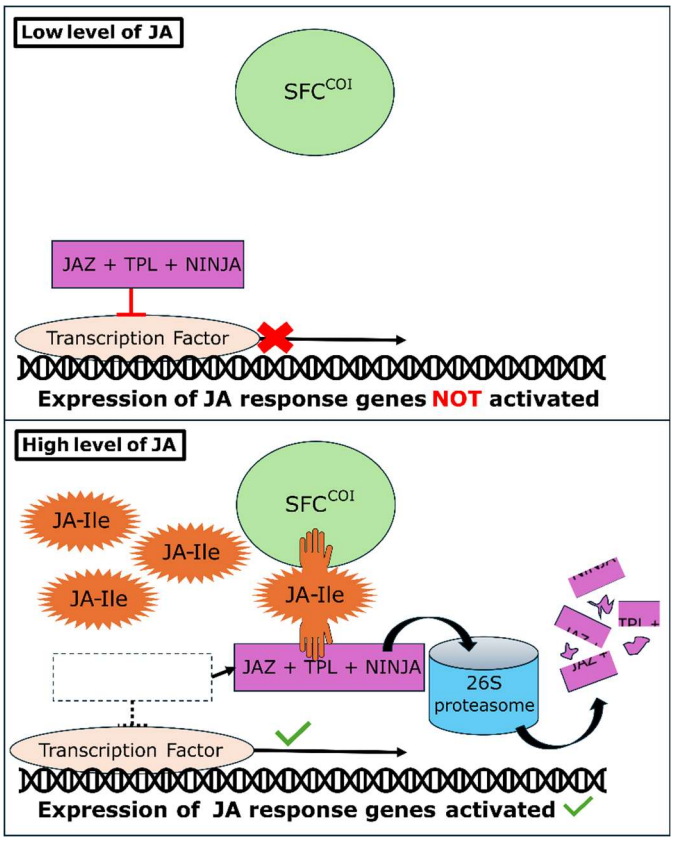

Figure 6.1. Summarised representation of the JA signalling pathway trigger; SCF: Skp1, Cullin and F-box proteins; COI: coronatine insensitive; JAZ: jasmonate ZIM-domain protein; TPL: Topless protein; NINJA: Novel interactor of JAZ, JA-Ile: jasmonoyl isoleucine

In this study, JA was applied exogenously to lemon plants that were subsequently infested with *T. erytreae*. The main hypothesis being tested was "Does an exogenous JA application induce lemon plants' defence for *T. erytreae* infestation?". Two other hypotheses were also being tested, namely i) "do the exogenous JA application and *T. erytreae* influence JA levels in the plant?" and ii) "do the exogenous JA application and *T. erytreae* influence the plants' VOCs profile?". These three hypotheses were tested in different time points after the exogenous JA application. JA levels in the plant were ascertained via enzyme-linked immunosorbent assay (ELISA). VOCs profiles were obtained via headspace solid phase microextraction gas chromatography coupled to mass spectrometry (HS-SPME-GC/MS). An integrative analysis of the results along the different time points was performed to better understand the effect of the exogenous JA application on the *C. ×limon–T. erytreae* interaction. The direct effect of the JA application and the indirect effect on the plants' VOCs profile have the potential to be used for further studies for the development of alternative control methods against *T. erytreae*.

6.4. Materials and methods

6.4.1. Plant and insect material

Certified two-year-old 'Eureka' lemon [*C.* ×*limon* (L.) Burm. f.] plants grafted on *Citrus macrophylla* Wester, from the same batch, were purchased in "Viveiros Citriplant" nursery. The plants, 0.8 to 1.0 m height, were cultivated in 5 L pots and were maintained in a climate chamber under controlled conditions [T= 23.0 ± 1°C, RH: 55 ± 5% and 13:11 h (L:D)]. Plants were irrigated according to their specific needs, and three weeks before the exogenous spray applications, all plants were pruned to induce new shoot growth.

The adults of *T. erytreae* preceeded from an established colony and were maintained on 'Eureka' lemon and 'Afín Verna 2' sour orange (*Citrus aurantium* L.) plants, in acrylic cages (40 x 30 x 43 cm) at controlled temperature [T= 21.0 ± 1°C, RH: 50 ± 5% and 16:8 h (L:D)].

6.4.2. Experiment design and phytohormone spray application

Four treatment groups were defined namely: "Control" plants sprayed with control spray mix and without *T. erytreae* infestation; "Psyll" plants sprayed with control spray mix and infested with *T. erytreae*; "JAsp" plants sprayed with JA spray mix and without *T. erytreae* infestation; and "JAsp_Psyll" plants sprayed with JA spray mix and infested with *T. erytreae*. Each group comprised five lemon plants (n = 5).

Control spray mix consisted of 0.025% Tween ® 20 and 1% ethanol in distilled water, while JA spray mix was the same as the control spray mix with the addition of 5 mM of methyl jasmonate.

Tween ® 20, ethanol and methyl jasmonate were purchased from Sigma-Aldrich® (San Louis, Missouri, USA) and. All plants were sprayed with a hand-held sprayer (500 mL in volume capacity) uniformly and to avoid dripping (approximately 30 mL per plant).

6.4.3. *Trioza erytreae* infestation

Plants from the Psyll and JAsp_Psyll treatments were infested 24h after the spray. Each plant was isolated within a cylindrical insect-proof net and infested with 10 adult *T. erytreae* specimens (five males and five females). After 96h, the adults were removed, and oviposition was evaluated by counting the eggs in each plant immediately. Insect

development was assessed every third day after oviposition evaluation up to 25 days after spray (DAS), registering the number of psyllids and their development stage.

6.4.4. Plants' endogenous JA concentrations

Leaves were sampled at four time periods: T0) prior to spray; T1) 24h after spray; T2) five DAS; and T3) at 25 DAS. Sampling consisted in removing the third leaf counting from the tip of a developed branch of the plant. Then 120 mg from the centre of this leaf, including its midrib vein, were excised and enwrapped in aluminium foil, frozen in liquid nitrogen, and then stored at -80 ºC. Homogenised and grounded into a fine powder in liquid nitrogen. Then 1mL of phosphate buffer saline (PBS) (pH 7.4) buffer solution was added to the samples. Samples were placed in a 1.5 mL microtube and centrifuged at 4 °C, 3,000 rpm, for 20 min. The supernatant was then used for plants' endogenous jasmonic acid concentrations evaluation by enzyme-linked immunosorbent assay (ELISA) using a plant jasmonic acid (JA) ELISA kit (Sunlong Biotech, Hangzhou, Zhejiang, China) following the manufacturers' instructions, with five biological replicates (n = 5), each of which with three technical replicates.

6.4.5. Volatile organic compound characterisation

Volatile organic compounds (VOCs) were analysed at each time of sampling periods (T0; T1; T2 and T3). The analysed plant material was not excised from the plant. Only new growth (growth that developed after pruning) was used for VOCs analysis. At T0, T1 and T2 a single young flush was used per plant, while at T3 a single young leaf was used. For each plant, the flush used for T0 was used for T1 and T2, and the leaf used in T3 was from the same flush used in the previous timings. Each treatment group was comprised of five plants (n = 5). The leaf area was measured for all plant material used in the VOCs analysis. Leaf areas were measured by positioning the leaves on a millimetric paper and photographing the leaf. The area was measured in cm^2 through scaling and area measurement tools on Inkscape (Inkscape Project, 2020) (Fig. S6.1 - Appendix). In the time points T0 through T2 all the areas of leaves on the flush were measured, in T3 the single leaf used for analysis was measured.

Volatile organic compounds were analysed by HS-SPME-GC/MS. A glass Schott bottle (100 mL capacity) with a small hole in the centre of the bottom sealed with a silicon

septum, was used to isolate the plant material. The bottle cap was removed, and the plant material (new flush or new leaf) was inserted into the Schott bottle through the cap side, which was then isolated with aluminium foil and scotch tape. Once placed it was left for 5 min to release volatile compounds, after which 5 µL of 4-methyl-2-Pentanol at a 0.127 mg/mL concentration (internal standard) were inserted into the Schott bottle, via syringe through the septum. Five minutes after the SPME fibre (divinylbenzene/carbonex/polydimethylsiloxane) (DVB/CAR/PDMS 50/30 µm) (Supelco, Bellefonte, PA, USA) was inserted through the septum of the bottle and exposed for 30 min for adsorption of the volatile compounds in the headspace. Volatile organic compounds were removed from the fibre by thermal desorption (220 °C) for 1 min in the chromatograph injection port. The fibre was kept in the injection port for 10 min for cleaning and conditioning for further analysis. The gas chromatograph used was a Shimadzu GC-2010 Plus equipped with a Shimadzu GC/MS-QP2010 SE mass spectrometer detector. A TRB-5MS column (30 m × 0.25 mm × 0.25 × m) (Teknokroma, Spain) was used. The injector was set at a temperature of 220 °C, and the manual injection was performed in spitless mode. The mobile phase consisted of helium 5.0 (Linde, Portugal), at a linear velocity of 30 cm/s and a 24.4 mL/min flow rate. The oven temperature was 40 °C for 1 min, followed by an increase of 2 °C /min until reaching 178 °C. A holding time of 5 min was used once the final temperature was reached, total run time was of 75 min. The ionisation source was maintained at 250 °C with an energy of 70 eV and a current of 0.125 kV. All mass spectra were obtained by electronic ionisation in the m/z range 35–500. Compounds were identified by comparing the mass spectra and through the Kovats index using databases such as NIST 69, PubChem and ChemSpider. Retention indices were obtained using a commercial n-alkane series, C7–C30 (Sigma-Aldrich, St. Louis, MS, USA), by direct spitless liquid injection (1 µL), while all further conditions of GC and MS were settled for the volatile analysis. Retention indices were calculated according to the Kovats index. The identified volatile compounds were expressed based on the areas determined by TIC (total ion chromatogram) integration.

The internal standard concentration was measured in relation to leaf area. Each VOC TIC area was multiplied by the internal standard concentration and then divided by

the mean TIC area of the internal standard. The mean TIC area of the internal standard was calculated based on 165 measurements.

6.4.6. Data analysis

Statistical tests were performed using Rstudio software R version 4.3.3 (R Core Team, 2020). The normality of all data was assessed using the Shapiro–Wilk test, while homoscedasticity was evaluated with Levene's test for normally distributed data and the Brown–Forsythe test for non-normally distributed data. All statistical tests *p*-value was set at < 0.05.

A Wilcoxon–rank sum test was employed to assess the differences in egg success ratio of *T. erytreae* on Psyll and JAsp_Psyll plants. A two-way mixed analysis of variance (ANOVA) was employed to examine the effect JA spray effect on the mean number of psyllids per plant over time (T2 and T3), with JA spray as a between-subjects factor and time point as a within-subjects factor. Post hoc Student's *t*-tests were conducted: paired *t*-tests for the within-subjects factor (time point) and unpaired *t*-tests for the between-subjects factor (JA spray).

To examine the time point effect on endogenous JA concentration, a one-way ANOVA was employed to each treatment group separately. Post hoc paired Student's *t*-tests were conducted.

To examine the treatment group effect on endogenous JA concentration for the T1 timing an unpaired Student's *t*-tests was conducted on JAsp and Control. For the T2 and T3 timings a two-way mixed ANOVA was employed with JA spray and infestation status as the two between-subjects factors. Post hoc pairwise multiple comparisons were run with unpaired Student's *t*-tests and a Bonferroni *p*-value adjustment.

To examine the time point effect on plants' VOCs, a one-way ANOVA was employed to each treatment group separately. To examine the treatment group effect on plants' VOCs was employed to each time point separately. VOCs ANOVAs were followed by the Tukey's post-hoc multi-comparison test. When only two groups were compared, a Student's *t*-test was applied.

To identify the most influential VOCs within this study, a Random Forest (RF) analysis was conducted using the randomForest package (Breiman et al., 2024). The importance of each VOC was determined based on the Gini Importance Measure (GIM),

where higher values indicate a greater impact of treatment and time on plant VOCs. VOCs with a GIM greater than 0.5 were selected for heatmap and principal component analysis PCA analysis (Table S6.1 – Appendix).

A heatmap was used to visualise the correlations between the most influential VOCs and the treatment groups, facilitating the identification of relationships between different parameters. This type of map, often combined with clustering algorithms, is useful for grouping similar data and visualising these groupings.

Principal component analysis (PCA) was applied to see if the most influential VOCs could be used to identify the treatment groups within each time point, and the timings within each treatment group. This analysis was visualised using a 2D-biplot graph, which simplifies the interpretation of multivariate data by projecting it in two dimensions to facilitate visualisation.

6.5. Results

6.5.1. *Trioza erytreae* infestation

Exogenous JA application significantly reduced the number of eggs and nymphs on lemon plants. Plants with no JA application (Psyll) harbouring 327 ± 25.8 [mean ± standard error mean (SEM)] eggs/plant and plants with JA application (JAsp_Psyll) harbouring 101.4 ± 16.6 eggs/plant (Fig. 6.2A). Exogenous JA application also reduced the egg success ratio, 94.6 ± 1.1 and 46.8 ± 17.8 for Psyll and JAsp_Psyll plants, respectively (Fig. 6.2B). The exogenous JA application affected *T. erytreae* oviposition behaviour and lowered the number of eggs/plant. Combined with the lower egg success rate, this results in a lower number of successfully developed fourth and fifth instar nymphs, namely 310.4 ± 25.4 and 54.4 ± 20.5 for Psyll and JAsp_Psyll plants, respectively (Fig. 6.2A).

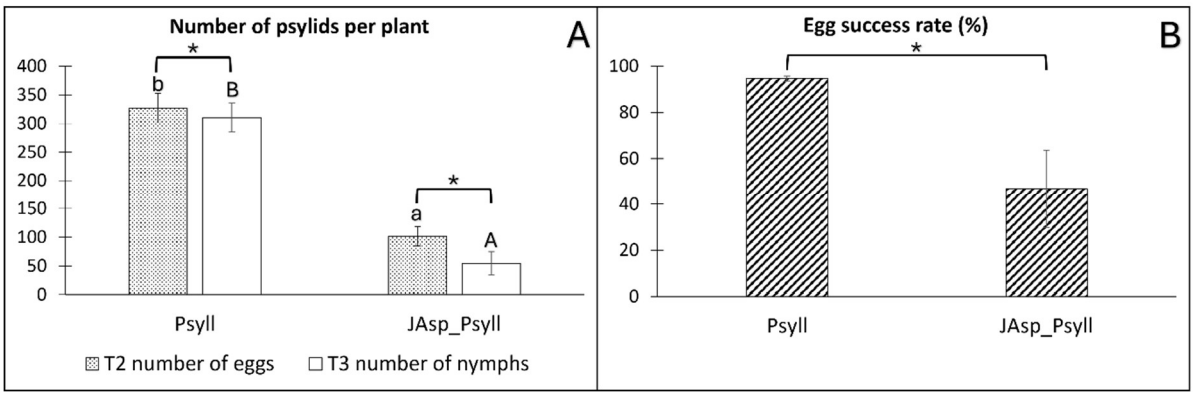

Figure 6.2. *Trioza erytreae* development on lemon plants treated with JA and without JA. Psyll: lemon trees sprayed with a 1% ethanol and 0.025% Tween in a water base solution and infested 24h after spray. JAsp_Psyll: lemon trees sprayed with a 5 mM of jasmonic acid and 0.025% Tween in a water base solution and infested 24h after spray. **A:** Mean number of eggs per tree five days after spray (DAS) (T2) and mean number of nymphs per tree 25 DAS (T3). Lower case letters represent the comparison between the two groups within the T2 time point. Upper case letters in the comparison between the two groups within the T3 time point. If treatments do not share a common letter in their respective comparisons, they are significantly different at $p < 0.05$. Asterisks represent the significantly different comparisons within the same group between the two time points (T2 and T3) at $p < 0.05$. **B:** Egg success rate, obtained by dividing the number of nymphs 25 DAS by the number of eggs at five DAS. Asterisk represents the significantly different comparison at $p < 0.05$. Standard error bars are shown for each bar chart.

The number of eggs is a major determinant of the final number of nymphs in this experiment. As a one-way ANOVA with no covariates indicated that there was a significant effect of the exogenous JA spray ($F = 49.24$, $p = 1.11 \times 10^{-4}$) on the number of nymphs. However, when egg number was included as a covariate, the effect of the exogenous JA spray became non-significant ($F = 0.06$, $p = 0.82$) and the egg number effect was significant ($F = 35.46$, $p = 5.67 \times 10^{-4}$).

Our results showed that the exogenous JA spray treatment negatively affected *T. erytreae* infestation. This treatment had its highest impact on the oviposition behaviour and a subsequent number of eggs laid. Moreover, the egg success ratio was also hindered, and both effects resulted in a significantly lower number of nymphs.

6.5.2. Exogenous JA application and *Trioza erytreae* infestation effect on plants' endogenous JA levels

Control plants' endogenous JA concentration did not differ at any time point. The short-term effect of the exogenous JA spray on the plants endogenous JA was negligible with no significant differences between control and JAsp plants at 1 DAS (T1). However, there was a clear tendency for higher values in all the timings in JAsp plants in relation to control, in particular at 5 DAS (T2) and at 25 DAS (T3) (Fig. 6.3). Furthermore, JAsp plants' endogenous JA concentration increased from 1 DAS (T1) to five days after exogenous JA spray (T2) (Fig. 6.3).

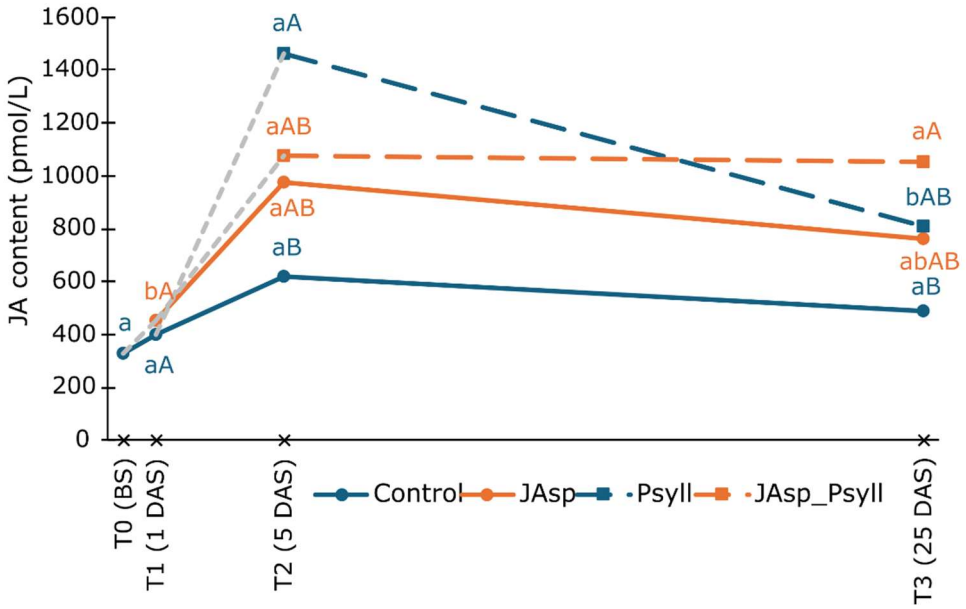

Figure 6.3. Plants' endogenous JA concentration measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Orange coloured lines represent plants treated with exogenous JA, blue coloured lines represent plants not treated with exogenous JA. Full lines with circular points represent plants that were not infested. Dashed lines with square points represent plants infested with *T. erytreae*. Gray dashed lines represent the theoretical evolution of the treatments after spray and infestation if the same start point was assumed. Lowercase letters represent the comparison within the groups and along the different time points (effect of time). Uppercase letters represent the comparison between the groups within the specific time point (treatment effect). When there is no common letter between points (compare letters within the same case type only), they are significantly different, at a Bonferroni adjusted $p < 0.05$.

Five DAS (T2) psyllid-infested plants without exogenous JA application (Psyll) showed a significantly higher endogenous JA concentration than the control plants. The

JA sprayed plants (JAsp and JAsp_Psyll) show a tendency for higher endogenous JA content in relation to the control plants in T2, albeit not significantly different. This would suggest that the plants with both exogenous JA application and infestation (JAsp_Psyll) should show the highest endogenous JA concentration of the four groups. However, this was not observed (Fig. 6.3). There was a significant decrease in plants' endogenous JA concentration in Psyll plants from 5 DAS (T2) to 25 DAS (T3), which was not observed in the JA_Psyll plants (Fig. 6.3).

At 25 DAS (T3), the plants that were infested with *T. erytreae* and had an exogenous JA application (JAsp_Psyll) had a higher endogenous JA concentration than control plants. Demonstrating that continuous stress induced by nymphal feeding and development coupled with the JA spray increases the plants' endogenous JA levels. While the isolated effect of exogenous JA application, or the isolated effect of continuous feeding did not significantly affect plants' endogenous JA levels (Fig. 6.3).

6.5.3. Volatile organic compound characterisation

A total of 132 volatile organic compounds were identified in this study, belonging to 17 compound families (Tables 6.1 and 6.2). A general decrease of total VOC intensity was observed over time in all treatments (Fig. 6.4A and Table 6.2). Monoterpenes were the most represented compound family in terms of percentage of the total profile in all treatments and timings (Fig. 6.4B). Monoterpenes also had the highest number of identified compounds, and D-limonene was the most abundant VOC (Table 6.1). A marked increase in the percentage of monoterpene aldehydes was evident in all treatment groups at 25 DAS (T3) (Fig. 6.4B).

Table 6.1. Volatile profile and treatment effect comparisons of lemon plants measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control- plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*; JAsp- plants sprayed with JA and not infested; Psyll- plants not sprayed with JA and infested; JA_Psyll- plants sprayed with JA and infested. The results are expressed as nanograms per cm^2 leaf area (mean ± standard error, n =5). LRI =Linear Retention Index. ND = Not detected. NA = Not applicable. Comparisons with significant differences (p-value< 0.05) are highlighted in bold. One-way ANOVA was employed, followed by the Tukey's post-hoc multi-comparison test. When only two groups were compared, a Student's *t*-test was applied.

		T0	T1			T2					T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
Monoterpenes															
Monoterpene hydrocarbons															
Thujene	977	22.50 ± 2.97	10.45 ± 1.93	10.26 ± 3.70	0.97	**5.03 ± 1.38 B**	**6.85 ± 1.84 B**	**27.79 ± 9.38 A**	**5.56 ± 0.91 B**	**0.01**	**2.26 ± 0.37 B**	**1.97 ± 0.16 B**	**3.62 ± 0.82 B**	**8.84 ± 2.36 A**	**4.75E-03**
alpha Pinene	982	24.27 ± 2.77	14.12 ± 1.73	16.11 ± 4.47	0.69	**6.46 ± 1.54 B**	**10.06 ± 2.13 B**	**30.04 ± 7.89 A**	**11.22 ± 2.90 B**	**7.10E-03**	**3.75 ± 0.51 B**	**4.45 ± 0.53 B**	**5.92 ± 1.06 AB**	**14.74 ± 4.51 A**	**0.02**
Camphene	994	0.93 ± 0.09	**ND B**	**0.71 ± 0.21 A**	**9.82E-03**	**0.26 ± 0.04 B**	**0.48 ± 0.09 AB**	**1.27 ± 0.28 A**	**0.67 ± 0.32 AB**	**0.03**	**0.18 ± 0.03 B**	**0.22 ± 0.02 B**	**0.27 ± 0.06 B**	**0.85 ± 0.21 A**	**1.70E-03**
beta Phellandrene	1014	59.32 ± 8.56	28.75 ± 7.67	28.46 ± 11.3	0.98	**16.62 ± 5.58 B**	**18.24 ± 3.55 B**	**76.07 ± 23.9 A**	**16.72 ± 2.64 B**	**8.24E-03**	**7.86 ± 0.41 B**	**ND B**	**14.07 ± 1.85 AB**	**28.60 ± 8.36 A**	**1.78E-03**
(-)-beta Pinene	1016	166.1 ± 25.6	88.98 ± 12.3	128.9 ± 44.0	0.41	**50.86 ± 13.4 B**	**76.33 ± 15.8 AB**	**180.5 ± 47.0 A**	**81.62 ± 15.8 AB**	**0.02**	**40.62 ± 5.50 B**	**50.54 ± 5.97 B**	**60.09 ± 9.18 AB**	**149.9 ± 46.4 A**	**0.02**
beta Myrcene	1028	149.7 ± 31.7	84.19 ± 17.4	52.40 ± 19.1	0.25	43.10 ± 10.9	52.55 ± 8.02	101.6 ± 28.2	52.20 ± 15.2	0.11	20.41 ± 3.18	34.54 ± 4.82	27.81 ± 2.72	48.04 ± 18.5	0.27
alpha Phellandrene	1050	24.21 ± 3.39	**ND B**	**8.36 ± 1.76 A**	**1.47E-03**	**4.05 ± 0.89 B**	**6.90 ± 1.26 AB**	**19.38 ± 5.73 A**	**9.58 ± 3.47 AB**	**0.03**	**1.91 ± 0.20 B**	**3.16 ± 0.30 B**	**6.80 ± 0.98 AB**	**12.12 ± 3.60 A**	**6.25E-03**
3-Carene	1057	113.8 ± 18.8	54.63 ± 8.23	30.75 ± 8.95	0.09	**15.34 ± 4.02 B**	**29.22 ± 5.72 B**	**107.6 ± 35.7 A**	**20.10 ± 3.00 B**	**7.74E-03**	6.95 ± 0.66	15.97 ± 2.56	22.27 ± 3.70	51.55 ± 25.1	0.12
alpha Terpinene	1063	9.19 ± 1.39	6.38 ± 2.40	4.76 ± 1.15	0.56	**3.19 ± 0.82 B**	**3.12 ± 0.53 B**	**9.99 ± 2.95 A**	**3.17 ± 1.05 B**	**0.02**	**0.88 ± 0.14 B**	**1.14 ± 0.13 B**	**ND B**	**8.40 ± 1.95 A**	**4.82E-05**
o-Cymene	1068	ND	**ND B**	**6.12 ± 0.80 A**	**6.05E-05**	**ND B**	**6.17 ± 1.80 B**	**44.60 ± 10.5 A**	**ND B**	**4.18E-05**	**ND B**	**ND B**	**5.55 ± 1.38 B**	**15.49 ± 2.83 A**	**7.32E-06**
D-Limonene	1078	2739 ± 482	1724 ± 256	1025 ± 344	0.14	**804 ± 160 B**	**1005 ± 184 B**	**2752 ± 701 A**	**916.5 ± 225 B**	**7.55E-03**	**207.4 ± 19.5 B**	**282.7 ± 38.3 B**	**534.3 ± 57.6 AB**	**868.6 ± 256 A**	**0.01**
trans beta Ocimene	1084	81.75 ± 22.0	71.46 ± 26.3	34.40 ± 16.4	0.27	34.80 ± 6.69	38.39 ± 5.37	54.82 ± 13.6	42.40 ± 19.5	0.70	7.66 ± 1.62	12.30 ± 2.58	8.91 ± 0.89	11.32 ± 2.60	0.38
cis beta Ocimene	1093	343.9 ± 88.3	348.3 ± 106	140.8 ± 67.7	0.14	158.8 ± 28.6	182.7 ± 25.2	227.0 ± 53.2	176.7 ± 76.3	0.80	31.23 ± 6.32	49.59 ± 11.1	45.51 ± 8.93	48.69 ± 9.71	0.48
gamma Terpinene	1101	22.13 ± 3.08	8.92 ± 1.88	11.47 ± 4.95	0.64	**5.79 ± 1.92 B**	**6.81 ± 1.96 AB**	**20.56 ± 6.36 A**	**8.92 ± 1.77 AB**	**0.04**	**3.93 ± 0.87 B**	**2.54 ± 0.27 B**	**4.00 ± 0.57 B**	**8.28 ± 1.80 A**	**8.03E-03**
1,3,8-p-Menthatriene	1118	0.66 ± 0.16	0.42 ± 0.06	0.44 ± 0.11	0.89	**0.27 ± 0.08 AB**	**0.35 ± 0.04 A**	**ND B**	**0.37 ± 0.12 A**	**0.01**	**0.05 ± 0.02 AB**	**0.10 ± 0.03 A**	**0.11 ± 0.01 A**	**ND B**	**1.89E-03**
4-Methyl-3-(1-methylethylidene)-1-cyclohexene	1120	ND	ND	ND	NA	**0.18 ± 0.02 BC**	**0.30 ± 0.04 AB**	**ND C**	**0.47 ± 0.13 A**	**1.36E-03**	0.07 ± 0.02	0.18 ± 0.04	ND	0.98 ± 0.53	0.06
Terpinolene	1125	21.63 ± 4.39	8.81 ± 1.86	5.78 ± 1.91	0.29	**3.87 ± 1.01 B**	**5.76 ± 1.05 AB**	**13.88 ± 4.44 A**	**5.18 ± 0.80 AB**	**0.03**	1.86 ± 0.29	4.46 ± 0.73	3.34 ± 0.56	8.43 ± 3.35	0.09
Cosmene	1167	1.86 ± 0.22	**1.39 ± 0.36 A**	**ND B**	**5.07E-03**	**0.61 ± 0.16 B**	**0.85 ± 0.08 AB**	**3.02 ± 0.85 A**	**1.67 ± 0.73 AB**	**0.03**	**ND B**	**0.21 ± 0.03 A**	**ND B**	**ND B**	**6.63E-09**

		T0	T1			T2					T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
Allo-Ocimene	1174	15.13 ± 3.85	13.35 ± 3.98	6.93 ± 2.87	0.23	6.01 ± 1.11	8.20 ± 1.13	15.65 ± 3.74	8.86 ± 3.02	0.08	1.44 ± 0.30	2.71 ± 0.61	2.25 ± 0.25	3.06 ± 0.56	0.11
Neo-allo-Ocimene	1185	12.34 ± 3.07	11.24 ± 2.77	5.76 ± 2.24	0.16	**4.69 ± 0.82 B**	**7.56 ± 1.13 AB**	**14.30 ± 3.24 A**	**8.84 ± 2.64 AB**	**0.05**	1.44 ± 0.31	3.12 ± 0.74	2.14 ± 0.31	3.10 ± 0.58	0.10
Total monoterpenes hydrocarbons	NA	3809 ± 694	2475 ± 418	1517 ± 503	0.18	**1165 ± 235 B**	**1466 ± 249 B**	**3700 ± 936 A**	**1371 ± 336 B**	**0.01**	**339.9 ± 39.4 B**	**469.9 ± 68.0 B**	**747.0 ± 74.3 AB**	**1291 ± 379 A**	**0.02**
Monoterpene alcohols															
Verbenol	1206	3.77 ± 0.67	1.15 ± 0.26	1.18 ± 0.37	0.95	0.77 ± 0.20	1.29 ± 0.16	2.70 ± 0.69	2.21 ± 0.73	0.07	**0.73 ± 0.12 B**	**1.07 ± 0.13 AB**	**1.21 ± 0.30 AB**	**2.62 ± 0.73 A**	**0.02**
trans-Sabinene hydrate	1107	2.42 ± 0.48	0.79 ± 0.25	0.92 ± 0.29	0.73	0.59 ± 0.15	0.69 ± 0.09	2.49 ± 1.03	0.77 ± 0.11	0.06	**0.20 ± 0.03 B**	**0.32 ± 0.06 B**	**0.44 ± 0.10 AB**	**0.83 ± 0.21 A**	**0.01**
beta Linalool	1146	53.44 ± 11.6	46.33 ± 10.1	24.38 ± 8.57	0.14	23.58 ± 3.10	32.60 ± 6.31	38.59 ± 10.5	32.36 ± 9.81	0.63	**4.25 ± 0.44 B**	**6.99 ± 1.47 B**	**13.11 ± 1.14 B**	**23.28 ± 4.53 A**	**2.50E-04**
trans-3-Caren-2-ol	1156	2.42 ± 0.57	**1.78 ± 0.52 A**	**0.41 ± 0.09 B**	**0.03**	**0.44 ± 0.17 B**	**0.59 ± 0.15 B**	**2.25 ± 0.63 A**	**0.52 ± 0.13 B**	**4.26E-03**	**0.07 ± 0.00 B**	**0.09 ± 0.01 B**	**0.78 ± 0.15 A**	**1.00 ± 0.21 A**	**8.11E-05**
Isocarveol	1177	ND	ND	ND	NA	**0.17 ± 0.05 A**	**0.11 ± 0.03 AB**	**ND B**	**0.22 ± 0.05 A**	**2.90E-03**	**0.04 ± 0.01 B**	**0.05 ± 0.00 B**	**0.18 ± 0.05 AB**	**0.33 ± 0.07 A**	**2.76E-04**
Levomenthol	1212	5.28 ± 1.68	3.94 ± 1.40	0.74 ± 0.20	0.05	**0.85 ± 0.36 B**	**0.53 ± 0.10 B**	**7.36 ± 2.61 A**	**0.54 ± 0.21 B**	**4.54E-03**	0.02 ± 0.01 A	0.03 ± 0.01 A	1.97 ± 0.74 A	1.89 ± 0.70 A	**0.02**
Terpinen-4-ol	1216	1.17 ± 0.12	**1.14 ± 0.27 A**	**0.35 ± 0.07 B**	**0.02**	0.24 ± 0.05	0.58 ± 0.15	1.22 ± 0.15	1.38 ± 0.56	0.05	0.10 ± 0.01	0.07 ± 0.01	1.74 ± 1.16	0.38 ± 0.10	0.18
Carveol	1217	1.78 ± 0.47	1.24 ± 0.59	0.64 ± 0.06	0.34	**0.16 ± 0.03 B**	**0.39 ± 0.10 B**	**1.49 ± 0.27 AB**	**4.55 ± 1.95 A**	**0.02**	**0.29 ± 0.08 AB**	**0.19 ± 0.04 B**	**0.52 ± 0.07 AB**	**1.61 ± 0.64 A**	**0.03**
alpha Terpineol	1227	38.40 ± 6.52	15.16 ± 3.32	12.43 ± 3.85	0.61	11.47 ± 3.15	10.53 ± 1.68	26.25 ± 11.6	15.04 ± 3.31	0.31	3.77 ± 0.67	3.35 ± 0.39	3.26 ± 0.60	5.43 ± 0.97	0.13
trans-Geraniol	1299	24.45 ± 7.21	10.26 ± 3.15	6.78 ± 2.74	0.43	6.00 ± 1.20	6.43 ± 0.81	6.12 ± 2.24	4.81 ± 0.97	0.86	**1.80 ± 0.51 B**	**8.94 ± 1.16 A**	**4.27 ± 1.28 B**	**1.91 ± 0.27 B**	**1.22E-04**
cis-Geraniol	1274	29.97 ± 9.13	11.69 ± 3.14	6.67 ± 2.48	0.25	6.64 ± 1.13	8.18 ± 1.05	5.66 ± 3.46	6.97 ± 1.46	0.85	**3.83 ± 1.08 B**	**14.83 ± 2.17 A**	**6.38 ± 1.51 B**	**2.62 ± 0.41 B**	**7.71E-05**
Total monoterpene alcohols	NA	163.1 ± 35.4	93.47 ± 20.0	54.51 ± 17.6	0.18	50.90 ± 8.89	61.91 ± 9.59	94.12 ± 28.8	69.38 ± 16.1	0.39	**15.11 ± 2.40 B**	**35.92 ± 5.00 A**	**33.85 ± 3.58 A**	**41.91 ± 4.19 A**	**1.14E-03**
Monoterpene aldehydes															
Citral	1188	9.86 ± 1.68	2.64 ± 0.56	3.07 ± 0.96	0.71	1.80 ± 0.51	3.57 ± 0.49	4.07 ± 1.31	4.59 ± 1.53	0.31	**1.54 ± 0.26 B**	**2.05 ± 0.26 B**	**2.44 ± 0.61 AB**	**5.89 ± 1.67 A**	**0.02**
(R)-(+)-Citronellal	1196	5.35 ± 1.16	1.84 ± 0.35	1.18 ± 0.39	0.24	1.29 ± 0.53	1.06 ± 0.14	2.20 ± 0.71	2.10 ± 0.58	0.36	**0.72 ± 0.15 B**	**1.18 ± 0.28 AB**	**1.89 ± 0.51 AB**	**2.88 ± 0.84 A**	**0.05**
beta Citral	1286	206.6 ± 34.51	59.76 ± 15.1	62.14 ± 20.2	0.93	43.88 ± 12.7	68.52 ± 8.57	96.88 ± 40.8	131.9 ± 43.1	0.25	**45.83 ± 8.46 B**	**62.40 ± 8.43 AB**	**76.18 ± 20.9 AB**	**161.2 ± 47.7 A**	**0.03**
Geranial	1313	374.3 ± 63.9	98.77 ± 23.6	120.3 ± 38.4	0.65	71.72 ± 21.0	136.2 ± 17.2	165.0 ± 75.1	211.1 ± 64.8	0.31	**84.57 ± 15.9 B**	**100.2 ± 13.9 AB**	**124.7 ± 34.6 AB**	**283.2 ± 81.2 A**	**0.03**
Total monoterpene aldehydes	NA	596.1 ± 101	163.0 ± 39.5	186.7 ± 59.8	0.75	118.7 ± 34.6	209.4 ± 26.3	268.1 ± 117	349.7 ± 109	0.29	**132.7 ± 24.7 B**	**165.8 ± 22.1 AB**	**205.3 ± 56.5 AB**	**453.2 ± 131 A**	**0.03**
Monoterpene esters															
Methyl geranate	1371	0.62 ± 0.09	**0.31 ± 0.08 A**	**ND B**	**3.17E-03**	**0.14 ± 0.02 B**	**0.19 ± 0.04 B**	**0.86 ± 0.29 A**	**0.17 ± 0.03 B**	**7.49E-03**	0.12 ± 0.03	0.23 ± 0.03	0.20 ± 0.03	0.49 ± 0.22	0.14
Isobornyl acetate	1326	0.66 ± 0.08	**0.51 ± 0.10 A**	**ND B**	**7.87E-04**	**0.11 ± 0.02 B**	**ND B**	**1.38 ± 0.21 A**	**0.29 ± 0.19 B**	**1.23E-05**	**ND B**	**ND B**	**0.28 ± 0.06 AB**	**0.58 ± 0.25 A**	**0.02**
Citronellyl propionate	1400	0.47 ± 0.10	0.36 ± 0.11	0.15 ± 0.03	0.10	0.20 ± 0.03	0.15 ± 0.02	0.27 ± 0.07	0.24 ± 0.08	0.53	0.07 ± 0.01	0.12 ± 0.02	0.15 ± 0.02	0.29 ± 0.11	0.10

Compound	LRI	T0	T1			T2					T3				
		Control	Control	JAsp	p-value	Control	JAsp	Psyll	JAsp_Psyll	p-value	Control	JAsp	Psyll	JAsp_Psyll	p-value
Nerol acetate	1410	9.90 ± 2.60	4.51 ± 1.81	4.93 ± 2.43	0.89	4.08 ± 1.30	7.42 ± 1.31	2.58 ± 1.03	6.69 ± 2.24	0.13	5.31 ± 0.67	6.03 ± 0.30	2.61 ± 0.86	5.43 ± 1.64	0.11
Geranyl acetate	1427	23.45 ± 5.70	8.07 ± 3.41	10.76 ± 4.01	0.62	6.81 ± 2.21	14.71 ± 2.65	6.99 ± 3.74	9.49 ± 3.05	0.24	**4.55 ± 0.81 B**	**7.36 ± 1.09 AB**	**3.30 ± 0.94 B**	**11.27 ± 2.82 A**	**0.02**
Total monoterpene esters	NA	35.11 ± 8.47	13.76 ± 5.37	15.83 ± 6.42	0.81	11.33 ± 3.56	22.47 ± 3.98	12.07 ± 5.10	16.88 ± 5.26	0.31	**10.05 ± 1.25 AB**	**13.74 ± 1.32 AB**	**6.55 ± 1.79 B**	**18.06 ± 4.05 A**	**0.02**
Monoterpene epoxides															
4,5-Epoxycarene	1221	7.45 ± 1.28	2.22 ± 0.50	2.44 ± 0.75	0.81	1.46 ± 0.43	2.78 ± 0.40	3.39 ± 1.37	5.05 ± 1.33	0.12	**1.37 ± 0.21 B**	**2.11 ± 0.27 B**	**2.33 ± 0.52 B**	**6.26 ± 1.22 A**	**5.06E-04**
Limonene 1,2-epoxide	1181	0.40 ± 0.03	**0.20 ± 0.02 B**	**0.13 ± 0.02 A**	**0.04**	**0.08 ± 0.02 B**	**0.23 ± 0.06 AB**	**0.40 ± 0.08 A**	**0.17 ± 0.05 AB**	**0.01**	ND	0.08 ± 0.01	0.10 ± 0.03	0.20 ± 0.09	0.05
Total monoterpene epoxides	NA	7.85 ± 1.28	2.43 ± 0.52	2.57 ± 0.76	0.88	1.54 ± 0.45	3.00 ± 0.42	3.78 ± 1.44	5.22 ± 1.35	0.13	**1.37 ± 0.21 B**	**2.19 ± 0.27 B**	**2.43 ± 0.51 B**	**6.47 ± 1.20 A**	**2.98E-04**
Monoterpene ketones															
2-Oxo-1,8-cineole	1259	0.73 ± 0.14	**0.51 ± 0.16 A**	**ND B**	**0.01**	**0.21 ± 0.08 AB**	**0.17 ± 0.04 B**	**1.10 ± 0.44 A**	**0.11 ± 0.01 B**	**0.02**	**0.05 ± 0.01 B**	**0.07 ± 0.02 AB**	**0.36 ± 0.15 A**	**0.19 ± 0.04 AB**	**0.04**
(-)-Carvone	1287	ND	ND	ND	NA	**ND B**	**ND B**	**3.95 ± 0.97 A**	**ND B**	**3.66E-05**	ND	ND	ND	ND	NA
Total monoterpene ketones	NA	0.73 ± 0.14	**0.51 ± 0.16 A**	**ND B**	**0.01**	**0.21 ± 0.08 B**	**0.17 ± 0.04 B**	**5.05 ± 1.19 A**	**0.11 ± 0.01 B**	**3.39E-05**	**0.05 ± 0.01 B**	**0.07 ± 0.02 AB**	**0.36 ± 0.15 A**	**0.19 ± 0.04 AB**	**0.04**
Total monoterpenes	NA	4612 ± 828	2748 ± 481	1777 ± 585	0.24	**1348 ± 282 B**	**1763 ± 281 AB**	**4083 ± 1087 A**	**1812 ± 354 AB**	**0.02**	**499.1 ± 41.7 B**	**687.6 ± 86.8 B**	**995.4 ± 78.7 AB**	**1811 ± 493 A**	**0.01**
Sesquiterpenes															
Sesquiterpene hydrocarbons															
alpha Cubenene	1396	1.03 ± 0.36	0.69 ± 0.23	1.16 ± 0.38	0.32	0.89 ± 0.40	0.58 ± 0.23	ND	0.20 ± 0.06	0.07	0.07 ± 0.02	0.13 ± 0.02	0.92 ± 0.49	0.64 ± 0.30	0.15
Copaene	1420	0.13 ± 0.01	ND	ND	NA	**0.07 ± 0.01 AB**	**0.06 ± 0.01 AB**	**ND B**	**0.11 ± 0.03 A**	**5.19E-03**	0.06 ± 0.02	0.10 ± 0.01	0.07 ± 0.01	0.36 ± 0.17	0.07
beta Elemene	1435	0.33 ± 0.08	0.30 ± 0.07	0.22 ± 0.10	0.54	0.21 ± 0.05	0.22 ± 0.02	0.31 ± 0.05	0.42 ± 0.13	0.20	0.12 ± 0.02	0.19 ± 0.02	0.13 ± 0.02	0.26 ± 0.11	0.30
4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	1458	0.89 ± 0.25	0.66 ± 0.21	0.21 ± 0.06	0.07	0.39 ± 0.17	0.28 ± 0.03	0.67 ± 0.07	0.51 ± 0.22	0.31	**0.06 ± 0.01 B**	**0.09 ± 0.02 B**	**0.45 ± 0.14 A**	**0.31 ± 0.01 AB**	**3.45E-03**
alpha Bergamotene	1467	3.87 ± 0.92	2.40 ± 1.01	0.86 ± 0.36	0.19	2.39 ± 0.45	2.01 ± 0.22	2.23 ± 0.52	1.93 ± 0.22	0.82	0.63 ± 0.10	1.04 ± 0.09	0.66 ± 0.13	0.73 ± 0.17	0.13
Caryophyllene	1471	52.80 ± 13.8	41.13 ± 17.8	39.87 ± 21.2	0.96	46.17 ± 8.30	45.72 ± 7.93	40.69 ± 8.07	73.12 ± 23.9	0.37	17.45 ± 2.17	28.28 ± 6.48	18.47 ± 3.21	22.27 ± 5.32	0.37
trans alpha Bergamotene	1487	58.82 ± 13.8	37.76 ± 15.6	22.29 ± 10.4	0.43	35.83 ± 6.45	30.64 ± 3.34	36.59 ± 8.50	42.08 ± 14.3	0.85	9.22 ± 1.34	18.65 ± 4.58	10.21 ± 2.00	10.50 ± 2.45	0.11
Alloaromadendrene	1489	0.85 ± 0.20	0.48 ± 0.13	0.37 ± 0.11	0.55	0.45 ± 0.17	0.37 ± 0.08	0.67 ± 0.14	0.34 ± 0.05	0.23	0.32 ± 0.05	0.47 ± 0.04	0.44 ± 0.10	0.71 ± 0.30	0.42
delta Guaiene	1492	0.32 ± 0.02	**0.13 ± 0.05 A**	**ND B**	**0.04**	**0.14 ± 0.01 A**	**0.14 ± 0.02 A**	**ND B**	**0.16 ± 0.04 A**	**9.81E-04**	0.10 ± 0.02	0.18 ± 0.03	0.07 ± 0.01	0.12 ± 0.05	0.13
Humulen-(v1)	1500	2.15 ± 0.86	**2.14 ± 0.70 A**	**0.13 ± 0.03 B**	**0.02**	0.62 ± 0.48	0.30 ± 0.16	ND	0.50 ± 0.18	0.41	**0.05 ± 0.01 AB**	**0.06 ± 0.01 AB**	**ND B**	**0.29 ± 0.12 A**	**0.02**

		T0	T1			T2					T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
alpha Humulene	1503	11.60 ± 3.11	9.35 ± 4.38	9.24 ± 5.07	0.99	10.82 ± 1.95	10.44 ± 1.69	8.29 ± 1.63	17.27 ± 5.66	0.27	4.22 ± 0.54	7.44 ± 1.68	3.85 ± 0.67	4.23 ± 0.96	0.09
beta Farnesene	1506	3.25 ± 0.79	2.38 ± 1.19	1.47 ± 0.56	0.51	2.21 ± 0.41	1.71 ± 0.17	1.07 ± 0.21	1.80 ± 0.62	0.27	0.36 ± 0.05	0.68 ± 0.20	0.36 ± 0.08	0.73 ± 0.31	0.38
gamma Muurolene	1524	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.02 ± 0.01 A**	**0.04 ± 0.00 A**	**ND B**	**ND B**	**1.56E-05**
beta Curcumene	1527	3.11 ± 1.12	2.38 ± 0.98	1.25 ± 0.33	0.31	1.69 ± 0.91	1.31 ± 0.38	2.07 ± 0.71	0.57 ± 0.14	0.38	**0.09 ± 0.01 B**	**0.17 ± 0.04 B**	**2.43 ± 1.08 A**	**1.00 ± 0.31 AB**	**0.03**
alpha Curcumene	1529	0.56 ± 0.10	0.38 ± 0.09	0.23 ± 0.07	0.21	**0.23 ± 0.04 B**	**0.28 ± 0.03 AB**	**0.52 ± 0.07 AB**	**0.67 ± 0.19 A**	**0.03**	**0.10 ± 0.01 B**	**0.22 ± 0.04 A**	**0.08 ± 0.00 B**	**0.14 ± 0.01 AB**	**4.13E-03**
beta Sesquiphellandrene	1532	2.43 ± 0.59	1.63 ± 0.69	0.88 ± 0.40	0.37	1.48 ± 0.29	1.24 ± 0.14	1.28 ± 0.32	1.32 ± 0.48	0.96	0.30 ± 0.04	0.66 ± 0.15	0.75 ± 0.25	0.38 ± 0.04	0.13
beta Chamigrene	1535	ND	**0.44 ± 0.14 A**	**ND B**	**0.02**	**0.07 ± 0.02 B**	**0.19 ± 0.03 A**	**ND C**	**0.04 ± 0.01 BC**	**7.42E-06**	**0.02 ± 0.00 B**	**0.04 ± 0.00 B**	**0.17 ± 0.04 A**	**ND B**	**3.16E-05**
Dysoxylonene	1537	ND	ND	ND	NA	**0.04 ± 0.01 A**	**ND B**	**ND B**	**0.05 ± 0.01 A**	**4.46E-05**	**0.03 ± 0.01 A**	**0.06 ± 0.01 A**	**0.06 ± 0.01 A**	**ND B**	**8.83E-05**
beta Germacrene	1542	3.74 ± 0.74	1.97 ± 0.63	2.02 ± 0.91	0.97	2.31 ± 0.61	2.02 ± 0.29	2.28 ± 0.78	3.22 ± 0.78	0.59	**2.07 ± 0.27 AB**	**3.52 ± 0.47 A**	**1.73 ± 0.41 B**	**1.36 ± 0.32 B**	**4.75E-03**
beta Bisabolene	1562	41.16 ± 9.48	34.43 ± 13.54	14.96 ± 6.61	0.23	24.46 ± 4.49	21.68 ± 1.93	17.65 ± 3.68	21.64 ± 6.06	0.74	4.43 ± 0.49	7.58 ± 1.44	5.22 ± 0.91	5.07 ± 0.48	0.12
(-)-Gamma-cadinene	1567	ND	**0.37 ± 0.11 A**	**ND B**	**0.01**	**0.20 ± 0.02 AB**	**0.17 ± 0.01 AB**	**ND B**	**0.50 ± 0.19 A**	**0.02**	**0.10 ± 0.03 A**	**0.16 ± 0.02 A**	**ND B**	**ND B**	**3.25E-06**
delta Cadinene	1576	0.40 ± 0.11	**0.75 ± 0.13 A**	**0.27 ± 0.03 B**	**7.33E-03**	0.25 ± 0.09	0.22 ± 0.03	0.33 ± 0.01	0.18 ± 0.04	0.24	**0.13 ± 0.03 AB**	**0.20 ± 0.02 A**	**ND B**	**0.17 ± 0.07 A**	**0.02**
alpha Muurolene	1590	0.16 ± 0.01	**0.35 ± 0.08 A**	**ND B**	**2.16E-03**	ND	ND	ND	ND	NA	**0.02 ± 0.01 BC**	**0.03 ± 0.00 AB**	**0.05 ± 0.01 A**	**ND C**	**4.83E-04**
Elixene	1385	0.88 ± 0.17	0.33 ± 0.09	0.55 ± 0.19	0.31	0.49 ± 0.12	0.48 ± 0.07	0.83 ± 0.16	0.65 ± 0.18	0.27	0.46 ± 0.06	0.79 ± 0.10	0.37 ± 0.10	0.76 ± 0.33	0.29
beta Santalene	1509	4.03 ± 0.93	2.56 ± 1.08	1.71 ± 0.58	0.51	2.40 ± 0.42	2.04 ± 0.20	2.64 ± 0.48	3.38 ± 0.91	0.42	0.63 ± 0.09	1.30 ± 0.30	0.65 ± 0.12	1.45 ± 0.61	0.25
alpha Bisabolene	1556	4.82 ± 1.17	**3.67 ± 1.55 A**	**ND B**	**0.05**	2.95 ± 0.50	2.42 ± 0.25	2.04 ± 0.52	2.73 ± 0.84	0.70	0.77 ± 0.10	1.47 ± 0.26	1.61 ± 0.49	0.75 ± 0.05	0.10
Total sesquiterpene hydrocarbons	NA	197.3 ± 46.0	146.7 ± 56.4	97.70 ± 46.4	0.52	136.8 ± 25.5	124.5 ± 14.7	120.2 ± 23.6	173.4 ± 53.3	0.66	41.85 ± 5.38	73.56 ± 16.0	48.77 ± 7.73	52.23 ± 7.74	0.18
Sesquiterpene alcohols															
cis-Nerolidol	1613	0.44 ± 0.08	**1.13 ± 0.25 A**	**ND B**	**1.98E-03**	ND	ND	ND	ND	NA	**0.02 ± 0.00 B**	**0.03 ± 0.01 B**	**0.15 ± 0.03 A**	**ND B**	**1.56E-05**
Epiglobulol	1668	ND	**0.10 ± 0.03 A**	**ND B**	**4.78E-03**	**0.08 ± 0.02 A**	**0.09 ± 0.01 A**	**ND B**	**ND B**	**6.46E-06**	0.02 ± 0.01	0.02 ± 0.00	0.05 ± 0.03	ND	0.09
alpha Bisabolol	1738	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.01 ± 0.00 A**	**0.01 ± 0.00 A**	**0.15 ± 0.07 A**	**ND A**	**0.03**
trans-Sesquisabinene hydrate	1585	0.36 ± 0.04	0.27 ± 0.08	0.20 ± 0.01	0.39	0.18 ± 0.06	0.13 ± 0.03	0.22 ± 0.03	0.15 ± 0.04	0.47	0.03 ± 0.01	0.06 ± 0.01	0.15 ± 0.05	0.21 ± 0.09	0.10
Total sesquiterpene alcohols	NA	0.80 ± 0.09	**1.50 ± 0.30 A**	**0.20 ± 0.01 B**	**2.43E-03**	0.26 ± 0.07	0.23 ± 0.03	0.22 ± 0.03	0.15 ± 0.04	0.46	**0.08 ± 0.01 B**	**0.12 ± 0.02 B**	**0.51 ± 0.15 A**	**0.21 ± 0.09 AB**	**0.02**

		T0	T1			T2				T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
Other sesquiterpenes															
3-Methyl-4-methylenebicyclo[3.2.1]oct-2-ene	1002	0.98 ± 0.10	ND	ND	NA	**0.25 ± 0.04 AB**	**0.46 ± 0.08 A**	**ND B**	**0.46 ± 0.11 A**	**1.00E-03**	**0.07 ± 0.02 BC**	**0.18 ± 0.04 AB**	**0.25 ± 0.04 A**	**ND C**	**1.86E-04**
Caryophyllene oxide	1632	0.24 ± 0.02	**ND B**	**0.10 ± 0.01 A**	**7.26E-06**	0.05 ± 0.01	0.08 ± 0.02	ND	0.27 ± 0.16	0.12	**0.03 ± 0.00 B**	**0.03 ± 0.00 B**	**0.07 ± 0.01 B**	**0.11 ± 0.02 A**	**9.61E-05**
Clovene	1403	ND	ND	ND	NA	**0.49 ± 0.23 A**	**ND A**	**ND A**	**0.29 ± 0.12 A**	**0.04**	**0.03 ± 0.00 B**	**0.03 ± 0.01 AB**	**ND B**	**0.32 ± 0.14 A**	**0.02**
(-)-Aristolene	1424	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.01 ± 0.00 B**	**0.03 ± 0.00 A**	**ND B**	**ND B**	**7.44E-08**
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)pent-1-en-3-one	1570	0.55 ± 0.08	ND	ND	NA	**ND B**	**ND B**	**ND B**	**0.27 ± 0.08 A**	**2.58E-04**	**ND B**	**ND B**	**0.32 ± 0.15 A**	**ND B**	**0.02**
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	1595	1.40 ± 0.33	1.08 ± 0.48	0.55 ± 0.19	0.34	0.78 ± 0.13	0.67 ± 0.07	0.59 ± 0.09	0.96 ± 0.22	0.33	0.20 ± 0.02	0.36 ± 0.06	0.13 ± 0.02	0.34 ± 0.15	0.17
Total other sequiterpenes	NA	3.17 ± 0.47	1.08 ± 0.48	0.65 ± 0.18	0.43	**1.57 ± 0.38 AB**	**1.22 ± 0.15 AB**	**0.59 ± 0.09 B**	**2.25 ± 0.51 A**	**0.02**	0.33 ± 0.04	0.64 ± 0.12	0.77 ± 0.15	0.77 ± 0.29	0.28
Total sesquiterpes	NA	201.3 ± 46.5	149.3 ± 56.8	98.56 ± 46.6	0.51	138.6 ± 25.9	126.0 ± 14.8	121.0 ± 23.7	175.8 ± 53.8	0.65	42.25 ± 5.44	74.31 ± 16.1	50.04 ± 7.79	53.20 ± 7.46	0.18
Acetates															
(Z)-3-Hexenol acetate	1055	67.20 ± 10.0	**61.46 ± 5.03 A**	**24.73 ± 4.89 B**	**7.85E-04**	**33.27 ± 6.67 A**	**3.54 ± 0.78 BC**	**20.31 ± 5.54 AB**	**ND C**	**1.75E-04**	**ND B**	**0.62 ± 0.17 B**	**13.18 ± 2.78 A**	**1.96 ± 0.22 B**	**1.24E-05**
4-tert-Butylcyclohexyl acetate	1333	7.66 ± 1.83	**5.91 ± 1.99 A**	**0.93 ± 0.29 B**	**0.04**	**1.45 ± 0.64 B**	**0.64 ± 0.15 B**	**12.89 ± 2.11 A**	**1.87 ± 0.99 B**	**4.78E-06**	**0.04 ± 0.01 B**	**0.03 ± 0.01 B**	**3.34 ± 1.16 AB**	**4.42 ± 1.20 A**	**2.62E-03**
Total acetates	NA	74.86 ± 9.65	**67.37 ± 4.13 A**	**25.65 ± 4.92 B**	**1.90E-04**	**34.72 ± 7.05 A**	**4.18 ± 0.76 B**	**33.20 ± 5.19 A**	**1.87 ± 0.99 B**	**3.91E-05**	**0.04 ± 0.01 B**	**0.65 ± 0.17 B**	**16.52 ± 3.32 A**	**6.38 ± 1.16 B**	**1.70E-05**
Alcohols															
2-n-Butoxyethanol	957	13.56 ± 4.25	10.76 ± 3.90	4.92 ± 0.35	0.17	3.70 ± 1.68	3.21 ± 0.71	32.70 ± 16.9	7.97 ± 3.14	0.08	**0.38 ± 0.05 B**	**0.52 ± 0.06 B**	**10.69 ± 3.77 A**	**7.24 ± 2.53 AB**	**0.01**
2-Methyl-6-methyleneoctan-2-ol	1111	21.87 ± 5.25	**17.07 ± 5.17 A**	**2.64 ± 0.73 B**	**0.02**	**3.11 ± 1.38 B**	**1.07 ± 0.21 B**	**22.89 ± 4.55 A**	**4.85 ± 1.96 B**	**5.89E-05**	**0.15 ± 0.04 B**	**0.16 ± 0.04 AB**	**7.72 ± 2.85 AB**	**9.30 ± 3.52 A**	**0.02**
(E)-2-Hexen-1-ol	916	5.07 ± 0.45	**18.20 ± 4.00 A**	**6.01 ± 0.92 B**	**0.02**	3.43 ± 0.27	2.28 ± 0.33	ND	6.20 ± 2.92	0.06	**0.44 ± 0.03 B**	**0.52 ± 0.06 B**	**4.10 ± 1.18 A**	**3.76 ± 1.02 A**	**4.26E-03**
1-Dodecanol	1521	0.97 ± 0.30	0.75 ± 0.29	0.40 ± 0.10	0.28	0.56 ± 0.33	0.55 ± 0.11	ND	0.20 ± 0.04	0.09	**0.09 ± 0.01 B**	**0.10 ± 0.02 B**	**0.59 ± 0.22 A**	**0.24 ± 0.02 AB**	**0.02**
(Z)-3-Hexen-1-ol	908	13.17 ± 1.68	**17.76 ± 2.98 A**	**4.08 ± 1.01 B**	**2.44E-03**	3.64 ± 0.42	1.37 ± 0.38	11.37 ± 6.69	1.93 ± 0.39	0.17	**0.04 ± 0.01 B**	**1.03 ± 0.44 AB**	**2.52 ± 0.66 A**	**1.28 ± 0.27 AB**	**6.20E-03**
Total alcohols	NA	54.65 ± 11.10	**64.54 ± 9.54 A**	**18.04 ± 1.85 B**	**1.38E-03**	**14.44 ± 3.67 B**	**8.48 ± 1.38 B**	**66.97 ± 21.1 A**	**21.16 ± 7.79 AB**	**9.08E-03**	**1.10 ± 0.06 B**	**2.32 ± 0.57 B**	**25.62 ± 7.98 A**	**21.82 ± 6.66 AB**	**5.89E-03**
Aldehydes															
(E)-2-Hexenal	906	0.70 ± 0.08	**1.05 ± 0.31 A**	**ND B**	**9.51E-03**	**0.24 ± 0.04 B**	**0.25 ± 0.03 B**	**1.22 ± 0.42 A**	**ND B**	**4.19E-03**	**ND B**	**ND B**	**0.05 ± 0.02 A**	**ND B**	**5.12E-03**
Heptanal	954	0.28 ± 0.06	0.35 ± 0.02	0.50 ± 0.07	0.07	**0.11 ± 0.01 B**	**0.33 ± 0.04 B**	**1.02 ± 0.20 A**	**ND B**	**1.28E-05**	**0.02 ± 0.00 BC**	**ND C**	**0.35 ± 0.06 AB**	**0.37 ± 0.15 A**	**6.24E-03**

		T0	T1			T2					T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
Nonanal	1149	7.47 ± 1.08	6.24 ± 1.84	2.02 ± 0.59	0.06	**1.50 ± 0.46 B**	**1.55 ± 0.56 B**	**15.11 ± 3.91 A**	**2.33 ± 1.10 B**	**4.72E-04**	**0.12 ± 0.02 B**	**0.14 ± 0.02 B**	**1.69 ± 0.47 A**	**3.06 ± 0.51 A**	**3.70E-05**
Decanal	1251	3.24 ± 0.61	**2.50 ± 0.79 A**	**0.59 ± 0.15 B**	**0.04**	**0.56 ± 0.21 B**	**0.44 ± 0.16 B**	**3.88 ± 1.00 A**	**1.32 ± 0.52 B**	**2.13E-03**	**0.08 ± 0.02 B**	**0.06 ± 0.01 B**	**0.84 ± 0.23 A**	**1.34 ± 0.11 A**	**3.59E-06**
Undecanal	1355	0.94 ± 0.19	0.62 ± 0.16	0.27 ± 0.05	0.07	**0.36 ± 0.16 AB**	**0.31 ± 0.06 B**	**0.94 ± 0.22 A**	**0.24 ± 0.06 B**	**0.01**	**0.07 ± 0.01 B**	**0.04 ± 0.01 B**	**0.40 ± 0.11 A**	**0.32 ± 0.04 A**	**8.77E-04**
Total aldehydes	NA	12.62 ± 1.87	**10.76 ± 2.80 A**	**3.38 ± 0.67 B**	**0.03**	**2.77 ± 0.83 B**	**2.88 ± 0.78 B**	**22.16 ± 5.09 A**	**3.89 ± 1.55 B**	**2.09E-04**	**0.29 ± 0.03 B**	**0.24 ± 0.03 B**	**3.34 ± 0.83 A**	**5.09 ± 0.74 A**	**2.06E-05**
Alkanes															
Decane	1047	4.08 ± 1.29	3.47 ± 0.56	2.30 ± 1.04	0.35	**1.25 ± 0.40 B**	**1.34 ± 0.50 B**	**6.11 ± 1.93 A**	**3.14 ± 1.11 AB**	**0.03**	**ND B**	**ND B**	**1.74 ± 0.51 B**	**4.19 ± 0.94 A**	**1.00E-04**
Dodecane	1247	2.11 ± 0.30	0.86 ± 0.14	0.69 ± 0.15	0.42	0.40 ± 0.06	0.93 ± 0.14	1.48 ± 0.31	1.74 ± 0.98	0.30	0.46 ± 0.11	0.34 ± 0.06	0.54 ± 0.08	0.61 ± 0.09	0.20
4,6-Dimethyldodecane	1322	0.30 ± 0.03	0.29 ± 0.05	0.20 ± 0.07	0.35	**0.12 ± 0.05 B**	**0.10 ± 0.02 B**	**0.47 ± 0.08 A**	**0.17 ± 0.10 B**	**5.40E-03**	**ND C**	**0.03 ± 0.00 BC**	**0.10 ± 0.02 AB**	**0.13 ± 0.03 A**	**2.31E-04**
Tridecane	1338	0.35 ± 0.03	0.26 ± 0.04	0.25 ± 0.04	0.79	**0.17 ± 0.04 B**	**0.15 ± 0.04 B**	**0.70 ± 0.13 A**	**0.12 ± 0.03 B**	**5.63E-05**	0.03 ± 0.01	0.07 ± 0.01	0.20 ± 0.06	0.19 ± 0.08	0.06
2,2,4,4,6,8,8-Heptamethylnonane	1368	2.53 ± 0.31	**2.65 ± 0.35 A**	**1.11 ± 0.21 B**	**5.28E-03**	**0.29 ± 0.04 B**	**ND B**	**5.88 ± 1.39 A**	**0.85 ± 0.43 B**	**7.80E-05**	**0.08 ± 0.02 B**	**0.04 ± 0.00 B**	**1.03 ± 0.35 AB**	**1.40 ± 0.39 A**	**3.41E-03**
Tetradecane	1375	0.56 ± 0.09	0.46 ± 0.13	0.30 ± 0.01	0.26	**0.23 ± 0.09 B**	**0.20 ± 0.03 B**	**0.77 ± 0.08 A**	**0.22 ± 0.14 B**	**1.28E-03**	**0.03 ± 0.00 B**	**0.05 ± 0.01 B**	**0.30 ± 0.07 A**	**0.18 ± 0.05 AB**	**9.55E-04**
Pentadecane	1450	1.39 ± 0.34	1.33 ± 0.53	0.58 ± 0.11	0.20	**0.43 ± 0.17 B**	**0.39 ± 0.13 B**	**1.33 ± 0.23 A**	**0.46 ± 0.18 B**	**5.38E-03**	**0.13 ± 0.03 B**	**0.19 ± 0.02 AB**	**0.27 ± 0.03 AB**	**0.33 ± 0.06 A**	**6.73E-03**
Hexadecane	1602	0.57 ± 0.11	**0.79 ± 0.30 A**	**ND B**	**0.03**	**0.09 ± 0.02 B**	**0.13 ± 0.02 B**	**0.42 ± 0.06 A**	**0.22 ± 0.07 B**	**8.32E-04**	**0.03 ± 0.01 B**	**0.01 ± 0.00 B**	**0.15 ± 0.02 A**	**0.11 ± 0.02 A**	**1.85E-05**
Heptadecane	1647	3.24 ± 0.82	4.58 ± 1.93	0.84 ± 0.30	0.09	**0.72 ± 0.31 AB**	**0.89 ± 0.38 AB**	**1.90 ± 0.20 A**	**0.55 ± 0.26 B**	**0.02**	**0.05 ± 0.01 B**	**0.04 ± 0.01 B**	**0.39 ± 0.05 A**	**0.58 ± 0.13 A**	**9.22E-05**
Octadecane	1810	0.48 ± 0.10	**0.67 ± 0.25 A**	**ND B**	**0.03**	**0.23 ± 0.09 AB**	**0.17 ± 0.02 B**	**0.45 ± 0.08 A**	**0.07 ± 0.01 B**	**3.17E-03**	**0.02 ± 0.00 B**	**0.01 ± 0.00 B**	**0.16 ± 0.04 A**	**0.12 ± 0.02 A**	**5.37E-04**
Nonadecane	1853	1.47 ± 0.33	**2.05 ± 0.78 A**	**0.22 ± 0.05 B**	**0.05**	0.53 ± 0.22	0.32 ± 0.07	0.65 ± 0.08	0.18 ± 0.07	0.08	**0.02 ± 0.00 B**	**0.01 ± 0.00 B**	**0.29 ± 0.05 A**	**0.31 ± 0.04 A**	**4.06E-06**
Total alkanes	NA	17.08 ± 2.13	17.41 ± 4.70	6.49 ± 1.16	0.05	**4.46 ± 1.37 B**	**4.62 ± 0.88 B**	**20.17 ± 4.12 A**	**7.73 ± 3.20 B**	**2.49E-03**	**0.85 ± 0.13 B**	**0.78 ± 0.10 B**	**5.17 ± 1.03 A**	**8.17 ± 1.40 A**	**3.19E-05**
Benzene derivatives															
p-Propyltoluene	1097	ND	ND	ND	NA	**ND B**	**ND B**	**2.03 ± 0.59 A**	**ND B**	**2.67E-04**	**0.09 ± 0.02 A**	**0.11 ± 0.01 A**	**ND B**	**ND B**	**5.26E-06**
Ethylbenzene	912	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.09 ± 0.01 A**	**ND B**	**ND B**	**ND B**	**1.18E-09**
o-Xylene	933	ND	ND	ND	NA	**ND B**	**0.47 ± 0.08 B**	**3.43 ± 0.47 A**	**1.09 ± 0.60 B**	**4.66E-05**	**0.10 ± 0.01 AB**	**0.08 ± 0.01 B**	**0.70 ± 0.19 A**	**0.70 ± 0.23 A**	**8.44E-03**
1,2,4-trimethylbenzene	1009	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.14 ± 0.03 AB**	**0.18 ± 0.02 A**	**0.28 ± 0.07 A**	**ND B**	**6.87E-04**
p-Diethylbenzene	1115	0.56 ± 0.10	0.18 ± 0.02	0.27 ± 0.06	0.24	**0.12 ± 0.05 B**	**0.25 ± 0.03 AB**	**0.64 ± 0.15 A**	**0.41 ± 0.18 AB**	**0.04**	0.14 ± 0.05	0.23 ± 0.07	0.32 ± 0.04	0.93 ± 0.39	0.06
4-Methylindane	1190	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.06 ± 0.01 B**	**0.12 ± 0.02 AB**	**0.28 ± 0.09 A**	**ND B**	**2.46E-03**
Prehnitene	1192	ND	ND	ND	NA	ND	ND	ND	ND	NA	**0.09 ± 0.02 B**	**0.18 ± 0.03 AB**	**ND B**	**0.55 ± 0.21 A**	**9.96E-03**
m-Xylene	1121	ND	**ND B**	**19.58 ± 7.96 A**	**0.04**	ND	ND	ND	ND	NA	**0.20 ± 0.05 B**	**0.38 ± 0.05 B**	**1.67 ± 0.37 A**	**ND B**	**3.77E-05**

		T0	T1			T2					T3				
Compound	**LRI**	**Control**	**Control**	**JAsp**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**	**Control**	**JAsp**	**Psyll**	**JAsp_Psyll**	**p-value**
Total Benzene derivatives	NA	0.56 ± 0.10	**0.18 ± 0.02 B**	**19.85 ± 7.93 A**	**0.04**	**0.12 ± 0.05 B**	**0.72 ± 0.11 B**	**6.10 ± 1.08 A**	**1.49 ± 0.78 B**	**3.51E-05**	**0.91 ± 0.19 B**	**1.29 ± 0.20 AB**	**3.27 ± 0.51 A**	**2.18 ± 0.83 AB**	**0.02**
Esters															
Methyl salicylate	1230	12.07 ± 3.35	**25.97 ± 4.11 A**	**0.90 ± 0.26 B**	**2.94E-04**	3.18 ± 1.14	0.40 ± 0.12	2.52 ± 0.81	1.42 ± 0.76	0.11	**0.06 ± 0.01 B**	**ND B**	**0.67 ± 0.07 A**	**ND B**	**3.14E-10**
Butyl Acrylate	935	ND	ND	ND	NA	**ND B**	**ND B**	**8.03 ± 2.04 A**	**3.72 ± 2.76 AB**	**0.01**	ND	ND	2.10 ± 1.16	1.30 ± 0.55	0.09
n-Butyl butanoate	1031	ND	ND	ND	NA	**ND B**	**ND B**	**1.01 ± 0.22 A**	**ND B**	**6.25E-06**	**ND A**	**ND A**	**0.73 ± 0.38 A**	**ND A**	**0.03**
Methyl benzoate	1129	30.58 ± 0.90	**35.60 ± 2.95 A**	**12.62 ± 1.32 B**	**1.01E-04**	8.31 ± 1.51	3.23 ± 0.57	6.99 ± 1.83	12.61 ± 7.54	0.44	**0.15 ± 0.02 B**	**0.30 ± 0.07 A**	**ND B**	**ND B**	**7.66E-05**
2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	1417	1.37 ± 0.53	1.04 ± 0.33	1.17 ± 0.48	0.83	0.87 ± 0.50	0.78 ± 0.28	1.02 ± 0.28	0.11 ± 0.03	0.23	**0.02 ± 0.00 A**	**0.10 ± 0.03 A**	**1.21 ± 0.58 A**	**0.54 ± 0.18 A**	**0.05**
Methyl ester octanoic acid	1170	0.85 ± 0.12	**1.47 ± 0.47 A**	**ND B**	**0.01**	**0.18 ± 0.04 AB**	**0.19 ± 0.03 AB**	**ND B**	**0.53 ± 0.20 A**	**0.01**	**ND B**	**ND B**	**0.25 ± 0.09 A**	**ND B**	**3.37E-03**
(3E)-3-Hexenyl butyrate	1224	ND	1.27 ± 0.29	1.02 ± 0.33	0.58	**0.06 ± 0.01 A**	**ND B**	**ND B**	**ND B**	**1.23E-08**	**ND B**	**0.05 ± 0.02 A**	**ND B**	**ND B**	**3.05E-03**
cis-3-Hexenyl-.alpha.-methylbutyrate	1278	0.35 ± 0.06	**0.65 ± 0.13 A**	**0.26 ± 0.08 B**	**0.03**	**0.08 ± 0.01 A**	**0.11 ± 0.02 A**	**ND B**	**0.15 ± 0.03 A**	**1.67E-04**	**ND B**	**ND B**	**0.08 ± 0.01 A**	**ND B**	**1.24E-07**
2-Methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl propanoate	1644	1.57 ± 0.32	0.98 ± 0.24	1.55 ± 0.37	0.23	**0.98 ± 0.45 A**	**0.85 ± 0.26 A**	**ND A**	**ND A**	**0.02**	ND	0.15 ± 0.04	1.41 ± 0.76	0.47 ± 0.08	0.08
Total esters	NA	46.79 ± 4.16	**66.98 ± 7.63 A**	**17.53 ± 2.09 B**	**2.46E-04**	13.66 ± 2.99	5.56 ± 0.57	19.56 ± 4.84	18.55 ± 9.69	0.31	**0.22 ± 0.03 B**	**0.60 ± 0.14 B**	**6.44 ± 1.76 A**	**2.31 ± 0.62 B**	**8.87E-04**
Ketones															
2-Hexanone	816	5.96 ± 1.01	3.07 ± 0.84	1.77 ± 0.28	0.18	**0.40 ± 0.08 B**	**ND B**	**14.98 ± 3.72 A**	**3.92 ± 1.68 B**	**2.58E-04**	0.70 ± 0.18	0.28 ± 0.05	4.03 ± 1.51	5.85 ± 2.66	0.06
6-Methyl-5-hepten-2-one	1024	3.14 ± 0.28	2.56 ± 0.59	0.98 ± 0.45	0.06	**0.40 ± 0.09 B**	**0.71 ± 0.24 B**	**8.38 ± 1.18 A**	**2.05 ± 1.35 B**	**3.11E-05**	**0.06 ± 0.01 B**	**0.07 ± 0.01 B**	**0.70 ± 0.11 AB**	**3.28 ± 1.51 A**	**0.02**
Bicyclo[3.3.0]octan-2-one, 7-ethylidene-	1264	4.04 ± 1.41	2.19 ± 0.80	2.25 ± 0.43	0.95	1.45 ± 0.64	1.72 ± 0.61	2.46 ± 0.75	0.95 ± 0.31	0.38	**0.21 ± 0.04 B**	**0.20 ± 0.02 B**	**0.85 ± 0.25 B**	**1.94 ± 0.29 A**	**2.31E-05**
Total ketones	NA	13.13 ± 2.46	7.83 ± 1.86	5.00 ± 0.70	0.19	**2.25 ± 0.70 B**	**2.43 ± 0.83 B**	**25.82 ± 4.18 A**	**6.93 ± 2.12 B**	**7.24E-06**	**0.98 ± 0.17 B**	**0.55 ± 0.06 B**	**5.58 ± 1.72 AB**	**11.08 ± 3.03 A**	**1.84E-03**
Miscelaneous															
Indole	1331	ND	**10.13 ± 1.70 A**	**4.55 ± 1.35 B**	**0.03**	**1.39 ± 0.32 B**	**4.81 ± 1.05 A**	**ND B**	**2.53 ± 0.88 AB**	**1.44E-03**	**ND B**	**0.02 ± 0.00 A**	**ND B**	**ND B**	**2.52E-06**
Geranyl nitrile	1162	28.15 ± 12.3	**94.62 ± 31.9 A**	**2.95 ± 1.50 B**	**0.02**	26.17 ± 5.67	21.42 ± 6.14	5.18 ± 1.57	20.42 ± 5.82	0.06	1.33 ± 0.94	0.43 ± 0.07	3.61 ± 2.26	3.21 ± 1.04	0.31
2-Methyl-4-pentenal	847	3.98 ± 0.96	2.42 ± 0.45	1.61 ± 0.56	0.29	**1.00 ± 0.08 B**	**0.61 ± 0.07 B**	**17.85 ± 4.56 A**	**0.91 ± 0.20 B**	**1.02E-04**	**ND B**	**ND B**	**0.85 ± 0.27 A**	**ND B**	**7.47E-04**
(3E)-4-Ethyl-3-nonen-5-yne	1159	2.60 ± 0.41	0.79 ± 0.13	1.17 ± 0.30	0.28	0.50 ± 0.20	1.03 ± 0.11	2.44 ± 1.02	1.86 ± 0.91	0.24	**0.38 ± 0.04 B**	**0.61 ± 0.04 B**	**1.20 ± 0.16 AB**	**1.93 ± 0.38 A**	**3.22E-04**
trans-(.+-.)-2-methyl-2-(4-methyl-3-	1267	3.05 ± 1.05	**1.85 ± 0.41 A**	**0.45 ± 0.13 B**	**0.01**	**0.25 ± 0.09 B**	**ND B**	**2.09 ± 0.18 A**	**0.58 ± 0.33 B**	**3.94E-06**	**0.04 ± 0.01 A**	**ND A**	**0.17 ± 0.02 A**	**1.39 ± 0.70 A**	**0.04**

		T0	T1			T2				T3				
Compound	LRI	Control	Control	JAsp	p-value	Control	JAsp	Psyll	JAsp_Psyll	p-value	Control	JAsp	Psyll	JAsp_Psyll	p-value
pentenyl)cyclopropanecarboxaldehyde															
3,3,7,11-tetramethyl-tricyclo[6.3.0.0(2,4)]undec-8-ene	1439	0.11 ± 0.02	**ND B**	**0.06 ± 0.01 A**	**2.09E-03**	**0.05 ± 0.01 B**	**0.05 ± 0.01 B**	**ND C**	**0.10 ± 0.02 A**	**5.30E-05**	**0.05 ± 0.01 B**	**0.09 ± 0.01 A**	**0.04 ± 0.01 B**	**ND C**	**2.62E-06**
4-methylene-2,8,8-trimethyl-2-vinyl-bicyclo[5.2.0]nonane	1462	ND	ND	ND	NA	**0.07 ± 0.01 B**	**0.10 ± 0.01 AB**	**ND B**	**0.20 ± 0.06 A**	**3.17E-03**	**0.04 ± 0.01 B**	**0.06 ± 0.01 A**	**ND C**	**ND C**	**3.98E-08**
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	1479	1.99 ± 0.52	1.51 ± 0.58	1.50 ± 0.73	1.00	1.54 ± 0.23	1.94 ± 0.33	0.76 ± 0.14	1.81 ± 0.65	0.18	0.49 ± 0.08	0.89 ± 0.20	0.40 ± 0.07	0.46 ± 0.10	0.05
Phenol	1022	2.88 ± 0.08	**1.65 ± 0.16 A**	**ND B**	**7.43E-06**	**ND B**	**ND B**	**12.20 ± 3.00 A**	**ND B**	**3.72E-05**	**ND B**	**ND B**	**1.61 ± 0.51 AB**	**3.48 ± 1.42 A**	**0.01**
Total miscelaneous	NA	42.75 ± 13.9	**113.0 ± 33.1 A**	**12.29 ± 3.80 B**	**0.02**	30.97 ± 6.23	29.97 ± 7.37	40.53 ± 7.28	28.42 ± 7.71	0.64	**2.33 ± 0.88 BC**	**2.11 ± 0.32 C**	**7.89 ± 1.96 AB**	**10.47 ± 1.82 A**	**1.26E-03**
Total VOC	NA	5076 ± 903	3246 ± 566	1983 ± 627	0.17	**1589 ± 324 B**	**1948 ± 302 AB**	**4439 ± 1132 A**	**2078 ± 411 AB**	**0.02**	**548.1 ± 46.3 B**	**770.5 ± 102 B**	**1119 ± 80.1 AB**	**1932 ± 491 A**	**7.69E-03**

Table 6.2. Volatile profile and time effect comparisons of lemon plants measured before spray (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control- plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*; JAsp- plants sprayed with JA and not infested; Psyll- plants not sprayed with JA and infested; JA_Psyll- plants sprayed with JA and infested. The results are expressed as nanograms per cm^2 leaf area (mean ±standard error, n =5). LRI =Linear Retention Index. ND = Not detected. NA = Not applicable. Comparisons with significant differences (p-value< 0.05) are highlighted in bold. One-way ANOVA was employed, followed by the Tukey's post-hoc multi-comparison test. When only two groups were compared, a Student's *t*-test was applied.

	Control					JAsp				Psyll			JAsp_Psyll		
Compound Family	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
Monoterpenes															
Monoterpene hydrocarbons	**3809 ± 694 a**	**2475 ± 418 ab**	**1165 ± 235 bc**	**339.9 ± 39.4 c**	**1.49E-04**	1517 ± 503	1466 ± 249	469.9 ± 68.0	0.07	**3700 ± 936 a**	**747.0 ± 74.3 b**	**0.04**	1371 ± 336	1291 ± 379	0.91
Monoterpene alcohols	**163.1 ± 35.4 a**	**93.47 ± 20.0 ab**	**50.90 ± 8.89 b**	**15.11 ± 2.40 b**	**8.46E-04**	54.51 ± 17.6	61.91 ± 9.59	35.92 ± 5.00	0.32	94.12 ± 28.8	33.85 ± 3.58	0.12	69.38 ± 16.1	41.91 ± 4.19	0.18
Monoterpene aldehydes	**596.1 ± 101 a**	**163.0 ± 39.5 b**	**118.7 ± 34.6 b**	**132.7 ± 24.7 b**	**5.34E-05**	186.7 ± 59.8	209.4 ± 26.3	165.8 ± 22.1	0.75	268.1 ± 118	205.3 ± 56.5	0.72	349.7 ± 110	453.1 ± 131	0.33
Monoterpene esters	**35.11 ± 8.47 a**	**13.76 ± 5.37 ab**	**11.33 ± 3.56 b**	**10.05 ± 1.25 b**	**0.01**	15.83 ± 6.42	22.47 ± 3.98	13.74 ± 1.32	0.38	12.07 ± 5.10	6.55 ± 1.79	0.43	16.88 ± 5.26	18.06 ± 4.05	0.56
Monoterpene epoxides	**7.85 ± 1.28 a**	**2.43 ± 0.52 b**	**1.54 ± 0.45 b**	**1.37 ± 0.21 b**	**2.65E-05**	2.57 ± 0.76	3.00 ± 0.42	2.19 ± 0.27	0.57	3.78 ± 1.44	2.43 ± 0.51	0.50	5.22 ± 1.35	6.47 ± 1.20	0.41
Monoterpene ketones	**0.73 ± 0.14 a**	**0.51 ± 0.16 ab**	**0.21 ± 0.08 b**	**0.05 ± 0.01 b**	**3.00E-03**	**NA b**	**0.17 ± 0.04 a**	**0.07 ± 0.02 ab**	**4.03E-03**	**5.05 ± 1.19 a**	**0.36 ± 0.15 b**	**0.01**	0.11 ± 0.01	0.19 ± 0.04	0.06
Total monoterpenes	**4612 ± 828 a**	**2748 ± 481 ab**	**1348 ± 282 bc**	**499.1 ± 41.7 c**	**1.50E-04**	1777 ± 585	1763 ± 281	687.6 ± 86.8	0.11	4083 ± 1087	995.4 ± 78.7	0.05	1812 ± 354	1811 ± 493	1.00
Sesquiterpenes															
Sesquiterpene hydrocarbons	197.3 ± 46.0	146.7 ± 56.4	136.8 ± 25.5	41.85 ± 5.38	0.07	97.70 ± 46.4	124.5 ± 14.7	73.56 ± 16.0	0.50	**120.2 ± 23.6 a**	**48.77 ± 7.73 b**	**0.05**	173.4 ± 53.3	52.23 ± 7.74	0.08
Sesquiterpene alcohols	**0.80 ± 0.09 b**	**1.50 ± 0.30 a**	**0.26 ± 0.07 bc**	**0.08 ± 0.01 c**	**4.71E-05**	**0.20 ± 0.01 ab**	**0.23 ± 0.03 a**	**0.12 ± 0.02 b**	**0.01**	0.22 ± 0.03	0.51 ± 0.15	0.09	0.15 ± 0.04	0.21 ± 0.09	0.62
Other sesquiterpenes	**3.17 ± 0.47 a**	**1.08 ± 0.48 b**	**1.57 ± 0.38 b**	**0.33 ± 0.04 b**	**7.06E-04**	**0.65 ± 0.18 ab**	**1.22 ± 0.15 a**	**0.64 ± 0.12 b**	**0.03**	0.59 ± 0.09	0.77 ± 0.15	0.12	2.25 ± 0.51	0.77 ± 0.29	0.10
Total sesquiterpenes	201.3 ± 46.5	149.3 ± 56.8	138.6 ± 25.9	42.25 ± 5.44	0.07	98.56 ± 46.6	125.96 ± 14.8	74.31 ± 16.1	0.49	**121.0 ± 23.7 a**	**50.04 ± 7.79 b**	**0.05**	175.8 ± 53.8	53.20 ± 7.46	0.08
Acetates	**74.86 ± 9.65 a**	**67.37 ± 4.13 a**	**34.72 ± 7.05 b**	**0.04 ± 0.01 c**	**9.73E-07**	**25.65 ± 4.92 a**	**4.18 ± 0.76 b**	**0.65 ± 0.17 b**	**9.44E-05**	**33.20 ± 5.19 a**	**16.52 ± 3.32 b**	**4.11E-03**	**1.87 ± 0.99 b**	**6.38 ± 1.16 a**	**0.04**
Alcohols	**54.65 ± 11.1 a**	**64.54 ± 9.54 a**	**14.44 ± 3.67 b**	**1.10 ± 0.06 b**	**3.70E-05**	**18.04 ± 1.85 a**	**8.48 ± 1.38 b**	**2.32 ± 0.57 c**	**1.29E-05**	**66.97 ± 21.1 a**	**25.62 ± 7.98 b**	**0.04**	21.16 ± 7.79	21.82 ± 6.66	0.94
Aldehydes	**12.62 ± 1.87 a**	**10.76 ± 2.80 a**	**2.77 ± 0.83 b**	**0.29 ± 0.03 b**	**2.31E-04**	**3.38 ± 0.67 a**	**2.88 ± 0.78 a**	**0.24 ± 0.03 b**	**6.11E-03**	**22.16 ± 5.09 a**	**3.34 ± 0.83 b**	**0.01**	3.89 ± 1.55	5.09 ± 0.74	0.52
Alkanes	**17.08 ± 2.13 a**	**17.41 ± 4.70 a**	**4.46 ± 1.37 b**	**0.85 ± 0.13 b**	**5.25E-04**	**6.49 ± 1.16 a**	**4.62 ± 0.88 a**	**0.78 ± 0.10 b**	**1.38E-03**	**20.17 ± 4.12 a**	**5.17 ± 1.03 b**	**0.02**	7.73 ± 3.20	8.17 ± 1.40	0.92
Benzene derivatives	**0.56 ± 0.10 ab**	**0.18 ± 0.02 b**	**0.12 ± 0.05 b**	**0.91 ± 0.19 a**	**3.79E-04**	**19.85 ± 7.93 a**	**0.72 ± 0.11 b**	**1.29 ± 0.20 b**	**0.02**	**6.10 ± 1.08 a**	**3.27 ± 0.51 b**	**0.02**	1.49 ± 0.78	2.18 ± 0.83	0.63
Esters	**46.79 ± 4.16 b**	**66.98 ± 7.63 a**	**13.66 ± 2.99 c**	**0.22 ± 0.03 c**	**5.91E-08**	**17.53 ± 2.09 a**	**5.56 ± 0.57 b**	**0.60 ± 0.14 c**	**1.89E-06**	**19.56 ± 4.84 a**	**6.44 ± 1.76 b**	**0.02**	18.55 ± 9.69	2.31 ± 0.62	0.17
Ketones	**13.13 ± 2.46 a**	**7.83 ± 1.86 ab**	**2.25 ± 0.70 bc**	**0.98 ± 0.17 c**	**1.90E-04**	**5.00 ± 0.70 a**	**2.43 ± 0.83 b**	**0.55 ± 0.06 b**	**1.15E-03**	**25.82 ± 4.18 a**	**5.58 ± 1.72 b**	**5.45E-03**	6.93 ± 2.12	11.08 ± 3.03	0.22
Miscelaneous	**42.75 ± 13.9 ab**	**113.0 ± 33.1 a**	**30.97 ± 6.23 b**	**2.33 ± 0.88 b**	**4.00E-03**	**12.29 ± 3.80 ab**	**29.97 ± 7.37 a**	**2.11 ± 0.32 b**	**4.69E-03**	**40.53 ± 7.28 a**	**7.89 ± 1.96 b**	**9.49E-03**	28.42 ± 7.71	10.47 ± 1.82	0.10
Total VOC	**5076 ± 903 a**	**3246 ± 566 ab**	**1589 ± 324 bc**	**548.1 ± 46.3 c**	**1.71E-04**	1983 ± 627	1948 ± 302	770.5 ± 102	0.09	**4439 ± 1132 a**	**1119 ± 80.1 b**	**0.05**	2078 ± 411	1932 ± 491	0.86

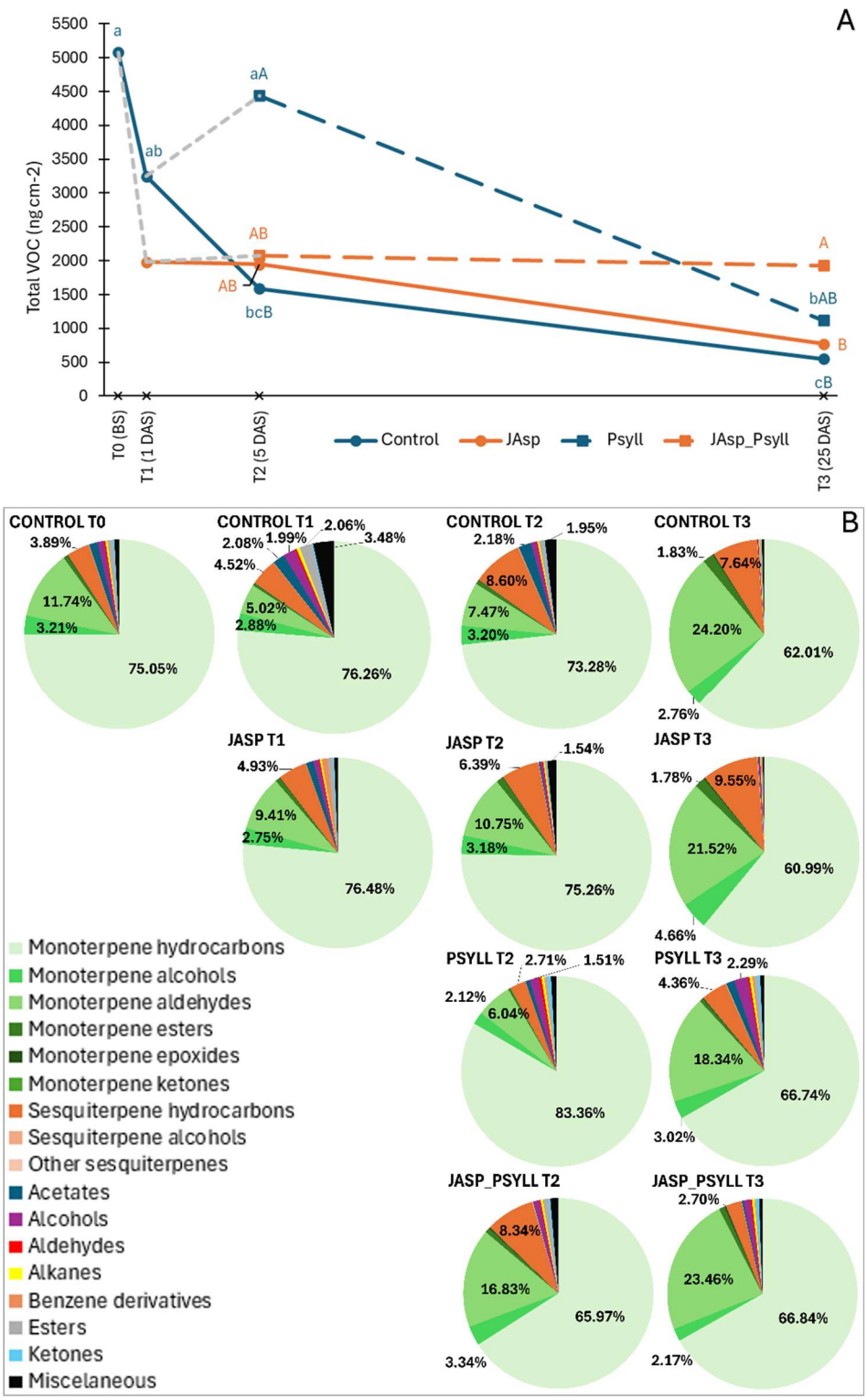

Figure 6.4. Plants' total VOCs emission and its profile. measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control- plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*; JAsp- plants sprayed with JA and not infested; Psyll- plants not sprayed with JA and infested; JA_Psyll- plants sprayed with JA and infested. **A:** Plants total VOCs emission. Orange coloured lines represent plants treated with exogenous JA; blue coloured lines represent plants not treated with exogenous JA. Full lines with circular points represent plants that were not infested. Dashed lines with square points represent plants infested with *Trioza erytreae*. Gray dashed lines represent the theoretical evolution of the treatments after spray and infestation if

the same start point was assumed. Lowercase letters represent the comparison within the groups and along the different time points (effect of time). Uppercase letters represent the comparison between the groups within the specific time point (treatment effect). When there is no common letter between points (compare letters within the same case type only), they are significantly different, at a Bonferroni adjusted $p < 0.05$. **B:** Pie charts for each VOC measurement group and timing, each slice represents a specific compound family described on the legend in the bottom left. The percentage values lower than 1.5% were not displayed in the figure.

6.5.3.1. Time effects on lemon plants' VOCs

In control plants, the majority of the VOCs families exhibited a tendency to decrease through time, being more pronounced from T0 to T2 (Fig. 6.4A and Table 6.2). Furthermore, within this group, only the monoterpene ketones and the benzene derivatives compound families did not differ significantly from T0 to T3 (Table 6.2). Within the control plants, the VOCs emissions in T2 and T3 are not separated by any of the principal axes in the PCA analysis. However, a clear separation was observed between T3 and T1 along the first principal component axis, which accounted for 51% of the variability in this comparison. Furthermore, T3 is separated from T0 along the first and second principal component axes that capture 73.2% variability for this comparison (Fig. 6.5). As the time frame increases, there is a concomitant rise in the differences in VOCs concentrations.

In the case of JAsp plants, a clear separation was observed between T3 and T1 along the first principal component axis, and between T3 and T2 along the second principal component axis. The first and second axis accounted for 44.1% and 31% variability for this comparison, respectively (Fig. 6.5). Consequently, in this treatment group, as in the control group, an increase in the differences in VOC concentrations was observed as the time frame increased.

In both the infested treatments, the T2 and T3 groups separated along the first principal component axis, which captured 67.8% and 46.4% variability within the Psyll and JAsp_Psyll comparison, respectively.

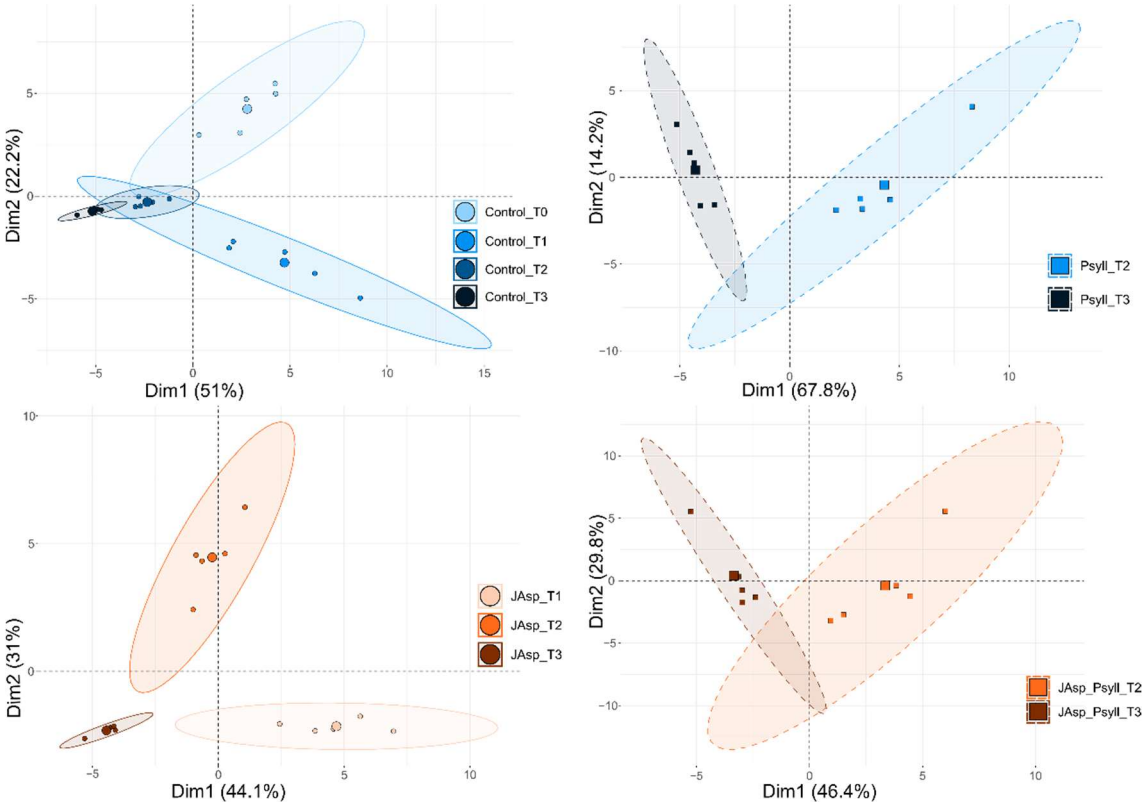

Figure 6.5. Principal component analysis (PCA) on the most influential VOCs data of lemon plants measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control represents plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*, JAsp represents plants sprayed with JA and not infested, Psyll represents plants not sprayed with JA and infested, JA_Psyll represent plants sprayed with JA and infested. The data is presented in the form of a two-dimensional plot, with biological replicates represented by circles (non-infested samples) and squares (infested samples). The mean value of each replicate group is represented by the largest circle or square. The orange colour represents the plants sprayed with JA. The blue colour represents the plants that were not sprayed with JA. The lighter colours represent the earlier timings, and the darker colours represent the later timings. The percentages on the axes show their contribution to explain variance.

The passage of time and plant development exerted an influence on the plants' VOCs emission in all of the treatments, albeit with slight differences between them. Ester levels were reduced with time in all groups, and this decreasing tendency was observed, but was not significantly different in JAsp_Psyll and in control plants from T2 to T3 (Table 6.2). Acetates demonstrated a significant decrease in all treatment groups, with the exception of the JAsp_Psyll group, where there was a significant increase in T3 (Table 6.2), highlighted by the (Z)-3-hexenol acetate (Table S6.2 - Appendix). From T2 to T3 the quantity of VOC remained relatively stable in all groups, with the exception of the Psyll group, which exhibited a marked decrease in the VOC levels across the majority of compound families (Fig.

6.4A and Table 6.2). The alcohols, aldehydes and alkanes decreased from T2 to T3 in both the Psyll and the JAsp treatment groups (Table 6.2).

6.5.3.2. Exogenous JA spray short-term effects on lemon plants VOC

Exogenous JA spray exhibited a clear short-term effect on the plant VOC profile [Control *vs* JAsp comparison in 1 DAS (T1)] (Fig. 6.6 and Table 6.1). Furthermore, the PCA analysis shows a clear separation between the two groups along the first principal component axis, which accounts for 66.2% of the variability in this comparison (Fig. 6.6). In relation to control plants, the JAsp plants exhibited reduced levels of monoterpene ketones, sesquiterpene alcohols, acetates, alcohols, aldehydes and esters. Of particular interest was the sesquiterpene alcohol cis-nerolidol that was exclusively detected in control plants (Fig. 6.7 and Table 6.1). Furthermore, we highlight the higher levels of methyl salicylate in control plants. In addition, a higher level of benzene derivatives was observed in plants from the JAsp group, particularly m-xylene. Additionally, the sesquiterpene caryophyllene oxide was only detected in JAsp plants (Fig. 6.7 and Table 6.1).

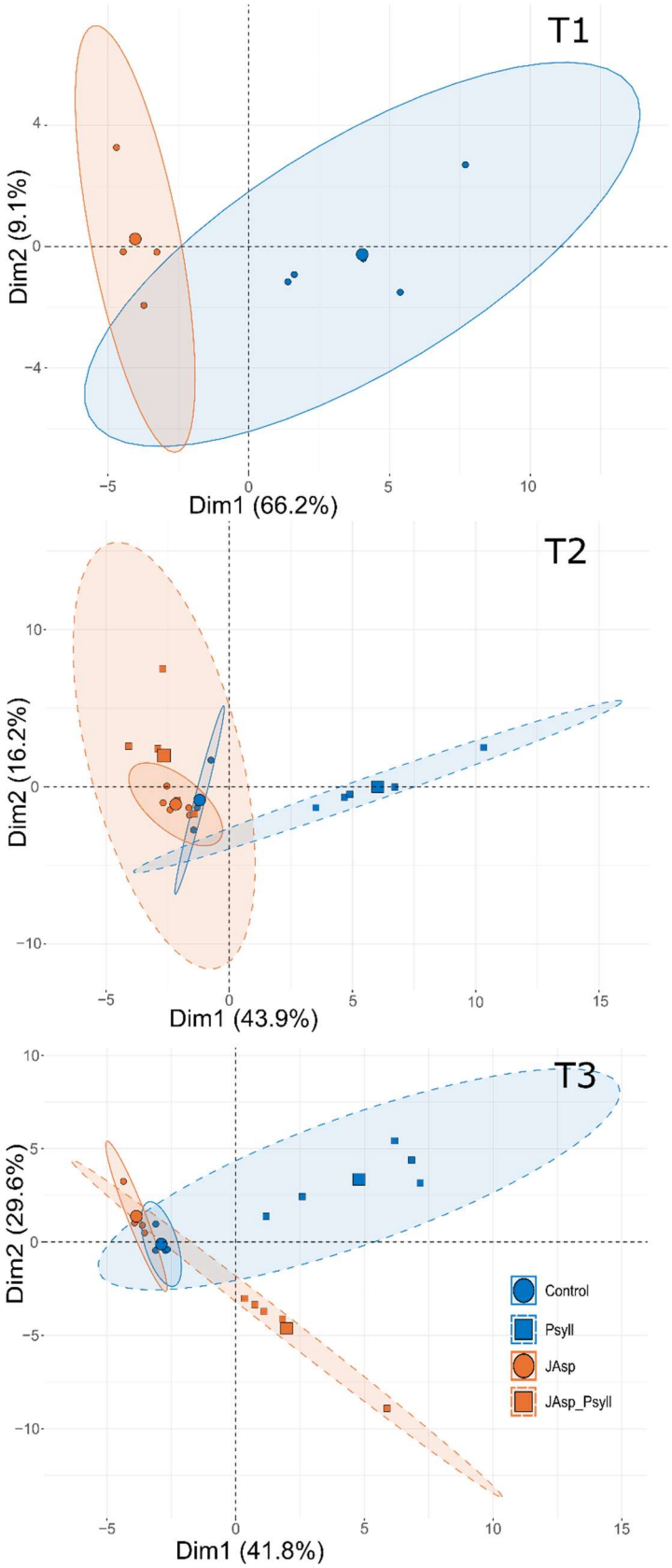

Figure 6.6. Principal component analysis (PCA) on the most influential VOCs data of lemon plants measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control represents plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*, JAsp represents plants sprayed with JA and not infested, Psyll represents plants not sprayed with JA and infested, JA_Psyll represent plants sprayed with JA and infested. The data is presented in the form

of a two-dimensional plot, with biological replicates represented by circles (non-infested samples) and squares (infested samples). The mean value of each replicate group is represented by the largest circle or square. The orange colour represents the plants sprayed with JA. The blue colour represents the plants that were not sprayed with JA. The lighter colours represent the earlier timings, and the darker colours represent the later timings. The percentages on the axes show their contribution to explain variance.

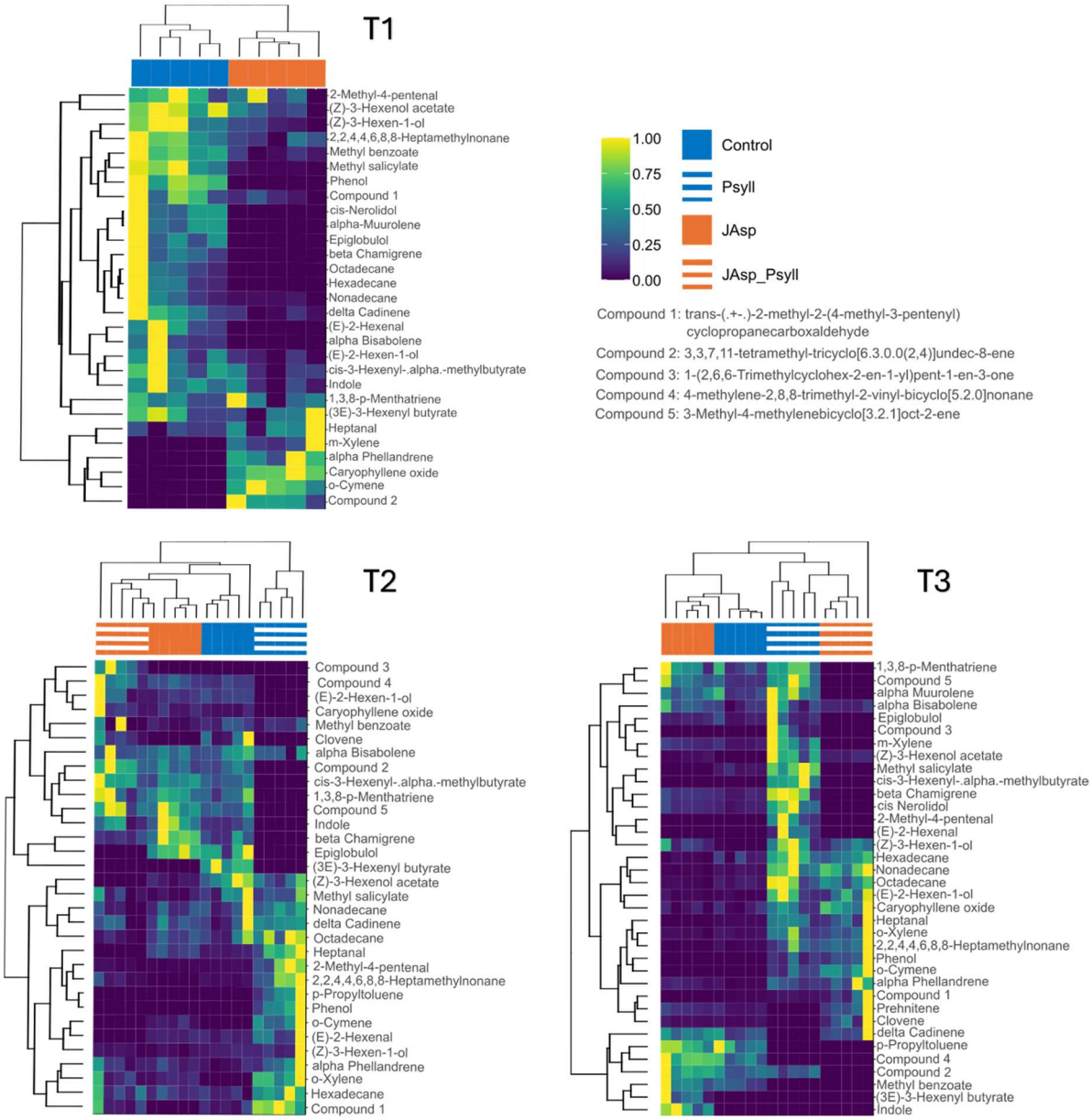

Figure 6.7. Heatmaps on the most influential VOCs data of lemon plants measured one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control represents plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*, JAsp represents plants sprayed with JA and not infested, Psyll represents plants not sprayed with JA and infested, JA_Psyll represent plants sprayed with JA and infested. The relative content heat map of volatile organic compounds (VOCs) the colour bar indicates the relative contents of VOCs. The brighter the colour, the higher the relative content.

6.5.3.3. Exogenous JA spray, *Trioza erytreae* adult feeding and oviposition effects on lemon plants VOCs

Five DAS (T2), the three non-control treatments affected plant VOCs emission. The impact *T. erytreae* adult feeding and oviposition on plants' VOCs was found to exceed that of the isolated JA spray treatment. The JA spray mainly hindered plants' acetate emission, as evidenced by the observation that both treatments devoid of JA spray (Control and Psyll plants) exhibited a higher quantity of acetate VOCs, in particular the (Z)-3-hexenol acetate (Fig. 6.7 and Table 6.1). In addition, the PCA analysis revealed that the VOCs adjustments in infested plants without JA spray treatment (Psyll) were the most pronounced, as evidenced by the clear separation of this group from the other three along the first principal component axis, which captured 43.9% of the variability (Fig. 6.6). In relation to control plants the total VOCs and the majority of the compound families were found to be higher on Psyll plants (Fig. 6.4A and Table 6.1).

The greater VOCs adjustment exhibited by the Psyll plants encompassed an elevated level of alcohols, aldehydes, alkanes, benzene derivatives, monoterpene hydrocarbons, monoterpene ketones and other ketones, in comparison to the other groups (Table 6.1). The monoterpene ketone - (-) carvone, was only detected in the Psyll treatment, and the ketone 6-methyl-5-hepten-2-one was significantly higher in this group. The benzene derivate o-xylene exhibited higher levels in the Psyll plants, in fact, this compound was not detected in control plants. Notably, phenol was only detected in the Psyll plants (Fig. 6.7 and Table 6.1).

The exogenous JA spray interfered with the plants VOC adjustment to *T. erytreae* adult feeding and oviposition, as evidenced by the higher levels of compounds of the “other sesquiterpenes” family in plants that were treated with JA and infested (JA_Psyll) in comparison to plants that were only infested (Psyll). In particular, caryophyllene oxide was not detected in Psyll plants (Fig. 6.7 and Table 6.1). Additionally, the monoterpene alcohol carveol was found to in higher abundance in JAsp_Psyll plants 5 DAS (T2) in relation to the non-infested treatment groups (Table 6.1).

6.5.3.4. Exogenous JA spray, *Trioza erytreae* nymphal feeding and development effects on lemon plants VOCs

All experimental treatments affected plant VOCs emission after 25 DAS (T3). However, the JA spray treatment (JAsp) with no *T. erytreae* nymphal feeding and development only showed significant differences in the monoterpene alcohol compound family when compared with control. This compound family group had lower levels in control plants in relation to all groups, in particular cis and trans Geraniol (Table 6.1). In contrast the *T. erytreae* nymphal feeding and development showed a high impact on plants VOCs, with the PCA analysis clearly separating both infested groups (Psyll and JAsp_Psyll) groups from the non-infested groups along the first principal component axis, which accounted for 41.8% of the variability for this comparison (Fig. 6.6). The infested groups exhibited higher levels of aldehydes and alkanes than the non-infested groups (Control and JAsp) (Table 6.1).

At 25 DAS the treatment with the greatest impact on the plants VOCs was the one involving both infestation and JA exogenous application (JAsp_Psyll). The PCA analysis revealed a clear separation between the JAsp and Psyll groups by the second principal component axis, which accounted for 29.6% of the variability for this comparison. Additionally, a distinction was observed between this group and the non-infested groups by the first principal component axis, as previously mentioned (Fig. 6.6). The JAsp_Psyll group showed higher levels of monoterpene epoxides than the remaining groups (Table 6.1). Furthermore, the JAsp_Psyll plants showed higher levels of total VOCs, monoterpenes, ketones and miscellaneous compounds than the non-infested groups (Control and JAsp). From these, we highlight the higher content of the ketone 6-methyl-5-heptene-2-one and phenol (Fig. 6.7 and Table 6.1). The monoterpene aldehydes were higher in JAsp_psyll in relation to control, namely citronellal. The exogenous JA spray had an effect on the plant's long-term VOCs response to *T. erytreae* nymphal feeding and development, as the JAsp_Psyll plants showed higher levels of monoterpene esters in relation to the plants that were only infested (Psyll), namely geranyl acetate (Table 6.1). The plants that were infested but not treated with exogenous JA (Psyll) exhibited higher levels of esters and acetates in relation to all the other treatments, from which we

highlight the acetate (Z)-3-hexenol acetate. Additionally, the sesquiterpene alcohol cis-nerolidol showed higher levels than the other groups, and it was not detected in the JAsp_Psyll group (Fig. 6.7 and Table 6.1). The Psyll treatment also exhibited higher levels of alcohols than the non-infested groups (Control and JAsp), namely (E)-2-hexen-1-ol. In relation to control plants the Psyll plants showed higher levels of benzene derivatives and monoterpene ketones, namely m-xylene and 2-oxo-1,8-cineole (Fig. 6.7 and Table 6.1).

6.6. Discussion

The exogenous JA spray treatment hindered *T. erytreae* infestation, greatly decreasing oviposition on lemon plants. The egg success ratio was also lowered, and a significantly lower number of successfully developed nymphs in JA sprayed infested plants (JAsp_Psyll) in comparison to only infested plants (Psyll) (Fig. 6.2). Exogenous JA spray was also observed to cause an alteration of *D. citri* infestation behaviour on 'Valencia' sweet orange, and reduced oviposition and nymphal development on mandarin plants (Patt et al., 2018; Rao et al., 2018a). In the present study *T. erytreae* adults contacted the plant at 1 DAS (T1) and proceeded with oviposition until they were removed 5 DAS (T2). The higher impact of the JA spray on the oviposition suggests that the JA spray effect on the plant endogenous JA and VOC profile at 1 DAS (T1) and of the four treatments at 5 DAS (T2) is of critical importance. The long-term effects of 25 DAS (T3) of all the treatments will help better understand the egg success ratio and the lower number of successfully hatched nymphs on JA-sprayed lemon plants.

6.6.1. Lemon plants general VOCs profile

In this study the majority of identified VOCs were mono and sesquiterpenes, which is in line with the fact that terpenes are one of the largest classes of plant secondary metabolites with many volatile representatives (Dudareva et al., 2004). In another HS-SPME-GC-MS study, limonene was shown to be the most represented volatile in 'Eureka' lemon and the monoterpene aldehyde citronellal percentage increased in mature leaves compared to young leaves (Azam et al., 2013). Both these observations are concordant with the present study, as D-limonene was the

major volatile compound and a general increase in percentage of monoterpene aldehydes was observed from T2 to T3.

The general decrease of total VOCs emissions in more mature leaves, as observed in this study, has been described in other citrus plants and poplar (*Populus tremula* L.) plants (Antwi-Agyakwa et al., 2019; Portillo-Estrada et al., 2017). This decrease may be associated with the reduction in the quantity of essential oil droplets (which contain concentrated forms of VOCs) in the secretory cavities of mature citrus leaves in relation to young citrus leaves (Mejri et al., 2022). The general decrease in VOCs has also been attributed to an evolutionary defence against herbivory, as younger leaves are usually more subjected to infestation and herbivory (Portillo-Estrada et al., 2017). The decrease of (Z)-3-hexenol acetate, a compound involved in plant defence against herbivory, observed in the more developed leaves of the present study, is consistent with this hypothesis (Schuman, 2023).

6.6.2. Exogenous JA spray short-term effect on lemon plants and its effect *Trioza erytreae* infestation

A tendency for higher endogenous JA levels in JAsp plants was observed. Furthermore, the psyllid infestation was hindered by the exogenous application of JA, and the VOCs profile in JAsp plants 1 DAS (T1) showed significant differences compared to control plants. These variations in VOCs may have influenced the psyllids' behaviour and subsequent infestation, as it was on 1 DAS (T1) that the plants were infested by the adult *T. erytreae*.

The sesquiterpene alcohol nerolidol has been shown to induce higher levels of plant endogenous JA (Chen et al., 2020). In the present study, the exogenous JA spray may have rendered this function of cis-nerolidol redundant, which may have led to a lower emission of this VOCs on JAsp plants (Fig. 6.7 and Table 6.1). The JA and salicylic acid (SA) signalling pathways are known to be mutually antagonistic, and methyl salicylate is a known agent of the SA signalling pathway (Kunkel and Brooks, 2002). The findings of the current study suggest that the exogenous JA spray induced of the JA signalling pathway, which in turn may have led to a reduction of methyl salicylate abundance observed in JAsp plants. Furthermore, methyl

salicylate has been shown to be a key attractant of *D. citri* to citrus hosts (Mann et al., 2012; Patt et al., 2018). Consequently, the lower abundance of methyl salicylate JAsp plants may have resulted in a diminished attractiveness of the lemon host for *T. erytreae*, potentially leading to reduced feeding and oviposition activity. The toxicity of certain benzene derivatives, such as m-xylene, has been demonstrated for insects (Adebambo et al., 2020). This compound has been identified in insect repellents and has been shown to affect insect attraction to plant hosts (Lu et al., 2020; Lv et al., 2024). Caryophyllene oxide has been shown to hinder insect activity and have effective insecticidal properties (Plata-Rueda et al., 2018; Yang et al., 2018). The m-xylene and caryophyllene oxide compounds were exclusively detected in the JA sprayed plants observed in this study. The detectable presence of these compounds may have induced a behavioural change in *T. erytreae* adults and contributed to the lower oviposition numbers observed in JA sprayed plants.

6.6.3. Exogenous JA spray, *Trioza erytreae* adult feeding and oviposition effect on lemon plants and their interaction with the psyllid

Five DAS (T2) the plants that were infested without JA spray (Psyll) had higher endogenous JA and VOC levels than the JA sprayed and infested plants (JAsp_Psyll). The exogenous JA application affected *T. erytreae* oviposition behaviour, leading to a lower egg number in the JAsp_Psyll plants than in the Psyll plants. We suggest that the exogenous JA application may have also affected the *T. erytreae* adult feeding behaviour and possibly lowered the number or intensity of probing and feeding in JAsp_Psyll plants relative to the Psyll plants. In a separate study, the exogenous application of JA resulted in a decrease in *D. citri* offspring, accompanied by a reduction in their probing and effective feeding time (Gao et al., 2023). If a similar effect took place in the current study, the lower activity of the *T. erytreae* adults could account for the lower content of endogenous JA and total VOC in the JAsp_Psyll in relation to the Psyll plants 5 DAS (T2). This hypothesis should be further investigated by implementing an electrical penetration graph (EPG) study, applying the same spray solutions.

At 5 DAS (T2), xylene (the previously discussed m-xylene or the isomer o-xylene) was only detected in defence induced plants (excluding the control plants). As discussed in the previous section xylene is known to affect insect attraction (Chen et al., 2017c; Lv et al., 2024). Our results suggest that xylene is activated in the lemon plant defence response and may have a behavioural impact on *T. erytreae*. In the current work, the previously mentioned and defence related (Z)-3-Hexenol acetate showed lower levels in exogenous JA treated plants (JAsp and JAsp_Psyll) in relation to plants without JA spray (Control and Psyll) (Fig. 6.7 and Table 6.1). In contrast, this compound emission has been shown to increase after applying JA to plants (Luo et al., 2023; Patt et al., 2018).

The monoterpene ketone - (-) carvone has been shown to affect oviposition in insects, namely a short-term effect of reducing oviposition but a long-term effect of increasing oviposition substantially, 1.5 to 1.9 times more in relation to insects not exposed to carvone (den Ouden et al., 1993). The compound 6-methyl-5-hepten-2-one was found to be induced by sap feeding aphids on eggplants (*Solanum melongena* L.) and was then shown to increase oviposition numbers of an aphid predator (Higashida et al., 2022). Carvone was only detected on Psyll plants at 5 DAS (T2) and 6-methyl-5-hepten-2-one content was higher in this group. Both of these compounds seem to have been induced by the feeding and oviposition activity of *T.erytreae* and could have promoted psyllid oviposition. This should be further investigated, by exposing *T. erytreae* adults to both compounds and observing the psyllid oviposition behaviour. The free form of phenol has been identified in different organs of plants including the leaves (Coen et al., 1995; Sheu et al., 2001). Phenolic compounds, comprising simple phenols, have a crucial role in plant response to herbivory (Luo et al., 2023). In the current study at 5 DAS (T2) phenol was exclusively identified in the Psyll plants, which could indicate an increase in phenolic compounds of lemon in response to *T. erytreae* adult feeding and oviposition.

A higher VOCs adjustment at 5 DAS (T2) was observed on Psyll plants, nevertheless the JA spray also affected the lemon plant VOC response to the psyllids and may help explain the differences in *T. erytreae* behaviour. For example,

caryophyllene oxide, discussed in the previous section, was not detected in Psyll plants at 5 DAS (T2), showing a higher content in JAsp_Psyll plants (Fig. 6.7 and Table 6.1). The insecticidal properties and the effect of lowering insect activity of caryophyllene oxide (Plata-Rueda et al., 2018; Yang et al., 2018) may be part of the reason for the lower oviposition numbers observed in JAsp_Psyll plants in relation to Psyll plants. The monoterpene alcohol carveol has shown to have a repelling effect on sap feeders and to attract pest predators (Ling et al., 2023). Carveol was higher in JAsp_Psyll plants in comparison to all the other treatment groups. This suggests that the JA spray priming and the *T. erytreae* feeding and oviposition have increased carveol emission, and it could have a repelling effect on the psyllid.

6.6.4. Exogenous JA spray, *Trioza erytreae* nymphal feeding and development effects on the lemon plants and their interaction with the psyllid

At 25 DAS (T3), the long-term isolated effect of JA spray priming and the isolated effect of long-term *T. erytreae* nymphal feeding and development did not significantly affect plants' endogenous JA levels nor the total VOCs emission in lemon plants. However, the conjunction of both effects, increased the plants' endogenous JA concentrations and total VOCs (Fig. 6.3 and Fig .6.4A). Interestingly the plants that were only infested (Psyll) showed a significant decrease in endogenous JA concentrations from 5 DAS (T2) to 25 DAS (T3), in contrast to the JA sprayed and infested plants (JAsp_Psyll), were this decrease was not observed. The JA signalling pathway is known to induce JA biosynthesis in a form of positive feedback loop (Ruan et al., 2019). The JA spray could have initiated this loop, and the continuous nymphal feeding may have perpetuated it maintaining the endogenous JA and total VOC levels higher than in the other groups.

Control and JAsp plants showed similar endogenous JA and VOCs compound levels at 25 DAS (T3) (Fig. 6.3 and Table 6.1), which indicates a loss of the primed state. However, there are differences between these two groups. Namely geraniol, known to have insecticidal and insect repellent effects, was higher in JAsp plants. JA spray has shown to increase geraniol levels in other plants (Chen and Viljoen, 2022; Khetsha et al., 2024). This suggests that in our experiment even after

25 days after the JA spray the priming effect is low, however, there are still some remnants of induced defence in lemon plants. At 25 DAS (T3) the JAsp_Psyll plants showed higher content of 6-methyl-5-hepten-2-one and phenol than the non-infested groups. Likewise, citronellal and geranyl acetate were higher in JAsp_Psyll plants than in control and Psyll plants, respectively. This indicates a heightened defence status in the JAsp_Psyll plants, as the 6-methyl-5-hepten-2-one acts as an insect pheromone known to attract natural predators and parasitoids (Higashida et al., 2022; Müller and Buchbauer, 2011). Phenol, as discussed in the previous section, is related with the phenolic compounds, crucial for the response against herbivory (Luo et al., 2023). Citronellal has been observed to increase in eureka lemon plants and has shown to have fungicidal and fungistatic properties (Azam et al., 2013; Yamasaki et al., 2007). Geranyl acetate was found to be correlated with citrus hosts that are more tolerant to HLB and has shown to have antibacterial properties (Dorman and Deans, 2000; Hijaz et al., 2016b). Methyl salicylate was not detected in any of the JA sprayed plants (JAsp and JAsp_Psyll) at 25 DAS. Suggesting an inhibition of methyl salicylate as a long-term effect of JA spray, and possibly an inhibition of the SA signalling pathway, which acts antagonistically from the JA signalling pathway (Kunkel and Brooks, 2002).

Even though Psyll plants showed a lower VOC adjustment than JAsp psyll plants at 25 DAS (T3), there were still particular defence related responses to be observed. Psyll plants showed higher content of (Z)-3-hexenol acetate and cis-nerolidol than the other plants and a higher content of (E)-2-hexen-1-ol in relation to non-infested plants. (Z)-3-Hexenol acetate is related with plant indirect response to herbivory, by attracting natural enemies, and its highly induced by wounding and insect feeding (Chehab et al., 2010; Schuman, 2023). Nerolidol increases JA content in the plants as discussed before, and it also increases the expression of plant defence genes (Chen et al., 2020). The fact that it was not detected in JAsp_Psyll plants (Fig. 6.7 and Table 6.1) suggests that the JA spray inhibits this specific response to *T. erytreae* nymphal feeding. (E)-2-Hexen-1-ol has shown to induce citrus general defence responses and genes related with the JA signalling pathway (Gomi et al., 2003). As 2-hexen-1-ol content was not statistically different in Psyll

and JAsp_Psyll plants it seems to be only attenuated by the JA spray, while showing a clear trend of being induced by *T. erytreae* nymphal feeding. The nymphal feeding effect and the adult feeding effect on Psyll plant volatiles have similarities, namely the increase in xylene content and in an increase in a monoterpene ketone, in relation to the control plants. M-xylene and the monoterpene ketone 2-oxo-1,8-cineole emission increased in Psyll plants at 25 DAS (T3). The higher levels of xylene in at least one of the non-control treatments throughout our experiment suggest it has an important role in lemon plants' response to psyllids feeding and JA spray, and it should be further investigated. The volatile 2-Oxo-1,8-cineole has shown to have a repelling effect on insects (Joubert et al., 2023), and could therefore be a tentative of the Psyll plants to avoid more *T. erytreae* infestation. This compound should be tested for its effect on the psyllid behaviour.

6.7. Conclusion

The exogenous spray with a solution of 5 mM of methyl jasmonate on lemon plants hindered *T. erytreae* infestation. The psyllid showed higher oviposition on lemon plants with no JA spray, and these plants showed greater endogenous JA and VOC adaptations right after the adult feeding and oviposition. In the long-term plant response, the combination of JA spray and continuous *T. erytreae* nymphal feeding had a higher impact on plant endogenous JA and VOCs. The lower amount, or non-detection, of xylene in control plants in different timings suggests that it is important in lemon plant defence activation by *T. erytreae* and JA spray. Caryophyllene oxide and cis-nerolidol were affected by JA spray in the short-term and adult feeding response. Geranyl acetate and methyl salicylate were affected by JA spray in the nymphal feeding response. Lemon VOCs response to *T. erytreae* infestation is complex, and this study suggests that the compounds 6-methyl-5-hepten-2-one, phenol, (Z)-3-hexenol acetate, 2-hexen-1-ol and carveol are crucial agents of this interaction. The VOCs highlighted in this work should be further investigated for their direct effect on *T. erytreae* behaviour. Moreover, the applicability and economic viability of an exogenous JA spray application for *T. erytreae* control should be tested in field and semi-field conditions.

Chapter 7. General discussion

European citriculture is on high alert due to the impending threat of HLB, a highly impactful citrus disease for which there is currently no viable curative method (Cocuzza et al., 2017; Ellis et al., 2025; Urbaneja-Bernat et al., 2020). There is an urgent need to improve the prevention and management practices for HLB, with a focus on the improvement of vector management and the development of novel vector control methods (Aidoo, 2023; Ayres et al., 2015). This thesis addressed this challenge by studying the interaction of *T. erytreae* with its citrus hosts, from a systems biology perspective. The objective of the thesis was to identify the key proteins, metabolites and/or metabolic pathways that were implicated in the interaction of the psyllid with lemon and orange plants using a proteomics and metabolomics approach.

For this thesis, host selection was informed by a comprehensive analysis of host suitability for *T. erytreae* (see host classifications in Table 2.1). Specifically, lemon plants (*C.* ×*limon*) which produce volatile organic compounds that attract *T. erytreae* (Antwi-Agyakwa et al., 2019) were chosen as hosts due to their high level of suitability. The other host plants were sweet orange plants (*C.* ×*sinensis*), which were chosen due to their good suitability, nonetheless, inferior to that of lemon trees. Both citrus hosts are of considerable significance in the context of global citriculture (FAO, 2025). The host suitability review (Chapter 2) describes that most studies on the host–*T. erytreae* interaction focused on adult preference and oviposition. The present studies focused on the plant–insect interaction when the psyllid was in the latter stages of nymphal development (fourth and fifth instars). Nymphs are characterised by a sedentary behaviour with high feeding activity (Van den Berg et al., 1991d), which informs on host responses subjected to a continuous and intense infestation.

With regard to the findings of this thesis, in the first trial conducted under its scope, the infestation by *T. erytreae* was observed in both lemon and sweet orange plants. The number of nymphs that developed on the sweet orange plants (the less suitable host) was found to be three times lower than that observed in the lemon plants.

The proteomic approach to compare the enriched vascular sap proteomes of infested and non-infested lemon and sweet orange plants revealed a significant modification of the proteome in the sweet orange plants upon infestation. This modification included the downregulation of pathways related to photosynthesis and the upregulation of pathways related to respiration. This is a common plant stress response, also observed in plants responding to herbivory (Coppola et al., 2013; Kerchev et al., 2012; Mozoruk et al., 2006). The hypersensitive response and the JA signalling pathway were found to be upregulated in the proteome of infested sweet orange plants. The latter pathway has also been observed to be induced in sweet orange plants in response to *D. citri* (Nehela et al., 2018). In contrast, the proteome modifications in lemon plants as a consequence of infestation were much less pronounced.

Several host responses were identified in both the proteomic and metabolomic analyses of sweet orange plants that had been infested with *T. erytreae*. A broader metabolic adjustment of this host plant was observed, including a resistance-type response. The metabolite JA was found to be upregulated, a finding that corresponded to the proteomic results. In contrast, modifications in the lemon plants' metabolome and proteome due to infestation exhibited minimal overlap. The metabolomic results in the lemon plants indicated a tolerance-type response, with the upregulation of defence-related phenylpropanoids. The phenylpropanoids are known to have an important role in plant response to herbivory (Li et al., 2016; Mathesius, 2018). The findings of the integrated metabolomic and proteomic analysis underscore the importance of examining the diverse layers of plant response in order to better understand the plant–insect interaction, in concordance with Barah and Bones (2015).

With respect to the proteomic approach that was employed on fourth and fifth instar nymphs that developed on lemon plants, the results revealed an increase in energy metabolism and translation factors. This finding was associated with the identification of growth and developmental pathways. The energy metabolism has also been observed to be induced in *D. citri* developing in highly suitable hosts (Ramsey et al., 2022). In contrast, nymphs developing in sweet orange exhibited the

upregulation of proteins associated with semi-sterile, abnormal development and behaviour. The effect of a shift of *T. erytreae* nymphs to a sucrose-only diet was also analysed, as insect development and fertility have been shown to decrease in less suitable diets (Chen et al., 2017b; Wang et al., 2013). The proteomes of nymphs that developed on lemon plants exhibited greater proteome adjustment in response to the transition to a sucrose-only diet than the nymphs developing on sweet orange plants. The present findings suggest that the diet of the lemon plant may offer a more effective means of supporting the development of *T. erytreae*.

The findings of the metabolomic and proteomic analysis prompted the formulation of the research question "Does the activation of the JA signalling pathway by the citrus host hinder *T. erytreae* development on them?". Consequently, a subsequent trial was performed in order to evaluate this hypothesis. For this trial, lemon plants were sprayed with a control solution or with exogenous JA, followed by infestation with *T. erytreae*.

The findings of the JA foliar spray analysis demonstrated that the exogenous JA application significantly hindered the psyllid infestation, reducing oviposition and affecting nymphal development. The exogenous JA application exhibited a pronounced impact on *T. erytreae* oviposition behaviour. It has been demonstrated that the JA phytohormone exerts a detrimental influence on the development of *D. citri* (Rao et al., 2018a). The identification of Potential new targets for the interference in this plant–insect interaction was achieved through the characterisation of the lemon plants volatile organic compound profiles. Lemon plants affected by exogenous application of JA and *T. erytreae* infestation produced higher emissions of 6-methyl-5-hepten-2-one and (Z)-3-hexenol acetate, compounds that are known to attract parasitoids and predators (Higashida et al., 2022; Schuman, 2023). Phenol, a pivotal component of the phenolic response to herbivory (Luo et al., 2023) and 2-hexen-1-ol, which induces the JA signalling pathway (Gomi et al., 2003), were both produced in greater quantities in lemon plants subjected to JA spray and *T. erytreae* infestation. Additionally, the induction of carveol in infested lemon plants was detected and is a compound that possesses insect repellent properties (Ling et al., 2023).

The present thesis characterises the interaction between the citrus hosts and *T.erytreae*, with a particular emphasis on characterising the role of proteins, metabolites and volatiles within this interaction. According to the findings, JA is a potential solution for the control of *T. erytreae* through the utilisation of exogenous JA sprays. Further studies are required to ascertain the efficacy of exogenous JA spray as a potential novel control strategy. To establish additional perspectives, further studies should employ choice tests and electrical penetration graph analysis to examine the direct effect of exogenous JA application, via foliar spray, on the host selection and feeding intensity of *T. erytreae*. In addition, larger-scale semi-field and field experiments, along with economic studies should be conducted, to ascertain the applicability and viability of exogenous JA spray applications in commercial citrus orchards. It is also important to investigate this plant host–*T. erytreae* interaction using less suitable hosts such as *Citrus trifoliata* or *Calodendrum capense* (see host classifications in Table 2.1). The study of their interaction could lead to the potential identification of highly detrimental biomolecules, chemicals and/or metabolic pathways for the psyllid. Further transcriptomic analysis would be a valuable addition to the research, with the aim of identifying the regulatory genes involved in this plant–insect interaction (Zogli et al., 2020). This analysis could broaden the identification of molecular targets that interfere in this interaction. Genetic engineering studies, transient expression systems (Åhman et al., 2019; Sun et al., 2019) and RNA interference represent other significant approaches with the potential to contribute to control strategies against *T. erytreae*. The efficacy of RNA interference techniques has already been demonstrated in the context of *D. citri* control (Kishk et al., 2017).

The present thesis underscores the importance of studying the plant–insect interactions in order to develop management strategies for the psyllid *T. erytreae*. Furthermore, the acquired knowledge may serve as a valuable foundation for the advancement of management and control strategies for the pest and HLB vector *T. erytreae*.

References

Abate, T. (1988). The identity and bionomics of insect vectors of tristeza and greening diseases of citrus in Ethiopia. *Tropical Pest Management* 34, 19–23. https://doi.org/10.1080/09670878809371198

Abd El-Ghany, N.M. (2019). Semiochemicals for controlling insect pests. *J Plant Prot Res*. 59 (1), 1-11 https://doi.org/10.24425/jppr.2019.126036

Abdalla, M.A., Sulieman, S., Mühling, K.H. (2020). Regulation of Selenium/Sulfur Interactions to Enhance Chemopreventive Effects: Lessons to Learn from Brassicaceae. *Molecules* 25, 5846. https://doi.org/10.3390/molecules25245846

Achor, D.S., Etxeberria, E., Wang, N., Folimonova, S.Y., Chung, K.R., Albrigo, L.G. (2010). Sequence of Anatomical Symptom Observations in Citrus Affected with Huanglongbing Disease. *Plant Pathol J* (Faisalabad) 9, 56–64. https://doi.org/10.3923/ppj.2010.56.64

Adebambo, T.H., Fox, D.T., Otitoloju, A.A. (2020). Toxicological Study and Genetic Basis of BTEX Susceptibility in *Drosophila melanogaster*. *Front Genet* 11. https://doi.org/10.3389/fgene.2020.594179

Agustí, M., Zaragoza, S., Bleiholder, H., Buhr, L., Hack, H., Klose, R., Staub, R. (1995). Escala BBCH para la descripción de los estadios fenológicos del desarrollo de los agrios (Gén. Citrus) [WWW Document]. *Levante Agrícola: Revista internacional de cítricos*. URL https://redivia.gva.es/handle/20.500.11939/7875 (accessed 18. 7.22).

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 Manag Sci* 70, 1728–1741. https://doi.org/10.1002/ps.3718

Åhman, I., Kim, S.-Y., Zhu, L.-H. (2019). Plant Genes Benefitting Aphids—Potential for Exploitation in Resistance Breeding. *Front Plant Sci* 10, 1–14. https://doi.org/10.3389/fpls.2019.01452

Aidoo, O.F. (2023). The African citrus psyllid *Trioza erytreae* (Hemiptera: Triozidae): Biology, management, and its role as a vector of huanglongbing. *Crop Protection* 172, 106348. https://doi.org/10.1016/j.cropro.2023.106348

Aidoo, O.F., Tanga, C.M., Azrag, A.G.A., Mohamed, S.A., Khamis, F.M., Rasowo, B.A., Ambajo, J., Sétamou, M., Ekesi, S., Borgemeister, C. (2022). Temperature-based phenology model of African citrus triozid (*Trioza erytreae* Del Guercio): Vector of citrus greening disease. *Journal of Applied Entomology* 146, 88–97. https://doi.org/10.1111/JEN.12942

Aidoo, O. F., Tanga, C.M., Khamis, F.M., Rasowo, B.A., Mohamed, S.A., Badii, B.K., Salifu, D., Sétamou, M., Ekesi, S., Borgemeister, C. (2019a). Host suitability and feeding preference of the African citrus triozid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae), natural vector of "*Candidatus* Liberibacter africanus." *Journal of Applied Entomology* 143, 262–270. https://doi.org/10.1111/jen.12581

Aidoo, O.F., Tanga, C.M., Mohamed, S.A., Khamis, F.M., Opisa, S., Rasowo, B.A., Kimemia, J.W., Ambajo, J., Sétamou, M., Ekesi, S., Borgemeister, C. (2021). The African citrus triozid *Trioza erytreae* Del Guercio (Hemiptera: Triozidae): temporal dynamics and susceptibility to entomopathogenic fungi in East Africa. *Int J Trop Insect Sci* 41, 563–573. https://doi.org/10.1007/s42690-020-00241-5

Aidoo, O. F., Tanga, C.M., Mohamed, S.A., Rasowo, B.A., Khamis, F.M., Rwomushana, I., Kimani, J., Agyakwa, A.K., Daisy, S., Sétamou, M., Ekesi, S., Borgemeister, C. (2019b). Distribution, degree of damage and risk of spread of *Trioza erytreae* (Hemiptera: Triozidae) in Kenya. *Journal of Applied Entomology* 143, 822–833. https://doi.org/10.1111/jen.12668

Aidoo, O. F., Tanga, C.M., Paris, T.M., Allan, S.A., Mohamed, S.A., Khamis, F.M., Sétamou, M., Borgemeister, C., Ekesi, S. (2019c). Size and shape analysis of *Trioza erytreae* Del Guercio (Hemiptera: Triozidae), vector of citrus huanglongbing disease. *Pest Manag Sci* 75, 760–771. https://doi.org/10.1002/ps.5176

Aki, T., Shigyo, M., Nakano, R., Yoneyama, T., Yanagisawa, S. (2008). Nano Scale Proteomics Revealed the Presence of Regulatory Proteins Including Three FT-Like proteins in Phloem and Xylem Saps from Rice. *Plant Cell Physiol* 49, 767–790. https://doi.org/10.1093/pcp/pcn049

Aleksander, S.A., Balhoff, J., Carbon, S., Cherry, J.M., Drabkin, H.J., Ebert, D., ... Westerfield, M. (2023). The Gene Ontology knowledgebase in 2023. *Genetics* 224. https://doi.org/10.1093/genetics/iyad031

Alquézar, B., Carmona, L., Bennici, S., Miranda, M.P., Bassanezi, R.B., Peña, L. (2022). Cultural Management of Huanglongbing: Current Status and Ongoing Research. *Phytopathology* 112, 11–25. https://doi.org/10.1094/PHYTO-08-21-0358-IA

Ameline, A., Karkach, A., Denoirjean, T., Grondin, M., Molinari, F., Turpin, P., Delatte, H., Reynaud, B. (2023). Bacterial plant pathogens affect the locomotor behavior of the insect vector: a case study of *Citrus volkameriana – Triozae erytreae – Candidatus* Liberibacter asiaticus system. *Insect Sci* 31, 901-910. https://doi.org/10.1111/1744-7917.13279

Annecke, D.P., Cilliers, C.J. (1963). The citrus Psylla, *Trioza erytreae* (Del Guercio), and its parasites in South Africa. *South African Journal of Agricultural Science* 6, 187–192. https://hdl.handle.net/10520/AJA05858860_600

Antwi-Agyakwa, A.K., Fombong, A.T., Deletre, E., Ekesi, S., Yusuf, A.A., Pirk, C., Torto, B. (2019). Lemon Terpenes Influence Behavior of the African Citrus Triozid *Trioza erytreae* (Hemiptera: Triozidae). *J Chem Ecol* 45, 934–945. https://doi.org/10.1007/s10886-019-01123-y

Antwi-Agyakwa, A.K., Yusuf, A.A., Pirk, C.W.W., Mohamed, S.A., Ekesi, S., Torto, B. (2021). Exploring non-host plant-based management strategy with lemongrass, garlic and guava volatiles for the African citrus triozid. *Journal of Applied Entomology* 145, 757–766. https://doi.org/10.1111/JEN.12884

Arimura, G., Tashiro, K., Kuhara, S., Nishioka, T., Ozawa, R., Takabayashi, J. (2000). Gene responses in bean leaves induced by herbivory and by Herbivore-Induced Volatiles. *Biochem Biophys Res Commun* 277, 305–310. https://doi.org/10.1006/bbrc.2000.3672

Arrese, E.L., Soulages, J.L. (2010). Insect Fat Body: Energy, Metabolism, and Regulation. *Annu Rev Entomol* 55, 207–225. https://doi.org/10.1146/annurev-ento-112408-085356

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., ... Sherlock, G. (2000). Gene Ontology: tool for the unification of biology. *Nat Genet* 25, 25-29. https://doi.org/10.1038/75556

Asters, M.C., Williams, W.P., Perkins, A.D., Mylroie, J.E., Windham, G.L., Shan, X. (2014). Relating significance and relations of differentially expressed genes in response to *Aspergillus flavus* infection in maize. *Scientific Reports* 4, 1–10. https://doi.org/10.1038/srep04815

Aubert, B. (1987). *Trioza erytreae* del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psylloidea), the two vectors of citrus greening disease: biological aspects and possible control strategies. *Fruits* 42, 149–162.

Aubert, B. Quilici, S. (1984). Biological Control of the African and Asian Citrus Psyllids (Homoptera: Psylloidea), Through Eulophid and Encyrtid Parasites (Hymenoptera: Chalcidoidea) in Reunion Island. In Garnsey, S.M., Timmer, L.W., Dodds J.A. (Eds.).

International Organization of Citrus Virologists Conference Proceedings 9, (pp.100-108).

Ayres, A.J., Belasque Jr., J., Bové, J.M. (2015). The experience with Huanglongbing management in Brazil. *Acta Hortic* 1065, 55–61. https://doi.org/10.17660/ActaHortic.2015.1065.4

Azam, M., Jiang, Q., Zhang, B., Xu, C., Chen, K. (2013). Citrus Leaf Volatiles as Affected by Developmental Stage and Genetic Type. *Int J Mol Sci* 14, 17744–17766. https://doi.org/10.3390/ijms140917744

Balint-Kurti, P. (2019). The plant hypersensitive response: concepts, control and consequences. *Mol Plant Pathol* 20, 1163–1178. https://doi.org/10.1111/mpp.12821

Balmer, A., Pastor, V., Gamir, J., Flors, V., Mauch-Mani, B. (2015). The "prime-ome": Towards a holistic approach to priming. *Trends Plant Sci* 7 443-452. https://doi.org/10.1016/j.tplants.2015.04.002

Banothu, V., Uma, A. (2022). Effect of Biotic and Abiotic Stresses on Plant Metabolic Pathways. In Badria, F. A. (Ed.). *Phenolic Compounds - Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications*. https://doi.org/10.5772/intechopen.99796

Barah, P., Bones, A.M. (2015). Multidimensional approaches for studying plant defence against insects: from ecology to omics and synthetic biology. *J Exp Bot* 66, 479–493. https://doi.org/10.1093/jxb/eru489

Barkley, P.B., Beattie, G. a C. (2008). Contingency Plans for Hlb (Huanglongbing) and His Vectors in Australia. *I Taller Internacional sobre Huanglongbing de los cítricos (Candidatus Liberibacterspp) y el psílido asiático de los cítricos (Diaphorina citri)* 1-15

Bassanezi, R.B., Lopes, S.A., de Miranda, M.P., Wulff, N.A., Volpe, H.X.L., Ayres, A.J. (2020). Overview of citrus huanglongbing spread and management strategies in Brazil. *Trop Plant Pathol* 45, 251–264. https://doi.org/10.1007/s40858-020-00343-y

Bassanezi, R.B., Montesino, L.H., Gimenes-Fernandes, N., Yamamoto, P.T., Gottwald, T.R., Amorim, L., Filho, A.B. (2013). Efficacy of area-wide inoculum reduction and vector control on temporal progress of huanglongbing in young sweet orange plantings. *Plant Dis* 97, 789–796. https://doi.org/10.1094/PDIS-03-12-0314-RE

Bekturova, A., Oshanova, D., Tiwari, P., Nurbekova, Z., Kurmanbayeva, A., Soltabayeva, A., Yarmolinsky, D., Srivastava, S., Turecková, V., Strnad, M., Sagi, M. (2021). Adenosine 5' phosphosulfate reductase and sulfite oxidase regulate sulfite-induced water loss in Arabidopsis. *J Exp Bot* 72, 6447–6466. https://doi.org/10.1093/jxb/erab249

Belasque, J., Bassanezi, R.B., Yamamoto, P.T., Ayres, A.J., Tachibana, A., Violante, A.R., Tank, A., Di Giorgi, F., Tersi, F.E.A., Menezes, G.M., Dragone, J., Jank, R.H., Bové, J.M. (2010). Lessons from huanglongbing management in São Paulo state, Brazil. *Journal of Plant Pathology* 92 285-302 https://www.jstor.org/stable/41998803

Bengoechea, L., Hernández, T., Quesada, C., Bartolomé, B., Estrella, I., Gómez-Cordovés, C. (1995). Structure of hydroxycinnamic acid derivatives established by high-performance liquid chromatography with photodiode-array detection. *Chromatographia* 41, 94–98. https://doi.org/10.1007/BF02688006

Benhadi-Marín, J., Fereres, A., Pereira, J.A. (2022). Potential areas of spread of *Trioza erytreae* over mainland Portugal and Spain. *J Pest Sci* 95, 67–78. https://doi.org/10.1007/s10340-021-01440-w

Benhadi-Marín, J., Fereres, A., Pereira, J.A. (2020). A model to predict the expansion of *Trioza erytreae* throughout the Iberian Peninsula using a pest risk analysis approach. *Insects* 11, 1–12. https://doi.org/10.3390/insects11090576

Benhadi-Marín, J., Garzo, E., Moreno, A., Pereira, J.A., Fereres, A. (2021). Host plant preference of *Trioza erytreae* on lemon and bitter orange plants. *Arthropod Plant Interact* 15, 887–896. https://doi.org/10.1007/s11829-021-09862-0

Benjamini, Y., Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 57, 289–300. https://doi.org/10.1111/J.2517-6161.1995.TB02031.X

Berardini, T.Z., Reiser, L., Li, D., Mezheritsky, Y., Muller, R., Strait, E., Huala, E. (2015). The arabidopsis information resource: Making and mining the "gold standard" annotated reference plant genome. *genesis* 53, 474–485. https://doi.org/10.1002/dvg.22877

Berg, M., Rogers, R., Muralla, R., Meinke, D. (2005). Requirement of aminoacyl-tRNA synthetases for gametogenesis and embryo development in Arabidopsis. *The Plant Journal* 44, 866–878. https://doi.org/10.1111/j.1365-313X.2005.02580.x

Berg, M.A. Van Den, Greenland, J. (2000). *Tamarixia dryi*, parasitoid of the citrus psylla, *Trioza erytreae*: a review. *Afr Plant Prot* 6, 25–28.

Berkey, C.D., Blow, N., Watnick, P.I. (2009). Genetic analysis of *Drosophila melanogaster* susceptibility to intestinal *Vibrio cholerae* infection. *Cell Microbiol* 11, 461–474. https://doi.org/10.1111/j.1462-5822.2008.01267.x

Bernsdorff, F., Döring, A.-C., Gruner, K., Schuck, S., Bräutigam, A., Zeier, J. (2016). Pipecolic Acid Orchestrates Plant Systemic Acquired Resistance and Defense Priming via Salicylic Acid-Dependent and -Independent Pathways. *Plant Cell* 28, 102–129. https://doi.org/10.1105/tpc.15.00496

Bordoloi, K.S., Krishnatreya, D.B., Baruah, P.M., Borah, A.K., Mondal, T.K., Agarwala, N. (2021). Genome-wide identification and expression profiling of chitinase genes in tea (*Camellia sinensis* (L.) O. Kuntze) under biotic stress conditions. *Physiology and Molecular Biology of Plants* 27, 369–385. https://doi.org/10.1007/s12298-021-00947-x

Bové, J. (2014). Keynote Address: Heat-tolerant Asian HLB meets heat-sensitive African HLB in the Arabian Peninsula! Why? *J Citrus Pathol* 1, 0–78. https://doi.org/10.5070/C411024569

Bové, J. (2006). Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. *J Citrus Pathol* 1, 7–37. https://doi.org/10.4454/jpp.v88i1.828

Breeschoten, T., Ros, V.I.D., Schranz, M.E., Simon, S. (2019). An influential meal: host plant dependent transcriptional variation in the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae). *BMC Genomics* 20, 845. https://doi.org/10.1186/s12864-019-6081-7

Breiman, L., Cutler, A., Liaw, A., Wiener, M. (2024). randomForest: Classification and regression based on a forest of trees using random inputs. R package version 4.7-1.2. CRAN: Contributed Packages. https://doi.org/10.32614/CRAN.package.randomForest

Broehan, G., Kroeger, T., Lorenzen, M., Merzendorfer, H. (2013). Functional analysis of the ATP-binding cassette (ABC) transporter gene family of *Tribolium castaneum*. *BMC Genomics* 14, 6. https://doi.org/10.1186/1471-2164-14-6

Broussard, L., Abadie, C., Lalande, J., Limami, A.M., Lothier, J., Tcherkez, G. (2023). Phloem Sap Composition: What Have We Learnt from Metabolomics? *Int J Mol Sci* 24, 6917. https://doi.org/10.3390/ijms24086917

Bu, D., Luo, H., Huo, P., Wang, Z., Zhang, S., He, Z., Wu, Y., Zhao, L., Liu, J., Guo, J., Fang, S., Cao, W., Yi, L., Zhao, Y., Kong, L., 2021. KOBAS-i: intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Res* 49, W317–W325. https://doi.org/10.1093/NAR/GKAB447

Bulley, S., Laing, W. (2016). The regulation of ascorbate biosynthesis. *Curr Opin Plant Biol* 33, 15–22. https://doi.org/10.1016/J.PBI.2016.04.010

Burkart, C., Qiu, F., Brendel, S., Benes, V., Hååg, P., Labeit, S., Leonard, K., Bullard, B. (2007). Modular Proteins from the *Drosophila sallimus* (sls) Gene and their Expression in Muscles with Different Extensibility. *J Mol Biol* 367, 953–969. https://doi.org/10.1016/j.jmb.2007.01.059

Canamasas, I., Debes, A., Natali, P.G., Kurzik-Dumke, U. (2003). Understanding Human Cancer Using Drosophila. *Journal of Biological Chemistry* 278, 30952–30960. https://doi.org/10.1074/jbc.M304225200

Cao, S., Li, H., Yao, X., Li, L., Jiang, L., Zhang, Q., Zhang, J., Liu, D., Lu, H. (2016). Enzymatic characterization of two acetyl-CoA synthetase genes from *Populus trichocarpa*. *Springerplus* 5, 818. https://doi.org/10.1186/s40064-016-2532-7

Capaldi, F.R., Gratão, P.L., Reis, A.R., Lima, L.W., Azevedo, R.A. (2015). Sulfur Metabolism and Stress Defense Responses in Plants. *Trop Plant Biol* 8, 60–73. https://doi.org/10.1007/s12042-015-9152-1

Carella, P., Wilson, D.C., Kempthorne, C.J., Cameron, R.K. (2016). Vascular Sap Proteomics: Providing Insight into Long-Distance Signaling during Stress. *Front Plant Sci* 7 651. https://doi.org/10.3389/fpls.2016.00651

Caretto, S., Linsalata, V., Colella, G., Mita, G., Lattanzio, V. (2015). Carbon Fluxes between Primary Metabolism and Phenolic Pathway in Plant Tissues under Stress. *Int J Mol Sci* 16, 26378–26394. https://doi.org/10.3390/ijms161125967

Carrillo, E., Rubiales, D., Castillejo, M.A. (2014). Proteomic Analysis of Pea (*Pisum sativum* L.) Response During Compatible and Incompatible Interactions with the Pea Aphid (*Acyrthosiphon pisum* H.). *Plant Mol Biol Report* 32, 697–718. https://doi.org/10.1007/s11105-013-0677-x

Carvalho, J.P. de, Aguiar, A.M.F. (1997). Pragas dos citrinos na Ilha da Madeira. Secretaria Regional da Agricultura Florestas e Pescas, Funchal.

Casas-Vila, N., Bluhm, A., Sayols, S., Dinges, N., Dejung, M., Altenhein, T., Kappei, D., Altenhein, B., Roignant, J.-Y., Butter, F. (2017). The developmental proteome of *Drosophila melanogaster*. *Genome Res* 27, 1273–1285. https://doi.org/10.1101/gr.213694.116

Catalani, E., Silvestri, F., Bongiorni, S., Taddei, A.R., Fanelli, G., Rinalducci, S., De Palma, C., Perrotta, C., Prantera, G., Cervia, D. (2021). Retinal damage in a new model of hyperglycemia induced by high-sucrose diets. *Pharmacol Res* 166, 105488. https://doi.org/10.1016/j.phrs.2021.105488

Catling, H.D. (1972). The bionomics of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). 6. Final population studies and a discussion of population dynamics. *J Entomol Soc South Afr* 35, 235–251. https://hdl.handle.net/10520/AJA00128789_2881

Catling, H.D. (1971). The bionomics of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae) 5. The influence of host plant quality. *J Entomol Soc South Afr* 34, 381–391. https://hdl.handle.net/10520/AJA00128789_2969

Catling, H.D. (1970). The bionomics of the South African citrus psylla *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae) 4. The influence of predators. *J Entomol Soc South Afr* 33, 342–348. https://hdl.handle.net/10520/AJA00128789_3140

Catling, H.D. (1969). The bionomics of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae) I. The influence of the flushing rhythm of citrus and factors which regulate flushing. *J Entomol Soc South Afr* 32, 191–208. https://hdl.handle.net/10520/AJA00128789_4003

Catling, H.D., Annecke, D.P. (1968). Ecology of citrus psylla in the Letaba district of Northern Transvaal. *S. Afr. Citrus J*. 410, 8–17.

Catling, H.D., Atkinson, P.R. (1974). Spread of Greening by *Trioza erytreae* (Del Guercio) in Swaziland. *International Organization of Citrus Virologists Conference Proceedings* 6, 33-39. https://doi.org/10.5070/c523k2f1d4

Chehab, E.W., Kaspi, R., Savchenko, T. V, Dehesh, K. (2010). Hexenyl Acetate Mediates Indirect Plant Defense Responses. *Proceedings of ANAS (Biological Sciences)* 65, 145–151.

Chen, E.-H., Hou, Q.-L., Wei, D.-D., Jiang, H.-B., Wang, J.-J. (2017a). Phenotypic plasticity, trade-offs and gene expression changes accompanying dietary restriction and switches in Bactrocera dorsalis (Hendel) (Diptera: Tephritidae). *Sci Rep* 7, 1988. https://doi.org/10.1038/s41598-017-02106-3

Chen, E.-H., Hou, Q.-L., Wei, D.-D., Jiang, H.-B., Wang, J.-J. (2017b). Phenotypes, antioxidant responses, and gene expression changes accompanying a sugar-only diet in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *BMC Evol Biol* 17, 194. https://doi.org/10.1186/s12862-017-1045-5

Chen, G., Su, Q., Shi, X., Liu, X., Peng, Z., Zheng, H., Xie, W., Xu, B., Wang, S., Wu, Q., Zhou, X., Zhang, Y. (2017c). Odor, Not Performance, Dictates *Bemisia tabaci*'s Selection between Healthy and Virus Infected Plants. *Front Physiol* 8. https://doi.org/10.3389/fphys.2017.00146

Chen, H., Gonzales-Vigil, E., Wilkerson, C.G., Howe, G.A. (2007). Stability of Plant Defense Proteins in the Gut of Insect Herbivores. *Plant Physiol* 143, 1954–1967. https://doi.org/10.1104/pp.107.095588

Chen, S., Zhang, L., Cai, X., Li, X., Bian, L., Luo, Z., Li, Z., Chen, Z., Xin, Z. (2020). (E)-Nerolidol is a volatile signal that induces defenses against insects and pathogens in tea plants. *Hortic Res* 7, 52. https://doi.org/10.1038/s41438-020-0275-7

Chen, W., Viljoen, A.M. (2022). Geraniol – A review update. *South African Journal of Botany* 150, 1205–1219. https://doi.org/10.1016/j.sajb.2022.09.012

Chen, X.D., Qureshi, J.A., Stelinski, L.L., (2022). Monitoring of *Diaphorina citri* populations from Florida reveals reduced susceptibility to cyantraniliprole and thiamethoxam. *Journal of Applied Entomology* 146, 725–733. https://doi.org/10.1111/jen.13011

Chin, E.L., Ramsey, J., Saha, S., Mishchuk, D., Chavez, J., Howe, K., Zhong, X., Flores-Gonzalez, M., Mitrovic, E., Polek, M., Godfrey, K., Mueller, L.A., Bruce, J., Heck, M., Slupsky, C.M. (2021). Multi-omics Comparison Reveals Landscape of *Citrus limon* and *Citrus sinensis* Response to '*Candidatus* Liberibacter asiaticus.' *PhytoFrontiers™* 1, 76–84. https://doi.org/10.1094/PHYTOFR-09-20-0018-R

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

Cifuentes-Arenas, J.C., de Goes, A., de Miranda, M.P., Beattie, G.A.C., Lopes, S.A. (2018). Citrus flush shoot ontogeny modulates biotic potential of *Diaphorina citri*. *PLoS One* 13, e0190563. https://doi.org/10.1371/journal.pone.0190563

Cifuentes-Arenas, J.C., de Oliveira, H.T., Raiol-Júnior, L.L., de Carvalho, E.V., Kharfan, D., Creste, A.L., Gastaminza, G., Salas, H., Bassanezi, R.B., Ayres, A.J., Lopes, S.A. (2022). Impacts of huanglongbing on fruit yield and quality and on flushing dynamics of Sicilian lemon trees. *Front Plant Sci* 13. https://doi.org/10.3389/fpls.2022.1005557

Cilia, M., Fish, T., Yang, X., Mclaughlin, M., Thannhauser, T.W., Gray, S. (2009). A comparison of protein extraction methods suitable for gel-based proteomic studies of aphid proteins. *Journal of Biomolecular Techniques* 20, 201–215. PMID: 19721822; PMCID: PMC2729484.

Cocuzza, G.E.M., Alberto, U., Hernández-Suárez, E., Siverio, F., Di Silvestro, S., Tena, A., Carmelo, R. (2017). A review on *Trioza erytreae* (African citrus psyllid), now in mainland Europe, and its potential risk as vector of huanglongbing (HLB) in citrus. *J Pest Sci* 90, 1–17. https://doi.org/10.1007/s10340-016-0804-1

Coen, M., Engel, R., Nahrstedt, A. (1995). Chavicol β-d-glucoside, a phenylpropanoid heteroside, benzyl-β-d-glucoside and glycosidically bound volatiles from subspecies of *Cedronella canariensis*. *Phytochemistry* 40, 149–155. https://doi.org/10.1016/0031-9422(95)00241-X

Cole, S.P.C. (2014). Multidrug Resistance Protein 1 (MRP1, ABCC1), a "Multitasking" ATP-binding Cassette (ABC) Transporter. Journal of Biological Chemistry 289, 30880–30888. https://doi.org/10.1074/jbc.R114.609248

Cook, G., Maqutu, V.Z., Van Vuuren, S.P. (2014). Population dynamics and seasonal fluctuation in the percentage infection of *Trioza erytreae* with "*Candidatus*" liberibacter africanus, the African citrus greening pathogen, in an orchard severely infected with african greening and transmission by field-collected *Trioza erytreae*. *African Entomology* 22, 127–135. https://doi.org/10.4001/003.022.0107

Coppola, V., Coppola, M., Rocco, M., Digilio, M.C., D'Ambrosio, C., Renzone, G., Martinelli, R., Scaloni, A., Pennacchio, F., Rao, R., Corrado, G. (2013). Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. *BMC Genomics* 14, 515. https://doi.org/10.1186/1471-2164-14-515

Corrado, G., Alagna, F., Rocco, M., Renzone, G., Varricchio, P., Coppola, V., Coppola, M., Garonna, A., Baldoni, L., Scaloni, A., Rao, R. (2012). Molecular interactions between the olive and the fruit fly *Bactrocera oleae*. *BMC Plant Biol* 12, 86. https://doi.org/10.1186/1471-2229-12-86

Coutinho-Abreu, I. V., Forster, L., Guda, T., Ray, A. (2014). Odorants for Surveillance and Control of the Asian Citrus Psyllid (*Diaphorina citri*). *PLoS One* 9, e109236. https://doi.org/10.1371/journal.pone.0109236

da Graça, J. V., Douhan, G.W., Halbert, S.E., Keremane, M.L., Lee, R.F., Vidalakis, G., Zhao, H., 2016. Huanglongbing: An overview of a complex pathosystem ravaging the world's citrus. *J Integr Plant Biol* 58, 373–387. https://doi.org/10.1111/jipb.12437

de Carvalho, D.U., Girardi, E.A., Pacheco, C. de A., Primiano, I.V., Kharfan, D., Moreira, A.S., Laranjeira, F.F., Bassanezi, R.B. (2024). Topping sweet orange trees as *Diaphorina citri* bait on the farm edge for huanglongbing management: Opportunities and limitations. *Sci Hortic* 338, 113612. https://doi.org/10.1016/j.scienta.2024.113612

de la Torre, F., Canas, R.A., Pascual, M.B., Avila, C., Canovas, F.M. (2014). Plastidic aspartate aminotransferases and the biosynthesis of essential amino acids in plants. *J Exp Bot* 65, 5527–5534. https://doi.org/10.1093/jxb/eru240

Dege, C., Hagman, J. (2014). Mi-2/Nu <scp>RD</scp> chromatin remodeling complexes regulate B and T-lymphocyte development and function. I*mmunol Rev* 261, 126–140. https://doi.org/10.1111/imr.12209

Del Guercio, G. (1918). Note ed osservazioni di entomologia agraria. Il cecidio delle foglie del limone ed il suo cecidozoo in Eritrea. *Agricoltura Coloniale* 12, 167–169.

Delisle, J., Hardy, M. (1997). Male larval nutrition influences the reproductive success of both sexes of the Spruce Budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Funct Ecol* 11, 451–463. https://doi.org/10.1046/j.1365-2435.1997.00114.x

den Ouden, H., Visser, J.H., Alkema, D.P.W., de Vlieger, J.J. s, Derks, P.S.M. (1993). Experiments with volatile substances in slow release formulations causing repellency for oviposition by the cabbage root fly, *Phorbia brassicae* Bché. (Dipt., Anthomyiidae). *Journal of Applied Entomology* 115, 307–312. https://doi.org/10.1111/j.1439-0418.1993.tb00395.x

Denslow, S.A., Walls, A.A., Daub, M.E. (2005). Regulation of biosynthetic genes and antioxidant properties of vitamin B6 vitamers during plant defense responses. *Physiol Mol Plant Pathol* 66, 244–255. https://doi.org/10.1016/J.PMPP.2005.09.004

DGAV, (2024). Despacho n.º 40/G/2024 Atualização da Zona Demarcada para *Trioza erytreae* [WWW Document] URL https://www.dgav.pt/wp-content/uploads/2024/07/Despacho40-ZD-Trioza_JUL2024.pdf (accessed 23. 8.22).

Dinant, S., Bonnemain, J.L., Girousse, C., Kehr, J. (2010). Phloem sap intricacy and interplay with aphid feeding. *C R Biol* 333, 504–515. https://doi.org/10.1016/J.CRVI.2010.03.008

Dinant, S., Lucas, W.J. (2012). Sieve Elements: Puzzling Activities Deciphered through Proteomics Studies, in Thompson, G.A., van Bel, A.J.E. (Eds.) *Phloem*. Wiley, (pp. 155–185). https://doi.org/10.1002/9781118382806.ch8

Do, C.-T., Pollet, B., Thévenin, J., Sibout, R., Denoue, D., Barrière, Y., Lapierre, C., Jouanin, L. (2007). Both caffeoyl Coenzyme A 3-O-methyltransferase 1 and caffeic acid O-methyltransferase 1 are involved in redundant functions for lignin, flavonoids and sinapoyl malate biosynthesis in Arabidopsis. *Planta* 226, 1117–1129. https://doi.org/10.1007/s00425-007-0558-3

Dong, Z., Zhang, J.-T. (2006). Initiation factor eIF3 and regulation of mRNA translation, cell growth, and cancer. *Crit Rev Oncol Hematol* 59, 169–180. https://doi.org/10.1016/j.critrevonc.2006.03.005

Dorman, H.J.D., Deans, S.G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol* 88, 308–316. https://doi.org/10.1046/j.1365-2672.2000.00969.x

Dorta, S. de O., Balbinotte, J., Monnerat, R., Lopes, J.R.S., da Cunha, T., Zanardi, O.Z., de Miranda, M.P., Machado, M.A., de Freitas-Astúa, J. (2020). Selection of *Bacillus thuringiensis* strains in citrus and their pathogenicity to *Diaphorina citri* (Hemiptera: Liviidae) nymphs. *Insect Sci* 27, 519–530. https://doi.org/10.1111/1744-7917.12654

Douglas, A.E. (2017). The B vitamin nutrition of insects: the contributions of diet, microbiome and horizontally acquired genes. *Curr Opin Insect Sci* 23, 65–69. https://doi.org/10.1016/J.COIS.2017.07.012

Douglas, A.E. (2006). Phloem-sap feeding by animals: problems and solutions. *J Exp Bot* 57, 747–754. https://doi.org/10.1093/JXB/ERJ067

Du, Ba, Wei, Z., Wang, Z., Wang, X., Peng, X., Du, Bo, Chen, R., Zhu, L., He, G. (2015). Phloem-exudate proteome analysis of response to insect brown plant-hopper in rice. *J Plant Physiol* 183, 13–22. https://doi.org/10.1016/j.jplph.2015.03.020

Du, J., Zhang, J., Su, Y., Liu, M., Ospina, J.K., Yang, S., Zhu, A.J. (2011). In Vivo RNAi Screen Reveals Neddylation Genes as Novel Regulators of Hedgehog Signaling. *PLoS One* 6, e24168. https://doi.org/10.1371/journal.pone.0024168

Duarte, B., Poeira, R., Magalhães, T., Paiva, P., Soares, C., Neto, L., Marques, N.T., Duarte, A. (2024). Current distribution of the African citrus psyllid *Trioza erytreae* in Portugal: relation to climatic conditions. *Acta Hortic* 423–428. https://doi.org/10.17660/ActaHortic.2024.1399.53

Dudareva, N., Pichersky, E., Gershenzon, J. (2004). Biochemistry of Plant Volatiles. *Plant Physiol* 135, 1893–1902. https://doi.org/10.1104/pp.104.049981

Dyer, L.A., Philbin, C.S., Ochsenrider, K.M., Richards, L.A., Massad, T.J., Smilanich, A.M., Forister, M.L., Parchman, T.L., Galland, L.M., Hurtado, P.J., Espeset, A.E., Glassmire, A.E., Harrison, J.G., Mo, C., Yoon, S., Pardikes, N.A., Muchoney, N.D., Jahner, J.P., Slinn, H.L., Shelef, O., Dodson, C.D., Kato, M.J., Yamaguchi, L.F., Jeffrey, C.S. (2018). Modern approaches to study plant–insect interactions in chemical ecology. *Nat Rev Chem* 2, 50–64. https://doi.org/10.1038/s41570-018-0009-7

Ebert, T.A., Backus, E.A., Shugart, H.J., Rogers, M.E. (2018). Behavioral Plasticity in Probing by *Diaphorina citri* (Hemiptera, Liviidae): Ingestion from Phloem Versus Xylem is Influenced by Leaf Age and Surface. *J Insect Behav* 31, 119–137. https://doi.org/10.1007/s10905-018-9666-0

Ellis, J., Lázaro, E., Duarte, B., Magalhães, T., Duarte, A., Benhadi-Marín, J., José, |, Pereira, A., Vicent, A., Parnell, S., Nik, |, Cunniffe, J. (2025). Developing epidemiological preparedness for a plant disease invasion: Modelling citrus huánglóngbìng in the European Union. *Plants, People, Planet* 1-21 https://doi.org/10.1002/PPP3.10643

El-Shesheny, I., El-Hawary, I., Mesbah, I., Killiny, N. (2016). Comparative proteomic analysis between fifth-instar nymphs and adults of Asian citrus psyllid *Diaphorina citri*. *Physiol Entomol* 41, 162–184. https://doi.org/10.1111/phen.12139

Enders, L., Begcy, K. (2021). Unconventional routes to developing insect-resistant crops. *Mol Plant* 14, 1439–1453. https://doi.org/10.1016/j.molp.2021.06.029

EPPO (2025). EPPO Global Database [WWW Document].URL https://gd.eppo.int/ (accessed 26.4.25).

EPPO (2023). First report of *Diaphorina citri* in Cyprus. [WWW Document]. EPPO Reporting Service no. 08 - 2023, Num. article: 2023/178. URL https://gd.eppo.int/reporting/article-7660 (accessed 26.4.24).

EPPO (2022). First report of *Diaphorina citri* in Israel. [WWW Document]. EPPO Reporting Service no. 02 - 2022, Num. article: 2022/032. URL https://gd.eppo.int/reporting/article-7262 (accessed 26.4.24).

EPPO (2021). Update on the situation of *Trioza erytreae* in Portugal. [WWW Document]. EPPO Reporting Service no. 11 – 2021 Num. article: 2021/244. URL https://gd.eppo.int/reporting/article-7215 (accessed 15.5.24).

EPPO (2015). *Trioza erytreae* occurs in mainland Portugal [WWW Document]. EPPO Reporting Service no. 11 – 2015 Num. article: 2015/204. URL https://gd.eppo.int/reporting/article-5151 (accessed 28.6.24).

Erb, M., Reymond, P. (2019). Molecular Interactions Between Plants and Insect Herbivores. *Annu Rev Plant Biol* 70, 527–557. https://doi.org/10.1146/annurev-arplant-050718-095910

Estruch, J.J., Warren, G.W., Mullins, M.A., Nye, G.J., Craig, J.A., Koziel, M.G. (1996). Vip3A, a novel Bacillus thuringiensis vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proceedings of the National Academy of Sciences* 93, 5389–5394. https://doi.org/10.1073/pnas.93.11.5389

Etienne, J., Aubert, B. (1980). Biological control of psyllid Vectors of greening disease on Reunion Island. *International Organization of Citrus Virologists Conference Proceedings* 8 118–121. https://doi.org/10.5070/C55v15k79k

EU (2008). Directive 2008/90 - Marketing of fruit plant propagating material and fruit plants intended for fruit production. [WWW Document]. URL http://data.europa.eu/eli/dir/2008/90/oj (accessed 9.2.25).

FAO (2025). FAOSTAT: Production: Crops and livestock products. [WWW Document]. URL https://www.fao.org/faostat/en/#data/QCL (accessed 9.2.25).

FAO (2021). Citrus Fruit Statistical Compendium 2020. [WWW Document]. URL http://www.fao.org/3/cb6492en/cb6492en.pdf (accessed 9.2.25).

Farmer, E.E., Gao, Y., Lenzoni, G., Wolfender, J., Wu, Q. (2020). Wound- and mechanostimulated electrical signals control hormone responses. *New Phytologist* 227, 1037–1050. https://doi.org/10.1111/nph.16646

Fiehn, O. (2003). Metabolic networks of *Cucurbita maxima* phloem. *Phytochemistry* 62, 875–886. https://doi.org/10.1016/S0031-9422(02)00715-X

Florencio-Ortiz, V., Sellés-Marchart, S., Casas, J.L. (2021). Proteome changes in pepper (*Capsicum annuum* L.) leaves induced by the green peach aphid (*Myzus persicae* Sulzer). *BMC Plant Biol* 21, 12. https://doi.org/10.1186/s12870-020-02749-x

Fraga, A., Ribeiro, L., Lobato, M., Santos, V., Silva, J.R., Gomes, H., da Cunha Moraes, J.L., de Souza Menezes, J., de Oliveira, C.J.L., Campos, E., da Fonseca, R.N. (2013). Glycogen and Glucose Metabolism Are Essential for Early Embryonic Development of the Red Flour Beetle *Tribolium castaneum*. *PLoS One* 8, e65125. https://doi.org/10.1371/journal.pone.0065125

Franco, J.Y., Thapa, S.P., Pang, Z., Gurung, F.B., Liebrand, T.W.H., Stevens, D.M., Ancona, V., Wang, N., Coaker, G. (2020). Citrus Vascular Proteomics Highlights the Role of

Peroxidases and Serine Proteases during Huanglongbing Disease Progression. *Molecular & Cellular Proteomics* 19, 1936–1952. https://doi.org/10.1074/mcp.RA120.002075

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 Physiol* 146, 818-824. https://doi.org/10.1104/pp.107.113027

Galvañ, A., Bassanezi, R.B., Luo, W., Vanaclocha, P., Vicent, A., Lázaro, E. (2023). Risk-based regionalization approach for area-wide management of HLB vectors in the Mediterranean Basin. *Front Plant Sci* 14. https://doi.org/10.3389/fpls.2023.1256935

Galvez-Sola, L., García-Sánchez, F., Pérez-Pérez, J.G., Gimeno, V., Navarro, J.M., Moral, R., Martínez-Nicolás, J.J., Nieves, M. (2015). Rapid estimation of nutritional elements on citrus leaves by near infrared reflectance spectroscopy. *Front Plant Sci* 6. https://doi.org/10.3389/fpls.2015.00571

Gao, J., Tao, T., Arthurs, S.P., Ye, F., An, X., Hussain, M., Mao, R. (2023). Plant jasmonic acid mediated contrasting effects of two citrus aphid species on *Diaphorina citri* Kuwayama. *Pest Manag Sci* 79, 811–820. https://doi.org/10.1002/ps.7249

Garbarino, J.E., Gibbons, I.R. (2002). Expression and genomic analysis of midasin, a novel and highly conserved AAA protein distantly related to dynein. *BMC Genomics* 3, 18. https://doi.org/10.1186/1471-2164-3-18

García-Méndez, V.H., Ortega-Arenas, L.D., Villanueva-Jiménez, J.A., Osorio-Acosta, F. (2019). Resistencia de *Diaphorina citri* Kuwayama a Insecticidas en Cinco Áreas Regionales de Control en México. *Southwestern Entomologist* 44, 947. https://doi.org/10.3958/059.044.0415

Godenschwege, T.A., Reisch, D., Diegelmann, S., Eberle, K., Funk, N., Heisenberg, M., Hoppe, V., Hoppe, J., Klagges, B.R.E., Martin, J., Nikitina, E.A., Putz, G., Reifegerste, R., Reisch, N., Rister, J., Schaupp, M., Scholz, H., Schwärzel, M., Werner, U., Zars, T.D., Buchner, S., Buchner, E. (2004). Flies lacking all synapsins are unexpectedly healthy but are impaired in complex behaviour. *European Journal of Neuroscience* 20, 611–622. https://doi.org/10.1111/j.1460-9568.2004.03527.x

Gomi, K., Yamasaki, Y., Yamamoto, H., Akimitsu, K. (2003). Characterization of a hydroperoxide lyase gene and effect of C6-volatiles on expression of genes of the oxylipin metabolism in Citrus. *J Plant Physiol* 160, 1219–1231. https://doi.org/10.1078/0176-1617-01177

González, E., Danehower, D., Daub, M.E. (2007). Vitamer Levels, Stress Response, Enzyme Activity, and Gene Regulation of Arabidopsis Lines Mutant in the Pyridoxine/Pyridoxamine 5'-Phosphate Oxidase (*PDX3*) and the Pyridoxal Kinase (*SOS4*) Genes Involved in the Vitamin B6 Salvage Pathway. *Plant Physiol* 145, 985–996. https://doi.org/10.1104/pp.107.105189

Goodman, L.D., Cope, H., Nil, Z., Ravenscroft, T.A., Charng, W.-L., Lu, S., ... Tan, Q.K.-G. (2021). TNPO2 variants associate with human developmental delays, neurologic deficits, and dysmorphic features and alter TNPO2 activity in Drosophila. *The American Journal of Human Genetics* 108, 1669–1691. https://doi.org/10.1016/j.ajhg.2021.06.019

Gottwald, T.R. (2010). Current Epidemiological Understanding of Citrus Huanglongbing. *Annu Rev Phytopathol* 48, 119–139. https://doi.org/10.1146/annurev-phyto-073009-114418

Gottwald, T.R., da Graça, J. V., Bassanezi, R. B. (2007). Citrus Huanglongbing: The Pathogen and Its Impact. *Plant Health Progress* 8, 31 https://doi.org/10.1094/PHP-2007-0906-01-RV

Gottwald, T.R., Hall, D.G., Beattie, G.A.C., Ichinose, K., Nguyen, M.C., Le, Q.D., Bar-Joseph, M., Lapointe, S., Stover, E., Parker, P.E., McCollum, G., Hilf, M.E. (2010). Investigations

of the Effect of Guava as a Possible Tool in the Control/Management of Huanglongbing. *International Organization of Citrus Virologists Conference Proceedings* 17. https://doi.org/10.5070/c51x89p04q

Guo, S., Duan, J., Qian, D., Wang, H., Tang, Y., Qian, Y., Wu, D., Su, S., Shang, E. (2013). Hydrophilic interaction ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry for determination of nucleotides, nucleosides and nucleobases in Ziziphus plants. *J Chromatogr A* 1301, 147–155. https://doi.org/10.1016/j.chroma.2013.05.074

Hall, D.G., Gottwald, T.R., Nguyen, N.C., Ichinose, K., Le, Q.D., Beattie, G.A.C., Stover, E. (2008). Greenhouse Investigations on the Effect of Guava on Infestations of Asian Citrus Psyllid in Grapefruit. *Proc Fla State Hort Soc* 121, 104–109.

Hall, D.G., Shatters, R.G., Carpenter, J.E., Shapiro, J.P. (2010). Research Toward an Artificial Diet for Adult Asian Citrus Psyllid. *Ann Entomol Soc Am* 103, 611–617. https://doi.org/10.1603/AN10004

Ham, B.-K., Lucas, W.J. (2017). Phloem-Mobile RNAs as Systemic Signaling Agents. *Annu Rev Plant Biol* 68, 173–195. https://doi.org/10.1146/annurev-arplant-042916-041139

Haroth, S., Feussner, K., Kelly, A.A., Zienkiewicz, K., Shaikhqasem, A., Herrfurth, C., Feussner, I. (2019). The glycosyltransferase UGT76E1 significantly contributes to 12-O-glucopyranosyl-jasmonic acid formation in wounded *Arabidopsis thaliana* leaves. *Journal of Biological Chemistry* 294, 9858–9872. https://doi.org/10.1074/jbc.RA119.007600

Havaux, M., Ksas, B., Szewczyk, A., Rumeau, D., Franck, F., Caffarri, S., Triantaphylidès, C. (2009). Vitamin B6 deficient plants display increased sensitivity to high light and photo-oxidative stress. *BMC Plant Biol* 9, 130. https://doi.org/10.1186/1471-2229-9-130

Henry, Y., Overgaard, J., Colinet, H. (2020). Dietary nutrient balance shapes phenotypic traits of *Drosophila melanogaster* in interaction with gut microbiota. *Comp Biochem Physiol A Mol Integr Physiol* 241, 110626. https://doi.org/10.1016/j.cbpa.2019.110626

Hernández-Suárez, E., Arjona-López, J.M., Rizza, R., Perera, S., Siverio, F., Hervalejo, A., Arenas-Arenas, F.J. (2023). Comparative efficacy of seven biorational insecticides to manage African citrus psyllid (*Trioza erytreae*) in European organic citriculture. *Biological Agriculture & Horticulture* 39, 194–206. https://doi.org/10.1080/01448765.2023.2197855

Hernández-Suárez, E., Suárez-Méndez, L., Parrilla, M., Arjona-López, J.M., Hervalejo, A., Arenas-Arenas, F.J. (2021). Feeding and oviposition behaviour of *Trioza erytreae* (Hemiptera: Triozidae) on different citrus rootstock material available in Europe. *Insects* 12, 623. https://doi.org/10.3390/insects12070623

Higashida, K., Yano, E., Takabayashi, J., Ozawa, R., Yoneya, K. (2022). Volatiles from eggplants infested by *Aphis gossypii* induce oviposition behavior in the aphidophagous gall midge *Aphidoletes aphidimyza*. *Arthropod Plant Interact* 16, 45–52. https://doi.org/10.1007/s11829-021-09882-w

Hijaz, F., Killiny, N. (2014a). Collection and Chemical Composition of Phloem Sap from *Citrus sinensis* L. Osbeck (Sweet Orange). *PLoS One* 9, e101830. https://doi.org/10.1371/journal.pone.0101830

Hijaz, F., Killiny, N. (2014b). Composition of citrus phloem sap and honeydew produced by the citrus phloem sap feeder, the Asian citrus psyllid, *Diaphorina citri* (Homopetera: Psyllidae). *J Citrus Pathol* 1, 157. https://doi.org/10.5070/C411024848

Hijaz, F., Lu, Z., Killiny, N. (2016a). Effect of host-plant and infection with '*Candidatus* Liberibacter asiaticus' on honeydew chemical composition of the Asian citrus psyllid, *Diaphorina citri*. *Entomol Exp Appl* 158, 34–43. https://doi.org/10.1111/eea.12377

Hijaz, F., Nehela, Y., Killiny, N. (2016b). Possible role of plant volatiles in tolerance against huanglongbing in citrus. *Plant Signal Behav* 11, e1138193. https://doi.org/10.1080/15592324.2016.1138193

Hollis, D. (1984). Afrotropical jumping plant lice of the family Triozidae (Homoptera: Psylloidea). *Bulletin of the British Museum (Natural History) Entomology* 49, 1–102. https://biostor.org/reference/159

Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., ... Nishioka, T. (2010). MassBank: a public repository for sharing mass spectral data for life sciences. *Journal of Mass Spectrometry* 45, 703–714. https://doi.org/10.1002/jms.1777

Horton, D.R., Krysan, J.L. (1990). Probing and Oviposition-Related Activity of Summerform Pear Psylla (Homoptera: Psyllidae) on Host and Nonhost Substrates. *Environ Entomol* 19, 1463–1468. https://doi.org/10.1093/ee/19.5.1463

Hosseinzadeh, S., Higgins, S.A., Ramsey, J., Howe, K., Griggs, M., Castrillo, L., Heck, M. (2021). Proteomic Polyphenism in Color Morphotypes of *Diaphorina citri*, Insect Vector of Citrus Greening Disease. *J Proteome Res* 20, 2851–2866. https://doi.org/10.1021/acs.jproteome.1c00089

Hu, Y., Comjean, A., Attrill, H., Antonazzo, G., Thurmond, J., Chen, W., Li, F., Chao, T., Mohr, S.E., Brown, N.H., Perrimon, N. (2023). PANGEA: a new gene set enrichment tool for *Drosophila* and common research organisms. *Nucleic Acids Res* 51, W419–W426. https://doi.org/10.1093/nar/gkad331

Huang, C.Y., Araujo, K., Sánchez, J.N., Kund, G., Trumble, J., Roper, C., Godfrey, K.E., Jin, H. (2021a). A stable antimicrobial peptide with dual functions of treating and preventing citrus Huanglongbing. *Proceedings of the National Academy of Sciences* 118. https://doi.org/10.1073/pnas.2019628118

Huang, C.Y., Niu, D., Kund, G., Jones, M., Albrecht, U., Nguyen, L., Bui, C., Ramadugu, C., Bowman, K.D., Trumble, J., Jin, H. (2021b). Identification of citrus immune regulators involved in defence against Huanglongbing using a new functional screening system. *Plant Biotechnol J* 19, 757–766. https://doi.org/10.1111/pbi.13502

Hughes, C.S., Moggridge, S., Müller, T., Sorensen, P.H., Morin, G.B., Krijgsveld, J. (2018). Single-pot, solid-phase-enhanced sample preparation for proteomics experiments. *Nature Protocols* 14, 68–85. https://doi.org/10.1038/s41596-018-0082-x

Hummel, T., Zipursky, S.L. (2004). Afferent Induction of Olfactory Glomeruli Requires N-Cadherin. *Neuron* 42, 77–88. https://doi.org/10.1016/S0896-6273(04)00158-8

Hunt, H., Brueggen, N., Galle, A., Vanderauwera, S., Frohberg, C., Fernie, A.R., Sonnewald, U., Sweetlove, L.J. (2023). Analysis of companion cell and phloem metabolism using a transcriptome-guided model of Arabidopsis metabolism. *Plant Physiol* 192, 1359–1377. https://doi.org/10.1093/plphys/kiad154

Hunter, W.B., Gonzalez, M.T., Andrade, E.C. (2017). Double stranded RNA compositions for reducing asian citrus psyllid infestation and methods of use. US 2017/0211082 Al.

Ibanez, F., Suh, J.H., Wang, Y., Stelinski, L.L. (2019). Long-term, sustained feeding by Asian citrus psyllid disrupts salicylic acid homeostasis in sweet orange. *BMC Plant Biol* 19, 493. https://doi.org/10.1186/s12870-019-2114-2

INE (2024). Instituto Nacional de Estatística - Estatísticas Agrícolas: 2023 [WWW Document].URL https://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_publicacoes&PUBLICACOESpub_boui=439500127&PUBLICACOESmodo=2 (accessed 17.09.24)

Inkscape Project (2020). Inkscape [WWW Document].URL https://inkscape.org/ (accessed 17.09.23)

Islam, S., Adam, Z., Akanda, J.H. (2024). Quinic and caffeic acids derivatives: Affecting antioxidant capacities and phenolics contents of certain therapeutic and specialty

crops employing water and ethanolic extracts. *Food Chemistry Advances* 4, 100693. https://doi.org/10.1016/j.focha.2024.100693

Jacinto, C., Matias, P., Oliveira, C., Duarte, A. (2024). Effect of heading cuts on branch growth of 'Encore' mandarin. *Acta Hortic* 1399, 241-246 https://doi.org/10.17660/ActaHortic.2024.1399.31

Jia, H., Liu, Y., Yan, W., Jia, J. (2009). PP4 and PP2A regulate Hedgehog signaling by controlling Smo and Ci phosphorylation. *Development* 136, 307–316. https://doi.org/10.1242/dev.030015

Jin, L.H., Shim, J., Yoon, J.S., Kim, B., Kim, J., Kim-Ha, J., Kim, Y.-J. (2008). Identification and Functional Analysis of Antifungal Immune Response Genes in Drosophila. *PLoS Pathog* 4, e1000168. https://doi.org/10.1371/journal.ppat.1000168

Jones, S.E., Killiny, N. (2021). Influence of Rootstock on the Leaf Volatile Organic Compounds of Citrus Scion Is More Pronounced after the Infestation with *Diaphorina citri*. *Plants* 10, 2422. https://doi.org/10.3390/plants10112422

Joubert, J., Sivparsad, B., Schröder, M., Germishuizen, I., Chen, J., Hurley, B., Allison, J.D., Hammerbacher, A. (2023). Susceptibility of *Eucalyptus* trees to defoliation by the *Eucalyptus* snout beetle, *Gonipterus* sp. n. 2, is enhanced by high foliar contents of 1,8-cineole, oxalic acid and sucrose and low contents of palmitic and shikimic acid. *Plant Cell Environ* 46, 3481–3500. https://doi.org/10.1111/pce.14696

Juan-Blasco, M., Qureshi, J.A., Urbaneja, A., Stansly, P.A. (2012). Predatory mite, *Amblyseius swirskii* (Acari: Phytoseiidae), for biological control of Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Florida Entomologist* 95, 543–551. https://doi.org/10.1653/024.095.0302

Kaiser, E., Morales, A., Harbinson, J., Heuvelink, E., Prinzenberg, A.E., Marcelis, L.F.M. (2016). Metabolic and diffusional limitations of photosynthesis in fluctuating irradiance in *Arabidopsis thaliana*. *Sci Rep* 6, 31252. https://doi.org/10.1038/srep31252

Kalyebi, A., Aisu, G., Ramathani, I., Ogwang, J., McOwen, N., Russell, P. (2016). Detection and identification of etiological agents (Liberibacter spp.) associated with citrus greening disease in Uganda. *Uganda Journal of Agricultural Sciences* 16, 43–54. https://doi.org/10.4314/ujas.v16i1.4

Kanehisa, M., Sato, Y., Kawashima, M. (2022). KEGG mapping tools for uncovering hidden features in biological data. *Protein Science* 31, 47–53. https://doi.org/10.1002/PRO.4172

Kariola, T., Brader, G., Li, J., Palva, E.T. (2005). Chlorophyllase 1, a Damage Control Enzyme, Affects the Balance between Defense Pathways in Plants. *Plant Cell* 17, 282–294. https://doi.org/10.1105/tpc.104.025817

Kassambra, A., Mundt, F. (2020). Extract and Visualize the Results of Multivariate Data Analyses [R package factoextra version 1.0.7] | Scinapse [WWW Document]. URL https://CRAN.R-project.org/package=factoextra (accessed 12.1.23).

Kehr, J. (2006). Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. *J Exp Bot* 57, 767–774. https://doi.org/10.1093/jxb/erj087

Kehr, J., Rep, M. (2007). Protein Extraction from Xylem and Phloem Sap, in Thellement, H., Zivy, M., Damerval, C., Méchin, V. (Eds.), *Plant Proteomics*. Humana Press, New Jersey, (pp. 27–36). https://doi.org/10.1385/1-59745-227-0:27

Kerchev, P.I., Fenton, B., Foyer, C.H., Hancock, R.D. (2012). Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. *Plant Cell Environ* 35, 441–453. https://doi.org/10.1111/j.1365-3040.2011.02399.x

Khadka, A., Allan, S.A., Cho, D., Weeks, E.N.I. (2020). Can the Addition of Odor and Visual Targets Enhance Attraction of the Asian Citrus Psyllid (Hemiptera: Liviidae) to Sticky Traps? *J Econ Entomol* 113, 2563–2567. https://doi.org/10.1093/jee/toaa184

Khetsha, Z., Moloantoa, K., Masowa, M., Sedibe, M. (2024). Comparative effects of two elicitors on the essential oil biosynthesis of simulated hail-damaged rose geranium (*Pelargonium graveolens* L'Hér. cv. 'BOURBON'). *African Journal of Food, Agriculture, Nutrition and Development* 24, 26441–26463. https://doi.org/10.18697/ajfand.130.24075

Kienow, L., Schneider, K., Bartsch, M., Stuible, H.-P., Weng, H., Miersch, O., Wasternack, C., Kombrink, E. (2008). Jasmonates meet fatty acids: functional analysis of a new acyl-coenzyme A synthetase family from *Arabidopsis thaliana*. *J Exp Bot* 59, 403–419. https://doi.org/10.1093/jxb/erm325

Killiny, N. (2017). Metabolite signature of the phloem sap of fourteen citrus varieties with different degrees of tolerance to *Candidatus* Liberibacter asiaticus. *Physiol Mol Plant* Pathol 97, 20–29. https://doi.org/10.1016/J.PMPP.2016.11.004

Killiny, N. (2016). Metabolomic comparative analysis of the phloem sap of curry leaf tree (*Bergera koenegii*), orange jasmine (*Murraya paniculata*), and Valencia sweet orange (*Citrus sinensis*) supports their differential responses to Huanglongbing. *Plant Signal Behav* 11, e1249080. https://doi.org/10.1080/15592324.2016.1249080

Killiny, N., Nehela, Y. (2017). Metabolomic response to huanglongbing: Role of carboxylic compounds in *Citrus sinensis* response to "*Candidatus* Liberibacter asiaticus" and its vector, *Diaphorina citri*. *Molecular Plant-Microbe Interactions* 30, 666–678. https://doi.org/10.1094/MPMI-05-17-0106-R

Kim, J., Felton, G.W. (2013). Priming of antiherbivore defensive responses in plants. *Insect Sci* 20, 273-285. https://doi.org/10.1111/j.1744-7917.2012.01584.x

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E. (2023). PubChem 2023 update. *Nucleic Acids Res* 51, D1373–D1380. https://doi.org/10.1093/nar/gkac956

Kishk, A., Anber, H.A.I., AbdEl-Raof, T.K., El-Sherbeni, A.D., Hamed, S., Gowda, S., Killiny, N. (2017). RNA interference of carboxyesterases causes nymph mortality in the Asian citrus psyllid, *Diaphorina citri*. *Arch Insect Biochem Physiol* 94, e21377. https://doi.org/10.1002/arch.21377

Kite, G.C., Hughes, M.J. (1997). Analysis of hydroxypipecolic acids by gas chromatography-mass spectrometry. *Phytochemical Analysis* 8, 294–301. https://doi.org/10.1002/(SICI)1099-1565(199711/12)8:6<294::AID-PCA372>3.0.CO;2-R

Klauser, D., Desurmont, G.A., Glauser, G., Vallat, A., Flury, P., Boller, T., Turlings, T.C.J., Bartels, S. (2015). The *Arabidopsis* Pep-PEPR system is induced by herbivore feeding and contributes to JA-mediated plant defence against herbivory. *J Exp Bot* 66, 5327–5336. https://doi.org/10.1093/jxb/erv250

Klodmann, J., Senkler, M., Rode, C., Braun, H.-P. (2011). Defining the Protein Complex Proteome of Plant Mitochondria. *Plant Physiol* 157, 587–598. https://doi.org/10.1104/pp.111.182352

Koo, Y.J., Yoon, E.S., Seo, J.S., Kim, J.-K., Choi, Y. Do (2013). Characterization of a methyl jasmonate specific esterase in arabidopsis. *J Korean Soc Appl Biol Chem* 56, 27–33. https://doi.org/10.1007/s13765-012-2201-7

Kosová, K., Vítámvás, P., Prášil, I.T., Renaut, J. (2011). Plant proteome changes under abiotic stress — Contribution of proteomics studies to understanding plant stress response. *J Proteomics* 74, 1301–1322. https://doi.org/10.1016/j.jprot.2011.02.006

Kumar, K., Debnath, P., Singh, S., Kumar, N. (2023). An Overview of Plant Phenolics and Their Involvement in Abiotic Stress Tolerance. *Stresses* 3, 570–585. https://doi.org/10.3390/stresses3030040

Kunkel, B.N., Brooks, D.M. (2002). Cross talk between signaling pathways in pathogen defense. *Curr Opin Plant Biol* 5, 325–331. https://doi.org/10.1016/S1369-5266(02)00275-3

Laemmli, U.K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature* 227, 680–685. https://doi.org/10.1038/227680a0

Lallemand, J., Fos, A., Bové, J.M. (1986). Transmission de la bactérie associée à la forme africaine de la maladie du "Greening" par le psylle asiatique *Diaphorina citri* Kuwayama. *Fruits* 41, 341–343.

Layalle, S., Arquier, N., Léopold, P. (2008). The TOR Pathway Couples Nutrition and Developmental Timing in Drosophila. *Dev Cell* 15, 568–577. https://doi.org/10.1016/j.devcel.2008.08.003

Lee, C.P., Eubel, H., O'Toole, N., Millar, A.H. (2008a). Heterogeneity of the Mitochondrial Proteome for Photosynthetic and Non-photosynthetic Arabidopsis Metabolism. *Molecular & Cellular Proteomics* 7, 1297–1316. https://doi.org/10.1074/mcp.M700535-MCP200

Lee, S.-J., Feldman, R., O'Farrell, P.H. (2008b). An RNA Interference Screen Identifies a Novel Regulator of Target of Rapamycin That Mediates Hypoxia Suppression of Translation in *Drosophila* S2 Cells. *Mol Biol Cell* 19, 4051–4061. https://doi.org/10.1091/mbc.e08-03-0265

Leimu, R., Koricheva, J. (2006). A meta-analysis of tradeoffs between plant tolerance and resistance to herbivores: combining the evidence from ecological and agricultural studies. *Oikos* 112, 1–9. https://doi.org/10.1111/j.0030-1299.2006.41023.x

Lelarge-Trouverie, C., Cohen, M., Trémulot, L., Van Breusegem, F., Mhamdi, A., Noctor, G. (2023). Metabolite modification in oxidative stress responses: A case study of two defense hormones. *Free Radic Biol Med* 196, 145–155. https://doi.org/10.1016/j.freeradbiomed.2023.01.007

Li, J., Li, L., Pang, Z., Kolbasov, V.G., Ehsani, R., Carter, E.W., Wang, N. (2019). Developing Citrus Huanglongbing (HLB) Management Strategies Based on the Severity of Symptoms in HLB-Endemic Citrus-Producing Regions. *Phytopathology* 109, 582–592. https://doi.org/10.1094/PHYTO-08-18-0287-R

Li, J., Zhu, L., Hull, J.J., Liang, S., Daniell, H., Jin, S., Zhang, X. (2016). Transcriptome analysis reveals a comprehensive insect resistance response mechanism in cotton to infestation by the phloem feeding insect *Bemisia tabaci* (whitefly). *Plant Biotechnol J* 14, 1956–1975. https://doi.org/10.1111/pbi.12554

Li, M., Nangong, Z. (2022). Precision trunk injection technology for treatment of huanglongbing (HLB)-affected citrus trees—a review. *Journal of Plant Diseases and Protection* 129, 15–34. https://doi.org/10.1007/s41348-021-00510-6

Li, Shuang, Cho, Y.S., Wang, B., Li, Shuangxi, Jiang, J. (2018). Regulation of Smoothened ubiquitylation and cell surface expression through a Cul4–DDB1–Gβ E3 ubiquitin ligase complex. *J Cell Sci* 131. https://doi.org/10.1242/jcs.218016

Limayem, A., Martin, E.M., Shankar, S. (2024). Study on the citrus greening disease: Current challenges and novel therapies. *Microb Pathog* 192, 106688. https://doi.org/10.1016/j.micpath.2024.106688

Lin, M.-K., Lee, Y.-J., Lough, T.J., Phinney, B.S., Lucas, W.J. (2009). Analysis of the Pumpkin Phloem Proteome Provides Insights into Angiosperm Sieve Tube Function. *Molecular & Cellular Proteomics* 8, 343–356. https://doi.org/10.1074/mcp.M800420-MCP200

Lin, X., Smagghe, G. (2019). Roles of the insulin signaling pathway in insect development and organ growth. *Peptides (N.Y.)* 122, 169923. https://doi.org/10.1016/j.peptides.2018.02.001

Ling, Z., Li, J., Dong, Y., Zhang, W., Bai, H., Li, S., Wang, S., Li, H., Shi, L. (2023). Terpene produced by coexpression of the TPS and P450 genes from *Lavandula angustifolia* protects plants from herbivore attacks during budding stages. *BMC Plant Biol* 23, 477. https://doi.org/10.1186/s12870-023-04490-7

Lisko, K.A., Aboobucker, S.I., Torres, R., Lorence, A. (2014). Engineering Elevated Vitamin C in Plants to Improve their Nutritional Content, Growth, and Tolerance to Abiotic Stress. In Jetter, R. (Eds.) *Phytochemicals – Biosynthesis, Function and Application* Springer, Cham. 44, 109–128. https://doi.org/10.1007/978-3-319-04045-5_6

Liu, J.X., Howell, S.H. (2010). Endoplasmic Reticulum Protein Quality Control and Its Relationship to Environmental Stress Responses in Plants. *Plant Cell* 22, 2930–2942. https://doi.org/10.1105/TPC.110.078154

Liu, L., Xu, X., Cheng, D., Yao, X., Pan, S. (2012). Structure–Activity Relationship of Citrus Polymethoxylated Flavones and Their Inhibitory Effects on *Aspergillus niger*. *J Agric Food Chem* 60, 4336–4341. https://doi.org/10.1021/jf3012163

Liu, Xinyu, Zou, Z., Zhang, C., Liu, Xian, Wang, J., Xin, T., Xia, B. (2020). Knockdown of the Trehalose-6-Phosphate Synthase Gene Using RNA Interference Inhibits Synthesis of Trehalose and Increases Lethality Rate in Asian Citrus Psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). *Insects* 11, 605. https://doi.org/10.3390/insects11090605

Lu, F., Li, S., Shen, B., Zhang, J., Liu, L., Shen, X., Zhao, R. (2020). The emission characteristic of VOCs and the toxicity of BTEX from different mosquito-repellent incenses. *J Hazard Mater* 384, 121428. https://doi.org/10.1016/j.jhazmat.2019.121428

Luan, J., Yao, D., Zhang, T., Walling, L.L., Yang, M., Wang, Y., Liu, S. (2013). Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. *Ecol Lett* 16, 390–398. https://doi.org/10.1111/ele.12055

Luo, C., Qiu, J., Zhang, Y., Li, M., Liu, P. (2023). Jasmonates Coordinate Secondary with Primary Metabolism. *Metabolites* 13, 1008. https://doi.org/10.3390/metabo13091008

Lv, J.-Y., Meng, Z.-J., Deng, Y.-N., Zhang, C.-W., Tao, M.-M., Yan, S.-C. (2024). Active volatile components of the preferred hosts are potential attractants to *Hyphantria cunea* adults. Pestic *Biochem Physiol* 202, 105910. https://doi.org/10.1016/j.pestbp.2024.105910

Ma, X., Yan, H., Yang, J., Liu, Y., Li, Z., Sheng, M., Cao, Y., Yu, X., Yi, X., Xu, W., Su, Z. (2022). PlantGSAD: a comprehensive gene set annotation database for plant species. *Nucleic Acids Res* 50, D1456–D1467. https://doi.org/10.1093/nar/gkab794

Mackenzie, S.M., Brooker, M.R., Gill, T.R., Cox, G.B., Howells, A.J., Ewart, G.D. (1999). Mutations in the white gene of *Drosophila melanogaster* affecting ABC transporters that determine eye colouration. *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1419, 173–185. https://doi.org/10.1016/S0005-2736(99)00064-4

MacLean, A., Legendre, F., Appanna, V.D. (2023). The tricarboxylic acid (TCA) cycle: a malleable metabolic network to counter cellular stress. *Crit Rev Biochem Mol Biol* 58, 81–97. https://doi.org/10.1080/10409238.2023.2201945

Magalhães, T., Dandlen, S.A., Anjos, L., Power, D.M., Pereira, J.A., Duarte, A., Marques, N.T. (2024). Comparing the response of *Citrus* × *limon* and *Citrus* × *sinensis* to *Trioza erytreae* infestation using a proteomic approach. *Acta Hortic* 379–386. https://doi.org/10.17660/ActaHortic.2024.1399.47

Magalhães, T., Duarte, A., Pereira, J.A., Marques, N.T. (2025). *Trioza erytreae* (Del Guercio, 1918) and the interaction with its hosts: a review. *Agriculture* 15, 101. https://doi.org/10.3390/agriculture15010101

Malik, N.S.A., Perez, J.L., Kunta, M., Olanya, M. (2015). Changes in Polyphenol Levels in Satsuma (*Citrus unshiu*) Leaves in Response to Asian Citrus Psyllid Infestation and Water Stress. *Open Agric J* 9, 1–5. https://doi.org/10.2174/1874331501509010001

Malik, N.S.A., Perez, J.L., Kunta, M., Patt, J.M., Mangan, R.L. (2014). Changes in free amino acids and polyamine levels in Satsuma leaves in response to Asian citrus psyllid infestation and water stress. *Insect Sci* 21, 707–716. https://doi.org/10.1111/1744-7917.12075

Mann, R.S., Ali, J.G., Hermann, S.L., Tiwari, S., Pelz-Stelinski, K.S., Alborn, H.T., Stelinski, L.L. (2012). Induced Release of a Plant-Defense Volatile 'Deceptively' Attracts Insect Vectors to Plants Infected with a Bacterial Pathogen. *PLoS Pathog* 8, e1002610. https://doi.org/10.1371/journal.ppat.1002610

Marygold, S.J., Roote, J., Reuter, G., Lambertsson, A., Ashburner, M., Millburn, G.H., Harrison, P.M., Yu, Z., Kenmochi, N., Kaufman, T.C., Leevers, S.J., Cook, K.R. (2007). The ribosomal protein genes and Minute loci of *Drosophila melanogaster*. *Genome Biol* 8, R216. https://doi.org/10.1186/gb-2007-8-10-r216

Mathesius, U. (2018). Flavonoid Functions in Plants and Their Interactions with Other Organisms. *Plants* 7, 30. https://doi.org/10.3390/plants7020030

Matias, P., Barrote, I., Azinheira, G., Continella, A., Duarte, A. (2023). Citrus Pruning in the Mediterranean Climate: A Review. *Plants* 12, 3360. https://doi.org/10.3390/plants12193360

Mauch-Mani, B., Baccelli, I., Luna, E., Flors, V. (2017). Defense Priming: An Adaptive Part of Induced Resistance. *Annu Rev Plant Biol* 68, 485–512. https://doi.org/10.1146/annurev-arplant-042916-041132

Mc Daniel, J.R., Moran, V.C. (1972). The parasitoid complex of the citrus psylla *Trioza erytreae* (Del Guercio) [Homoptera: Psyllidae]. *Entomophaga* 17, 297–317. https://doi.org/10.1007/BF02371184

Mejri, H., Khetatfa, T., Aidi Wannes, W., Smaoui, A., Saidani Tounsi, M. (2022). Histochemistry, chemical composition and antioxidant activity of *Citrus aurantium* L. essential oil during leaf development. *Journal of Essential Oil Research* 34, 329–338. https://doi.org/10.1080/10412905.2022.2067255

Micheloud, N.G., Castro, D.C., Buyatti, M.A., Gabriel, P.M., Gariglio, N.F. (2018). Factors affecting phenology of different Citrus varieties under the temperate climate conditions of Santa Fe, Argentina. *Rev Bras Frutic* 40. https://doi.org/10.1590/0100-29452018315

Milacic, M., Beavers, D., Conley, P., Gong, C., Gillespie, M., Griss, J., … D'Eustachio, P. (2024). The Reactome Pathway Knowledgebase 2024. *Nucleic Acids Res* 52, D672–D678. https://doi.org/10.1093/nar/gkad1025

Mito, T., Nakamura, T., Noji, S. (2010). Evolution of insect development: to the hemimetabolous paradigm. *Curr Opin Genet Dev* 20, 355–361. https://doi.org/10.1016/j.gde.2010.04.005

Molassiotis, A., Fotopoulos, V. (2011). Oxidative and nitrosative signaling in plants. *Plant Signal Behav* 6, 210–214. https://doi.org/10.4161/psb.6.2.14878

Moormann, J., Heinemann, B., Hildebrandt, T.M. (2022). News about amino acid metabolism in plant–microbe interactions. *Trends Biochem Sci* 47, 839–850. https://doi.org/10.1016/J.TIBS.2022.07.001

Moran, V.C. (1968a). Preliminary observations on the choice of host plants by adults of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: *J Entomol Soc South Afr* 31, 404–410. https://doi.org/10.10520/AJA00128789_3113

Moran, V.C. (1968b). The development of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae), on *Citrus limon* and four indigenous host plants. *J Entomol Soc South Afr* 31, 391–402. https://hdl.handle.net/10520/AJA00128789_3112

Moran, V.C., Blowers, J.R. (1967). On the biology of the South African citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). *J Entomol Soc South* 30, 96–106. https://doi.org/10.10520/AJA00128789_2702

Moran, V.C., Brown, R.P. (1973). The antennae, host plant chemoreception and probing activity of the citrus psylla, *Trioza erytreae* (Del Guercio) (Homoptera: Psyllidae). *J Entomol Soc South* Afr 36, 191–202. https://doi.org/10.10520/AJA00128789_3434

Moran, V.C., Buchan, P.R. (1975). Oviposition by the Citrus Psylla, *Trioza erytreae* (Homoptera: Psyllidae), in relation to leaf hardiness. *Entomol Exp Appl* 18, 96–104. https://doi.org/10.1111/j.1570-7458.1975.tb00390.x

Mozoruk, J., Hunnicutt, L.E., Cave, R.D., Hunter, W.B., Bausher, M.G. (2006). Profiling transcriptional changes in *Citrus sinensis* (L.) Osbeck challenged by herbivory from the xylem-feeding leafhopper *Homalodisca coagulata* (Say) by cDNA macroarray analysis. *Plant Science* 170, 1068–1080. https://doi.org/10.1016/j.plantsci.2006.01.014

Müller, M., Buchbauer, G. (2011). Essential oil components as pheromones. A review. *Flavour Fragr J* 26, 357–377. https://doi.org/10.1002/ffj.2055

Munir, S., Ahmed, A., Li, Y., He, Pengbo, Singh, B.K., He, Pengfei, Li, X., Asad, S., Wu, Y., He, Y. (2021). The hidden treasures of citrus: finding Huanglongbing cure where it was lost. *Crit Rev Biotechnol* 42, 634-649. https://doi.org/10.1080/07388551.2021.1942780

Mustafa, T., Horton, D.R., Cooper, W.R., Swisher, K.D., Zack, R.S., Munyaneza, J.E. (2015). Interhaplotype Fertility and Effects of Host Plant on Reproductive Traits of Three Haplotypes of *Bactericera cockerelli* (Hemiptera: Triozidae). *Environ Entomol* 44, 300–308. https://doi.org/10.1093/ee/nvu029

Nagarajan, S., Grewal, S.S. (2014). An investigation of nutrient-dependent mRNA translation in *Drosophila* larvae. *Biol Open* 3, 1020–1031. https://doi.org/10.1242/bio.20149407

Nehela, Y., Hijaz, F., Elzaawely, A.A., El-Zahaby, H.M., Killiny, N. (2018). Citrus phytohormonal response to *Candidatus* Liberibacter asiaticus and its vector *Diaphorina citri*. *Physiol Mol Plant Pathol* 102, 24–35. https://doi.org/10.1016/j.pmpp.2017.11.004

Nehela, Y., Killiny, N. (2019). '*Candidatus* Liberibacter asiaticus' and Its Vector, *Diaphorina citri*, Augment the Tricarboxylic Acid Cycle of Their Host via the γ-Aminobutyric Acid Shunt and Polyamines Pathway. *Molecular Plant-Microbe Interactions®* 32, 413–427. https://doi.org/10.1094/MPMI-09-18-0238-R

Nunes, P., Branco, M., Franco, J.C., Santos, M. (2025). Patterns, processes and scales shaping invasive pest species dynamics within agricultural landscapes: Modelling the spread of the African citrus psyllid in European lemon orchards. *Agric Syst* 226, 104295. https://doi.org/10.1016/j.agsy.2025.104295

Nykoll, L., Bottoms, J., Meghji, M., Hart, H., Que, Q., Pulliam, D. (2012). Corn event MIR162. US 8232456 B2.

Oates, C., Denby, K., Myburg, A., Slippers, B., Naidoo, S. (2016). Insect Gallers and Their Plant Hosts: From Omics Data to Systems Biology. *Int J Mol Sci* 17, 1891. https://doi.org/10.3390/ijms17111891

Oliveira, D.F., Benhadi-Marín, J., Neto, J., Sanz, L., Garzo, E., Aguiar, A., Fereres, A., Pereira, J.A. (2022). Kaolin particle films disrupt landing, settling behavior and feeding of *Trioza erytrae* on lemon plants. *Pest Manag Sci* 78, 4753–4763. https://doi.org/10.1002/ps.7095

Oliveros, J.C. (2015). Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html.

Osório, H., Silva, C., Ferreira, M., Gullo, I., Máximo, V., Barros, R., Mendonça, F., Oliveira, C., Carneiro, F. (2021). Proteomics Analysis of Gastric Cancer Patients with Diabetes Mellitus. *J Clin Med* 10, 1–14. https://doi.org/10.3390/JCM10030407

Ostendorp, A., Pahlow, S., Krüßel, L., Hanhart, P., Garbe, M.Y., Deke, J., Giavalisco, P., Kehr, J. (2017). Functional analysis of *Brassica napus* phloem protein and ribonucleoprotein complexes. *New Phytologist* 214, 1188–1197. https://doi.org/10.1111/nph.14405

Öztürk-Çolak, A., Marygold, S.J., Antonazzo, G., Attrill, H., Goutte-Gattat, D., Jenkins, V.K, ... Lovato, T. (2024). FlyBase: updates to the *Drosophila* genes and genomes database. *Genetics* 227. https://doi.org/10.1093/genetics/iyad211

Paiva, P.E.B., Cota, T., Neto, L., Soares, C., Tomás, J.C., Duarte, A. (2020). Water Vapor Pressure Deficit in Portugal and Implications for the Development of the Invasive African Citrus Psyllid *Trioza erytreae*. *Insects* 11, 229. https://doi.org/10.3390/insects11040229

Panchal, P., Miller, A.J., Giri, J. (2021). Organic acids: versatile stress-response roles in plants. *J Exp Bot* 72, 4038–4052. https://doi.org/10.1093/jxb/erab019

Pang, Z., Lu, Y., Zhou, G., Hui, F., Xu, L., Viau, C., Spigelman, A.F., MacDonald, P.E., Wishart, D.S., Li, S., Xia, J. (2024). MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Res* 55, W398-W406. https://doi.org/10.1093/nar/gkae253

Paré, P.W., Tumlinson, J.H. (1999). Plant Volatiles as a Defense against Insect Herbivores. *Plant Physiol* 121, 325–332. https://doi.org/10.1104/pp.121.2.325

Paris, T.M., Allan, S.A., Hall, D.G., Hentz, M.G., Hetesy, G., Stansly, P.A. (2016). Host plant affects morphometric variation of *Diaphorina citri* (Hemiptera: Liviidae). *PeerJ* 4, e2663. https://doi.org/10.7717/peerj.2663

Parthasarathy, R., Palli, S.R. (2011). Molecular analysis of nutritional and hormonal regulation of female reproduction in the red flour beetle, *Tribolium castaneum*. *Insect Biochem Mol Biol* 41, 294–305. https://doi.org/10.1016/j.ibmb.2011.01.006

Patil, I. (2021). Visualizations with statistical details: The “ggstatsplot” approach. *J Open Source Softw* 6, 3167. https://doi.org/10.21105/joss.03167

Patt, J.M., Robbins, P.S., Niedz, R., McCollum, G., Alessandro, R. (2018). Exogenous application of the plant signalers methyl jasmonate and salicylic acid induces changes in volatile emissions from citrus foliage and influences the aggregation behavior of Asian citrus psyllid (*Diaphorina citri*), vector of Huanglongbing. *PLoS One* 13, e0193724. https://doi.org/10.1371/journal.pone.0193724

Paul, S., Das, S. (2021). Natural insecticidal proteins, the promising bio-control compounds for future crop protection. *The Nucleus* 64, 7–20. https://doi.org/10.1007/s13237-020-00316-1

Peng, A., Zou, X., He, Y., Chen, S., Liu, X., Zhang, J., Zhang, Q., Xie, Z., Long, J., Zhao, X. (2021). Overexpressing a NPR1-like gene from *Citrus paradisi* enhanced Huanglongbing resistance in *C. sinensis*. *Plant Cell Rep* 40, 529–541. https://doi.org/10.1007/S00299-020-02648-3

Pérez-Hedo, M., Hoddle, M.S., Alferez, F., Batista, L., Beattie, G.A.C., Chakravarty, S., ... Urbaneja, A. (2025). Global strategies to manage huanglongbing (HLB) and its vectors: insights and implications for the Mediterranean region. *Entomologia Generalis* 45, 1–16. https://doi.org/10.1127/entomologia/2024/2877

Pérez-Otero, R., Pérez-Turco, R., Neto, J., Fereres, A. (2024). The African Psyllid *Trioza erytreae* Del Guercio (1918) Is Very Sensitive to Low Relative Humidity and High Temperatures. *Insects* 15, 62. https://doi.org/10.3390/insects15010062

Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D.J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., Vizcaíno, J.A. (2022). The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Res* 50, D543–D552. https://doi.org/10.1093/nar/gkab1038

Pitino, M., Sturgeon, K., Dorado, C., Cano, L.M., Manthey, J.A., Shatters, R.G., Rossi, L. (2020). *Quercus* leaf extracts display curative effects against *Candidatus* Liberibacter asiaticus that restore leaf physiological parameters in HLB-affected citrus trees. *Plant Physiology and Biochemistry* 148, 70–79. https://doi.org/10.1016/j.plaphy.2020.01.013

Plata-Rueda, A., Campos, J.M., da Silva Rolim, G., Martínez, L.C., Dos Santos, M.H., Fernandes, F.L., Serrão, J.E., Zanuncio, J.C. (2018). Terpenoid constituents of cinnamon and clove essential oils cause toxic effects and behavior repellency response on granary weevil, *Sitophilus granarius*. *Ecotoxicol Environ Saf* 156, 263–270. https://doi.org/10.1016/j.ecoenv.2018.03.033

Pollard, D.G. (1971). The use of polyporus for the investigation of stylet behaviour in the hemiptera. *Entomol Exp Appl* 14, 283–296. https://doi.org/10.1111/j.1570-7458.1971.tb00166.x

Portillo-Estrada, M., Kazantsev, T., Niinemets, Ü. (2017). Fading of wound-induced volatile release during *Populus tremula* leaf expansion. *J Plant Res* 130, 157–165. https://doi.org/10.1007/s10265-016-0880-6

Primo-Millo, E., Agustí, M. (2020). Vegetative growth, in Talon, M., Caruso, M., Gmitter, F.G. *The Genus Citrus*. Elsevier, (pp. 193–217). https://doi.org/10.1016/B978-0-12-812163-4.00010-3

Pullock, D.A., Krüger, K., Manrakhan, A., Yusuf, A.A., Weldon, C.W. (2024). Addition of Selected Plant-Derived Semiochemicals to Yellow Sticky Traps Does Not Improve Citrus Psyllid Captures. *J Chem Ecol* 50, 701-713. https://doi.org/10.1007/s10886-024-01491-0

QGIS Development Team, 2020. QGIS Geographic Information System [WWW Document] URL https://qgis.org/ (accessed 18. 7.22).

Quintana-González de Chaves, M., Montero-Gomez, N., Álvarez-Acosta, C., Hernández-Suárez, E., Hervalejo, A., Arjona-López, J.M., Arenas-Arenas, F.J. (2024). The Combination of Citrus Rootstock and Scion Cultivar Influences *Trioza erytreae* (Hemiptera: Triozidae) Survival, Preference Choice and Oviposition. *Insects* 15, 363. https://doi.org/10.3390/insects15050363

Quintana-González De Chaves, M., Teresani, G.R., Hernández-Suárez, E., Bertolini, E., Moreno, A., Fereres, A., Cambra, M., Siverio, F. (2020). "*Candidatus* Liberibacter solanacearum" Is Unlikely to Be Transmitted Spontaneously from Infected Carrot Plants to Citrus Plants by *Trioza Erytreae*. *Insects* 11, 514. https://doi.org/10.3390/insects11080514

R Core Team (2020). R: A Language and Environment for Statistical Computing. Vienna, Austria. [WWW Document] URL https://www.R-project.org (accessed 18. 7.22).

Radwanski, E.R., Last, R.L. (1995). Tryptophan biosynthesis and metabolism: biochemical and molecular genetics. *Plant Cell* 7, 921–934. https://doi.org/10.1105/tpc.7.7.921

Ramsey, J.S., Ammar, E.-D., Mahoney, J.E., Rivera, K., Johnson, R., Igwe, D.O., Thannhauser, T.W., MacCoss, M.J., Hall, D.G., Heck, M. (2022). Host Plant Adaptation Drives Changes in *Diaphorina citri* Proteome Regulation, Proteoform Expression, and Transmission of '*Candidatus* Liberibacter asiaticus', the Citrus Greening Pathogen. *Phytopathology* 112, 101–115. https://doi.org/10.1094/PHYTO-06-21-0275-R

Ramsey, J.S., Johnson, R.S., Hoki, J.S., Kruse, A., Mahoney, J., Hilf, M.E., Hunter, W.B., Hall, D.G., Schroeder, F.C., MacCoss, M.J., Cilia, M. (2015). Metabolic interplay between the asian citrus psyllid and its profftella symbiont: An achilles' heel of the citrus greening insect vector. *PLoS One* 10, e0140826. https://doi.org/10.1371/journal.pone.0140826

Rani, L., Saini, S., Shukla, N., Chowdhuri, D.K., Gautam, N.K. (2020). High sucrose diet induces morphological, structural and functional impairments in the renal tubules of *Drosophila melanogaster*: A model for studying type-2 diabetes mediated renal

tubular dysfunction. *Insect Biochem Mol Biol* 125, 103441. https://doi.org/10.1016/j.ibmb.2020.103441

Rao, C., George, A., Rahangadale, S. (2018a). Exogenous Phytohormones Induced Resistance in *Citrus reticulata* Blanco against *Diaphorina citri* Kuwayama. *Pesticide Research Journal* 30, 224-229. https://doi.org/10.5958/2249-524X.2018.00035.3

Rao, M.J., Ding, F., Wang, N., Deng, X., Xu, Q. (2018b). Metabolic Mechanisms of Host Species Against Citrus Huanglongbing (Greening Disease). *CRC Crit Rev Plant Sci* 37, 496–511. https://doi.org/10.1080/07352689.2018.1544843

Rasool, B., Karpinska, B., Pascual, J., Kangasjärvi, S., Foyer, C.H. (2020). Catalase, glutathione, and protein phosphatase 2A-dependent organellar redox signalling regulate aphid fecundity under moderate and high irradiance. *Plant Cell Environ* 43, 209–222. https://doi.org/10.1111/pce.13669

Rawat, N., Kiran, S.P., Du, D., Gmitter, F.G., Deng, Z. (2015). Comprehensive meta-analysis, co-expression, and miRNA nested network analysis identifies gene candidates in citrus against Huanglongbing disease. *BMC Plant Biol* 15, 184. https://doi.org/10.1186/s12870-015-0568-4

Reynaud, B., Turpin, P., Molinari, F.M., Grondin, M., Roque, S., Chiroleu, F., Fereres, A., Delatte, H. (2022). The African citrus psyllid *Trioza erytreae*: An efficient vector of *Candidatus* Liberibacter asiaticus. *Front Plant Sci* 13. https://doi.org/10.3389/FPLS.2022.1089762

Riaz, U., Kharal, M.A., Murtaza, G., Zaman, Q. uz, Javaid, S., Malik, H.A., Aziz, H., Abbas, Z. (2018). Prospective Roles and Mechanisms of Caffeic Acid in Counter Plant Stress: A Mini Review. *Pakistan Journal of Agricultural Research* 32, 8-19. https://doi.org/10.17582/journal.pjar/2019/32.1.8.19

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 43, e47–e47. https://doi.org/10.1093/nar/gkv007

Rizzi, Y.S., Monteoliva, M.I., Fabro, G., Grosso, C.L., LarÃ³vere, L.E., Alvarez, M.E. (2015). P5CDH affects the pathways contributing to Pro synthesis after ProDH activation by biotic and abiotic stress conditions. *Front Plant Sci* 6. https://doi.org/10.3389/fpls.2015.00572

Roberts, D.B. (2006). *Drosophila melanogaster*: the model organism. *Entomol Exp Appl* 121, 93–103. https://doi.org/10.1111/j.1570-8703.2006.00474.x

Roberts, R., Cook, G., Grout, T.G., Khamis, F., Rwomushana, I., Nderitu, P.W., Seguni, Z., Materu, C.L., Steyn, C., Pietersen, G., Ekesi, S., le Roux, H.F. (2017). Resolution of the Identity of '*Candidatus* Liberibacter' Species from Huanglongbing-Affected Citrus in East Africa. *Plant Dis* 101, 1481–1488. https://doi.org/10.1094/PDIS-11-16-1655-RE

Rodríguez-Celma, J., Ceballos-Laita, L., Grusak, M.A., Abadía, J., López-Millán, A.-F. (2016). Plant fluid proteomics: Delving into the xylem sap, phloem sap and apoplastic fluid proteomes. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* 1864, 991–1002. https://doi.org/10.1016/j.bbapap.2016.03.014

Roy, A., Gupta, S., Hess, D., Das, K.P., Das, S. (2014). Binding of insecticidal lectin *Colocasia esculenta* tuber agglutinin (CEA) to midgut receptors of *Bemisia tabaci* and *Lipaphis erysimi* provides clues to its insecticidal potential. *Proteomics* 14, 1646–1659. https://doi.org/10.1002/pmic.201300408

Ruan, J., Zhou, Y., Zhou, M., Yan, J., Khurshid, M., Weng, W., Cheng, J., Zhang, K. (2019). Jasmonic Acid Signaling Pathway in Plants. *Int J Mol Sci* 20, 2479. https://doi.org/10.3390/ijms20102479

Russell, C.W., Pelz-Stelinski, K.S. (2015). Development of an artificial diet and feeding system for juvenile stages of the Asian citrus psyllid, *Diaphorina citri*. *Entomol Exp Appl* 154, 171–176. https://doi.org/10.1111/eea.12268

Salvucci, M.E., Rosell, R.C., Brown, J.K. (1998). Uptake and metabolism of leaf proteins by the silverleaf whitefly. *Insect Biochemistry and Physiology* 39, 155–165. https://doi.org/10.1002/(SICI)1520-6327(1998)39:4<155::AID-ARCH3>3.0.CO;2-%23

Samways, M.J., Manicom, B.Q. (1983). Immigration, Frequency Distributions and Dispersion Patterns of the Psyllid *Trioza erytreae* (Del Guercio) in a Citrus Orchard. *J Appl Ecol* 20, 463. https://doi.org/10.2307/2403520

Saplaoura, E., Kragler, F. (2016). Mobile Transcripts and Intercellular Communication in Plants. In Lin, C., Luan, S. (Eds.) *The Enzymes* Elsevier, (pp. 1–29). https://doi.org/10.1016/bs.enz.2016.07.001

Schultz, J.C., Appel, H.M., Ferrieri, A.P., Arnold, T.M. (2013). Flexible resource allocation during plant defense responses. *Front Plant Sci* 4, 324. https://doi.org/10.3389/fpls.2013.00324

Schuman, M.C. (2023). Where, When, and Why Do Plant Volatiles Mediate Ecological Signaling? The Answer Is Blowing in the Wind. *Annu Rev Plant Biol* 74, 609–633. https://doi.org/10.1146/annurev-arplant-040121-114908

Schuman, M.C., Baldwin, I.T. (2016). The Layers of Plant Responses to Insect Herbivores. *Annu Rev Entomol* 61, 373–394. https://doi.org/10.1146/annurev-ento-010715-023851

Shafqat, W., Jaskani, M.J., Maqbool, R., Khan, A.S., Naqvi, S.A., Ali, Z., Khan, I.A. (2020). Genome Wide Analysis of *Citrus sinensis* Heat Shock Proteins. *Iran J Biotechnol* 18, 29–38. https://doi.org/10.30498/IJB.2020.2529

Sharma, A., Ference, C.M., Shantharaj, D., Baldwin, E.A., Manthey, J.A., Jones, J.B. (2021). Transcriptomic analysis of changes in *Citrus* × *microcarpa* gene expression post *Xanthomonas citri* subsp. *citri* infection. *European Journal of Plant Pathology* 162, 163–181. https://doi.org/10.1007/S10658-021-02394-6

Sharma, A., Raman, A. (2017). Feeding biology and nutritional physiology of Psylloidea (Insecta: Hemiptera): implications in host–plant relations. *Curr Sci* 113, 1543–1552. http://dx.doi.org/10.18520/cs/v113/i08/1543-1552

Sharma, P., Dubey, R.S. (2016). Protein synthesis by plants under stressful conditions. In Pessarkli, M. (Ed.) *Handbook of Plant and Crop Stress* (pp. 465–518). https://doi.org/10.1201/9781351104609-22

Shashank, P.R., Bollineni, H. (2014). Insect proteomics: present and future prospective. *Current Biotica* 7, 336–342. https://www.cabidigitallibrary.org/doi/epdf/10.5555/20143146171

Sheu, S.-J., Chieh, C.-L., Weng, W.-C. (2001). Capillary electrophoretic determination of the constituents of *Artemisiae Capillaris* Herba. *J Chromatogr* A 911, 285–293. https://doi.org/10.1016/S0021-9673(01)00513-1

Shi, H., Xiong, L., Stevenson, B., Lu, T., Zhu, J.K. (2002). The Arabidopsis salt overly sensitive 4 Mutants Uncover a Critical Role for Vitamin B6 in Plant Salt Tolerance. *Plant Cell* 14, 575–588. https://doi.org/10.1105/TPC.010417

Shi, Q., Febres, V.J., Jones, J.B., Moore, G.A. (2015). Responsiveness of different citrus genotypes to the *Xanthomonas citri* ssp. *citri* -derived pathogen-associated molecular pattern (PAMP) flg22 correlates with resistance to citrus canker. *Mol Plant Pathol* 16, 507–520. https://doi.org/10.1111/mpp.12206

Shi, Q., George, J., Krystel, J., Zhang, S., Lapointe, S.L., Stelinski, L.L., Stover, E. (2019). Hexaacetyl-chitohexaose, a chitin-derived oligosaccharide, transiently activates citrus defenses and alters the feeding behavior of Asian citrus psyllid. *Hortic Res* 6, 1–10. https://doi.org/10.1038/s41438-019-0158-y

Shi, Q., Han, Y., Jiang, J. (2014). Suppressor of fused impedes Ci/Gli nuclear import by opposing Trn/Kapβ2 in Hedgehog signaling. *J Cell Sci* 127, 1092-1103. https://doi.org/10.1242/jcs.142828

Shukla, E., Thorat, L.J., Nath, B.B., Gaikwad, S.M. (2015). Insect trehalase: Physiological significance and potential applications. *Glycobiology* 25, 357–367. https://doi.org/10.1093/glycob/cwu125

Si, W., Wang, Q., Li, Y., Dong, D. (2020). Label-free quantitative proteomic analysis of insect larval and metamorphic molts. *BMC Dev Biol* 20, 24. https://doi.org/10.1186/s12861-020-00227-z

Sibout, R., Eudes, A., Mouille, G., Pollet, B., Lapierre, C., Jouanin, L., Séguin, A. (2005). *CINNAMYL ALCOHOL DEHYDROGENASE-C* and *-D* Are the Primary Genes Involved in Lignin Biosynthesis in the Floral Stem of Arabidopsis. *Plant Cell* 17, 2059–2076. https://doi.org/10.1105/tpc.105.030767

Simms, E.L., Triplett, J. (1994). Costs and Benefits of Plant Responses to Disease: Resistance and Tolerance. *Evolution* 48, 1973-1985. https://doi.org/10.2307/2410521

Singerman, A., Lence, S.H., Useche, P. (2017). Is area-wide pest management useful? The case of citrus greening. *Appl Econ Perspect Policy* 39, 609–634. https://doi.org/10.1093/aepp/ppx030

Singerman, A., Rogers, M.E. (2020). The Economic Challenges of Dealing with Citrus Greening: The Case of Florida. *J Integr Pest Manag* 11, 3–4. https://doi.org/10.1093/jipm/pmz037

Singh, A.K., Lakhotia, S.C. (2012). The hnRNP A1 homolog Hrp36 is essential for normal development, female fecundity, omega speckle formation and stress tolerance in *Drosophila melanogaster*. *J Biosci* 37, 659–678. https://doi.org/10.1007/s12038-012-9239-x

Squarr, A.J., Brinkmann, K., Chen, B., Steinbacher, T., Ebnet, K., Rosen, M.K., Bogdan, S. (2016). Fat2 acts through the WAVE regulatory complex to drive collective cell migration during tissue rotation. *Journal of Cell Biology* 212, 591–603. https://doi.org/10.1083/jcb.201508081

Stockton, D.G., Pescitelli, L.E., Ebert, T.A., Martini, X., Stelinski, L.L. (2017). Induced Preference Improves Offspring Fitness in a Phytopathogen Vector. *Environ Entomol* 46, 1090–1097. https://doi.org/10.1093/ee/nvx135

Su, Y., Ospina, J.K., Zhang, J., Michelson, A.P., Schoen, A.M., Zhu, A.J. (2011). Sequential Phosphorylation of Smoothened Transduces Graded Hedgehog Signaling. *Sci Signal* 4. https://doi.org/10.1126/scisignal.2001747

Sun, L., Nasrullah, Ke, F., Nie, Z., Wang, P., Xu, J. (2019). Citrus genetic engineering for disease resistance: past, present and future. *Int J Mol Sci* 20, 5256. https://doi.org/10.3390/ijms20215256

Sun, X., Yu, T., Bin, M., Hu, C., Bi, F., Peng, X., Yi, G., Zhang, X. (2022). Transcriptomic analysis reveals the defense mechanisms of citrus infested with *Diaphorina citri*. *Hortic Plant J*. https://doi.org/10.1016/J.HPJ.2022.07.008

Surano, A., del Grosso, C., Musio, B., Todisco, S., Giampetruzzi, A., Altamura, G., Saponari, M., Gallo, V., Mastrorilli, P., Boscia, D., Saldarelli, P. (2024). Exploring the xylem-sap to unravel biological features of *Xylella fastidiosa* subspecies pauca ST53 in immune, resistant and susceptible crop species through metabolomics and in vitro studies. *Front Plant Sci* 14. https://doi.org/10.3389/fpls.2023.1343876

Suzuki, K., Okamoto, T., Kidokoro, Y. (2002). Biphasic modulation of synaptic transmission by hypertonicity at the embryonic *Drosophila* neuromuscular junction. *J Physiol* 545, 119–131. https://doi.org/10.1113/jphysiol.2002.026898

Szczesny, D., Bartosińska, E., Jacyna, J., Patejko, M., Siluk, D., Kaliszan, R. (2018). Quantitative determination of trigonelline in mouse serum by means of hydrophilic interaction liquid chromatography–MS/MS analysis: Application to a pharmacokinetic study. *Biomedical Chromatography* 32, e4054. https://doi.org/10.1002/bmc.4054

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N.T., Legeay, M., Fang, T., Bork, P., Jensen, L.J., von Mering, C. (2021). The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 49, D605–D612. https://doi.org/10.1093/NAR/GKAA1074

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A.L., Fang, T., Doncheva, N.T., Pyysalo, S., Bork, P., Jensen, L.J., von Mering, C. (2023). The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res* 51, D638–D646. https://doi.org/10.1093/nar/gkac1000

Taamalli, A., Arráez-Román, D., Abaza, L., Iswaldi, I., Fernández-Gutiérrez, A., Zarrouk, M., Segura-Carretero, A. (2015). LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species *Mentha pulegium* and *Origanum majorana*. *Phytochemical Analysis* 26, 320–330. https://doi.org/10.1002/pca.2566

Tamesse, J.L. (2000). Réceptivité à *Trioza erytreae* (Del Guercio) de variétés d'agrumes au Cameroun. Fruits 55, 389–400. https://revues.cirad.fr/index.php/fruits/article/view/35719/35462

Tamesse, J.L., Messi, J. (2004). Facteurs influençant la dynamique des populations du psylle Africain des agrumes *Trioza erytreae* Del Guercio (Hemiptera: Triozidae) au Cameroun. *Int J Trop Insect Sci* 24, 213–227. https://doi.org/10.1079/IJT200429

Tamesse, J.L., Messi, J. (2002). Incidence de *Trioza erytreae* (Del Guercio) (Homoptera: Triozidae), psylle vecteur du greening sur la sensibilité des plantules d'agrumes dans une pépinière au Cameroun. *Insect Science and its Application* 22, 97–103. https://doi.org/10.1017/s1742758400015174

Tang, H., Rompani, S.B., Atkins, J.B., Zhou, Y., Osterwalder, T., Zhong, W. (2005). Numb Proteins Specify Asymmetric Cell Fates via an Endocytosis- and Proteasome-Independent Pathway. *Mol Cell Biol* 25, 2899–2909. https://doi.org/10.1128/MCB.25.8.2899-2909.2005

Tansey, J.A., Jones, M.M., Vanaclocha, P., Robertson, J., Stansly, P.A. (2015). Costs and benefits of frequent low-volume applications of horticultural mineral oil for management of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Crop Protection* 76, 59–67. https://doi.org/10.1016/j.cropro.2015.06.011

Teixeira, A., Noronha, H., Frusciante, S., Diretto, G., Gerós, H. (2023). The grapevine metabolite profile of phloem sap is modified by flavescence dorée. *OENO One* 57, 307–320. https://doi.org/10.20870/oeno-one.2023.57.1.7302

Thiele, B., Füllner, K., Stein, N., Oldiges, M., Kuhn, A.J., Hofmann, D. (2008). Analysis of amino acids without derivatization in barley extracts by LC-MS-MS. *Anal Bioanal Chem* 391, 2663–2672. https://doi.org/10.1007/s00216-008-2167-9

Tholl, D., Lee, S. (2011). Terpene Specialized Metabolism in *Arabidopsis thaliana*. *Arabidopsis Book* 9, e0143. https://doi.org/10.1199/tab.0143

Thompson, G.A., Goggin, F.L. (2006). Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *J Exp Bot* 57, 755–766. https://doi.org/10.1093/JXB/ERJ135

Tine, Y., Renucci, F., Costa, J., Wélé, A., Paolini, J. (2017). A Method for LC-MS/MS Profiling of Coumarins in *Zanthoxylum zanthoxyloides* (Lam.) B. Zepernich and Timler Extracts and Essential Oils. *Molecules* 22, 174. https://doi.org/10.3390/molecules22010174

Tlak Gajger, I., Dar, S.A. (2021). Plant Allelochemicals as Sources of Insecticides. *Insects* 12, 189. https://doi.org/10.3390/insects12030189

Trippe, R.C., Pilon-Smits, E.A.H. (2021). Selenium transport and metabolism in plants: Phytoremediation and biofortification implications. *J Hazard Mater* 404, 124178. https://doi.org/10.1016/j.jhazmat.2020.124178

Tsang, W.Y., Lemire, B.D. (2003). Mitochondrial ATP synthase controls larval development cell nonautonomously in *Caenorhabditis elegans*. *Developmental Dynamics* 226, 719–726. https://doi.org/10.1002/dvdy.10272

Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M.Y., Geiger, T., Mann, M., Cox, J. (2016). The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods* 2016 13, 731–740. https://doi.org/10.1038/nmeth.3901

Upchurch, R.G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters* 30, 967–977. https://doi.org/10.1007/S10529-008-9639-Z

Urbaneja-Bernat, P., González-Cabrera, J., Hernández-Suárez, E., Tena, A. (2023). Honeydew of HLB vector, *Trioza erytreae*, increases longevity, egg load and parasitism of its main parasitoid *Tamarixia dryi*. *Biological Control* 179, 105169. https://doi.org/10.1016/j.biocontrol.2023.105169

Urbaneja-Bernat, P., Hernández-Suárez, E., Tena, A., Urbaneja, A. (2020). Preventive measures to limit the spread of *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae) in mainland Europe. *Journal of Applied Entomology* 144, 553–559. https://doi.org/10.1111/jen.12771

Urbaneja-Bernat, P., Pérez-Rodríguez, J., Krüger, K., Catalán, J., Rizza, R., Hernández-Suárez, E., Urbaneja, A., Tena, A. (2019). Host range testing of *Tamarixia dryi* (Hymenoptera: Eulophidae) sourced from South Africa for classical biological control of *Trioza erytreae* (Hemiptera: Psyllidae) in Europe. *Biological Control* 135, 110–116. https://doi.org/10.1016/j.biocontrol.2019.04.018

Uzun, A., Yesiloglu, T. (2012). Genetic Diversity in Citrus, in Çalişkan, M. (Ed.) *Genetic Diversity in Plants* InTech (pp. 213- 230). https://doi.org/10.5772/32885

Vaishnav, D., Chowdhury, P. (2023). Types and Function of Phytohormone and Their Role in Stress. In Hussain, S., Awan, T.H., Waraich, E.A., Iqbal, M. (Eds.) *Plant Abiotic Stress Responses and Tolerance Mechanisms* IntechOpen https://doi.org/10.5772/intechopen.109325

Valterová, I., Nehlin, G., Borg-Karlson, A.K. (1997). Host plant chemistry and preferences in egg-laying *Trioza apicalis* (Homoptera, Psylloidea). *Biochem Syst Ecol* 25, 477–491. https://doi.org/10.1016/S0305-1978(97)00028-8

Van den Berg, M. (1992). Developmental biology and population studies on the citrus psylla *Trioza erytreae* (Del Guercio) (Hemiptera: Triozidae). Fruits 47, 583–589.

Van den Berg, M., Deacon, V.E. (1988). Dispersal of the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae), in the absence of its host plants. *Phytophylactica* 20, 361–368. https://doi.org/10.10520/AJA03701263_1288

Van den Berg, M.A. (1990). The citrus pyslla, *Trioza erytreae* (Del Guercio) (hemiptera: Triozidae): A review. *Agric Ecosyst Environ* 30, 171–194. https://doi.org/10.1016/0167-8809(90)90104-L

Van den Berg, M.A. (1986). Effects of citrus cultivars and reduced irrigation on availability of new growth for Citrus psylla breeding. Fruits 41, 597–604.

Van den Berg, M.A., Anderson, S.H., Deacon, V.E. (1991a). Population studies of the citrus psylla, *Trioza erytreae*: Factors influencing population size. *Phytoparasitica* 19, 183–193. https://doi.org/10.1007/BF02981116

Van den Berg, M.A., Anderson, S.H., Deacon, V.E. (1991b). Population studies of the citrus psylla, *Trioza erytreae*: Factors influencing dispersal. Phytoparasitica 19, 283–289. https://doi.org/10.1007/BF02980962

Van den Berg, M.A., Deacon, V.E., De Jager, K. (1990). Ecology of the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae) 1. Daily activities and habits of adults. *Phytophylactica* 22, 323–328. https://doi.org/10.10520/AJA03701263_1351

Van den Berg, M.A., Deacon, V.E., Steenekamp, P.J. (1991c). Dispersal within and between citrus orchards and native hosts, and nymphal mortality of citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae). *Agric Ecosyst Environ* 35, 297–309. https://doi.org/10.1016/0167-8809(91)90080-H

Van den Berg, M.A., Deacon, V.E., Thomas, C.D. (1991d). Ecology of the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae). 4. Settling and general behaviour of nymphs. Phytophylactica 23, 201–206.

Van den Wildenberg, S.M.J.L., Tao, L., Kapitein, L.C., Schmidt, C.F., Scholey, J.M., Peterman, E.J.G. (2008). The Homotetrameric Kinesin-5 KLP61F Preferentially Crosslinks Microtubules into Antiparallel Orientations. *Current Biology* 18, 1860–1864. https://doi.org/10.1016/j.cub.2008.10.026

Van Der Merwe, C.P. (1923). The Citrus Psylla (*TRIOZA MERWEI*, Pettey.). *Journal of the Department of Agriculture, (Division of Entomology, Durban)* 7, 135–141. https://hdl.handle.net/10520/AJA0000020_1462

Villarreal, C.M., Darakananda, K., Wang, V.R., Jayaprakash, P.M., Suzuki, Y. (2015). Hedgehog signaling regulates imaginal cell differentiation in a basally branching holometabolous insect. *Dev Biol* 404, 125–135. https://doi.org/10.1016/j.ydbio.2015.05.020

Viola, R.E. (2000). L-Aspartase: New Tricks from an Old Enzyme. In Purich, D.L. (Ed.) *Advances in Enzymology and Related Areas of Molecular Biology* Wiley (pp. 295–341). https://doi.org/10.1002/9780470123201.ch7

Viquez, N.M., Li, C.R., Wairkar, Y.P., DiAntonio, A. (2006). The B' Protein Phosphatase 2A Regulatory Subunit *well-rounded* Regulates Synaptic Growth and Cytoskeletal Stability at the *Drosophila* Neuromuscular Junction. *The Journal of Neuroscience* 26, 9293–9303. https://doi.org/10.1523/JNEUROSCI.1740-06.2006

Vranova, V., Lojkova, L., Rejsek, K., Formanek, P. (2013). Significance of the Natural Occurrence of L- Versus D-Pipecolic Acid: A Review. *Chirality* 25, 823–831. https://doi.org/10.1002/chir.22237

Walker, G.P. (2022). Sieve element occlusion: Interactions with phloem sap-feeding insects. A review. *J Plant Physiol* 269, 153582. https://doi.org/10.1016/J.JPLPH.2021.153582

Walling, L.L. (2008). Avoiding Effective Defenses: Strategies Employed by Phloem-Feeding Insects. *Plant Physiol* 146, 859–866. https://doi.org/10.1104/PP.107.113142

Walsh, K., Schena, M., Flint, A.J., Koshland, D.E. (1987). Compensatory regulation in metabolic pathways--responses to increases and decreases in citrate synthase levels. *Biochem Soc Symp* 54, 183–95. PMID: 3332995.

Wang, J., Constabel, C.P. (2004). Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). *Planta* 220, 87–96. https://doi.org/10.1007/s00425-004-1327-1

Wang, P., Lu, P.-F., Zheng, X.-L., Chen, L.-Z., Lei, C.-L., Wang, X.-P. (2013). New Artificial Diet for Continuous Rearing of the Bean Pod Borer, *Maruca vitrata*. *Journal of Insect Science* 13, 1–11. https://doi.org/10.1673/031.013.12101

War, A.R., Paulraj, M.G., Ahmad, T., Buhroo, A.A., Hussain, B., Ignacimuthu, S., Sharma, H.C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signal Behav* 7, 1306–1320. https://doi.org/10.4161/psb.21663

Wei, X., Mira, A., Yu, Q., Gmitter, F.G. (2021). The Mechanism of Citrus Host Defense Response Repression at Early Stages of Infection by Feeding of *Diaphorina citri* Transmitting *Candidatus* Liberibacter asiaticus. *Front Plant Sci* 12, 971. https://doi.org/10.3389/fpls.2021.635153

White, T.C.R. (1968). Uptake of water by eggs of *Cardiaspina densitexta* (Homoptera: Psyllidae) from leaf of host plant. *J Insect Physiol* 14, 1669–1683. https://doi.org/10.1016/0022-1910(68)90100-5

Wickham, H. (2016). ggplot2. In Gentleman, R., Hornik, K., Parmigiani, G. *Use R!* Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-24277-4

Will, T., Furch, A.C.U., Zimmermann, M.R. (2013). How phloem-feeding insects face the challenge of phloem-located defenses. *Front Plant Sci* 4, 336. https://doi.org/10.3389/FPLS.2013.00336/BIBTEX

Wishart, D.S., Tzur, D., Knox, C., Eisner, R., Guo, A.C., Young, N., … H.J., Querengesser, L. (2007). HMDB: the Human Metabolome Database. *Nucleic Acids Res* 35, D521–D526. https://doi.org/10.1093/nar/gkl923

Wong, L.C., Costa, A., McLeod, I., Sarkeshik, A., Yates, J., Kyin, S., Perlman, D., Schedl, P. (2011). The Functioning of the Drosophila CPEB Protein Orb Is Regulated by Phosphorylation and Requires Casein Kinase 2 Activity. *PLoS One* 6, e24355. https://doi.org/10.1371/journal.pone.0024355

Wu, X., Yan, J., Wu, Y., Zhang, H., Mo, S., Xu, X., Zhou, F., Ding, H. (2019). Proteomic analysis by iTRAQ-PRM provides integrated insight into mechanisms of resistance in pepper to *Bemisia tabaci* (Gennadius). *BMC Plant Biol* 19, 270. https://doi.org/10.1186/s12870-019-1849-0

Wulansari, A.D., Hayati, D., Long, D.X., Choi, K., Hong, J. (2023). Hydroxycinnamic acid derivatives for UV-selective and visibly transparent dye-sensitized solar cells. *Sci Rep* 13, 3235. https://doi.org/10.1038/s41598-022-17236-6

Xia, F.-N., Zeng, B., Liu, H.-S., Qi, H., Xie, L.-J., Yu, L.-J., Chen, Q.-F., Li, J.-F., Chen, Y.-Q., Jiang, L., Xiao, S. (2020). SINAT E3 Ubiquitin Ligases Mediate FREE1 and VPS23A Degradation to Modulate Abscisic Acid Signaling. *Plant Cell* 32, 3290–3310. https://doi.org/10.1105/tpc.20.00267

Xu, Y., Fu, X. (2022). Reprogramming of Plant Central Metabolism in Response to Abiotic Stresses: A Metabolomics View. *Int J Mol Sci* 23, 5716. https://doi.org/10.3390/ijms23105716

Yamanaka, N., Rewitz, K.F., O'Connor, M.B. (2013). Ecdysone Control of Developmental Transitions: Lessons from *Drosophila* Research. *Annu Rev Entomol* 58, 497–516. https://doi.org/10.1146/annurev-ento-120811-153608

Yamasaki, Y., Kunoh, H., Yamamoto, H., Akimitsu, K. (2007). Biological roles of monoterpene volatiles derived from rough lemon (*Citrus jambhiri* Lush) in citrus defense. *Journal of General Plant Pathology* 73, 168–179. https://doi.org/10.1007/s10327-007-0013-0

Yan, S., Liu, Q., Li, W., Yan, J., Fernie, A.R. (2022). Raffinose Family Oligosaccharides: Crucial Regulators of Plant Development and Stress Responses. *CRC Crit Rev Plant Sci* 41, 286–303. https://doi.org/10.1080/07352689.2022.2111756

Yang, C., Ou, D., Guo, W., Lü, J., Guo, C., Qiu, B., Pan, H. (2020a). De Novo Assembly of the Asian Citrus Psyllid *Diaphorina citri* (Hemiptera: Psyllidae) Transcriptome across Developmental Stages. *Int J Mol Sci* 21, 4974. https://doi.org/10.3390/ijms21144974

Yang, J., Wang, X., Xie, M., Wang, G., Li, Z., Zhang, Y., Wu, L., Zhang, G., Ma, Z. (2020b). Proteomic analyses on xylem sap provides insights into the defense response of *Gossypium hirsutum* against *Verticillium dahliae*. *J Proteomics* 213, 103599. https://doi.org/10.1016/j.jprot.2019.103599

Yang, Q., Zhao, D., Liu, Q. (2020c). Connections Between Amino Acid Metabolisms in Plants: Lysine as an Example. *Front Plant Sci* 11. https://doi.org/10.3389/fpls.2020.00928

Yang, S. young, Lim, D. jung, Kim, Y.H., Kim, I.S. (2018). Screening of Plant Extracts and Identification of their Insecticidal Metabolites against *Myzus persicae*. *Korean Journal of Environmental Agriculture* 37, 125–134. https://doi.org/10.5338/KJEA.2018.37.2.19

Yi, F., Wang, Zhuo, Liu, J., Zhang, Y., Wang, Zhijun, Xu, H., Li, X., Bai, N., Cao, L., Song, X. (2017). Structural Maintenance of Chromosomes protein 1: Role in Genome Stability and Tumorigenesis. *Int J Biol Sci* 13, 1092–1099. https://doi.org/10.7150/ijbs.21206

Yildiz, I., Mantz, M., Hartmann, M., Zeier, T., Kessel, J., Thurow, C., Gatz, C., Petzsch, P., Köhrer, K., Zeier, J. (2021). The mobile SAR signal N-hydroxypipecolic acid induces NPR1-dependent transcriptional reprogramming and immune priming. *Plant Physiol* 186, 1679–1705. https://doi.org/10.1093/plphys/kiab166

Yoo, H., Widhalm, J.R., Qian, Y., Maeda, H., Cooper, B.R., Jannasch, A.S., Gonda, I., Lewinsohn, E., Rhodes, D., Dudareva, N. (2013). An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine:phenylpyruvate aminotransferase. *Nat Commun* 4, 2833. https://doi.org/10.1038/ncomms3833

Yu, Q., Dai, F., Russo, R., Guha, A., Pierre, M., Zhuo, X., Wang, Y.Z., Vincent, C., Gmitter, F.G. (2022). Phenotypic and Genetic Variation in Morphophysiological Traits in Huanglongbing-Affected Mandarin Hybrid Populations. *Plants* 12, 42. https://doi.org/10.3390/plants12010042

Zadražnik, T., Hollung, K., Egge-Jacobsen, W., Meglič, V., Šuštar-Vozlič, J. (2013). Differential proteomic analysis of drought stress response in leaves of common bean (*Phaseolus vulgaris* L.). *J Proteomics* 78, 254–272. https://doi.org/10.1016/j.jprot.2012.09.021

Zanardi, O.Z., Volpe, H.X.L., Luvizotto, R.A.G., Magnani, R.F., Gonzalez, F., Calvo, C., Oehlschlager, C.A., Lehan, B.J., Esperança, V., Delfino, J.Y., de Freitas, R., de Carvalho, R.I., Mulinari, T.A., Miranda, M.P., Bento, J.M.S., Leal, W.S. (2019). Laboratory and field evaluation of acetic acid-based lures for male Asian citrus psyllid, *Diaphorina citri*. *Sci Rep* 9, 12920. https://doi.org/10.1038/s41598-019-49469-3

Zeng, W., Sun, Z., Cai, Z., Chen, H., Lai, Z., Yang, S., Tang, X. (2017). Proteomic analysis by iTRAQ-MRM of soybean resistance to *Lamprosema Indicate*. *BMC Genomics* 18, 444. https://doi.org/10.1186/s12864-017-3825-0

Zhang, M., Wang, J., Luo, Q., Yang, C., Yang, H., Cheng, Y. (2021). CsMYB96 enhances citrus fruit resistance against fungal pathogen by activating salicylic acid biosynthesis and facilitating defense metabolite accumulation. *J Plant Physiol* 264, 153472. https://doi.org/10.1016/J.JPLPH.2021.153472

Zhang, Y.-M., Ye, D.-X., Liu, Y., Zhang, X.-Y., Zhou, Y.-L., Zhang, L., Yang, X.-L. (2023). Peptides, new tools for plant protection in eco-agriculture. *Advanced Agrochem* 2, 58–78. https://doi.org/10.1016/j.aac.2023.01.003

Zhao, H., Sun, X., Xue, M., Zhang, X., Li, Q. (2016). Antioxidant Enzyme Responses Induced by Whiteflies in Tobacco Plants in Defense against Aphids: Catalase May Play a Dominant Role. *PLoS One* 11, e0165454. https://doi.org/10.1371/journal.pone.0165454

Zheng, L., Xu, Q., Gong, G., Liao, Y., Yu, M., Shabala, S., Chen, W., Wu, W. (2023). *Nicotiana tabacum* as a dead-end trap for adult *Diaphorina citri*: A potential biological tactic for protecting citrus orchards. *Front Plant Sci* 13. https://doi.org/10.3389/fpls.2022.1081663

Zhong, Y., Shtineman-Kotler, A., Nguyen, L., Iliadi, K.G., Boulianne, G.L., Rotin, D. (2011). A Splice Isoform of DNedd4, DNedd4-Long, Negatively Regulates Neuromuscular Synaptogenesis and Viability in *Drosophila*. *PLoS One* 6, e27007. https://doi.org/10.1371/journal.pone.0027007

Zhong, Z.F., Zhou, X.J., Lin, J.B., Liu, X.J., Shao, J., Zhong, B.L., Peng, T. (2019). Effects of leaf colorness, pigment contents and allelochemicals on the orientation of the Asian citrus psyllid among four Rutaceae host plants. *BMC Plant Biol* 19, 1–21. https://doi.org/10.1186/S12870-019-1818-7

Zhu, F., Xi, D.-H., Yuan, S., Xu, F., Zhang, D.-W., Lin, H.-H. (2014). Salicylic Acid and Jasmonic Acid Are Essential for Systemic Resistance Against *Tobacco mosaic virus* in *Nicotiana*

benthamiana. *Molecular Plant-Microbe Interactions®* 27, 567–577. https://doi.org/10.1094/MPMI-11-13-0349-R

Zogli, P., Pingault, L., Grover, S., Louis, J. (2020). Ento(o)mics: the intersection of 'omic' approaches to decipher plant defense against sap-sucking insect pests. *Curr Opin Plant Biol* 56, 153–161. https://doi.org/10.1016/j.pbi.2020.06.002

Appendix

Appendix Chapter 3

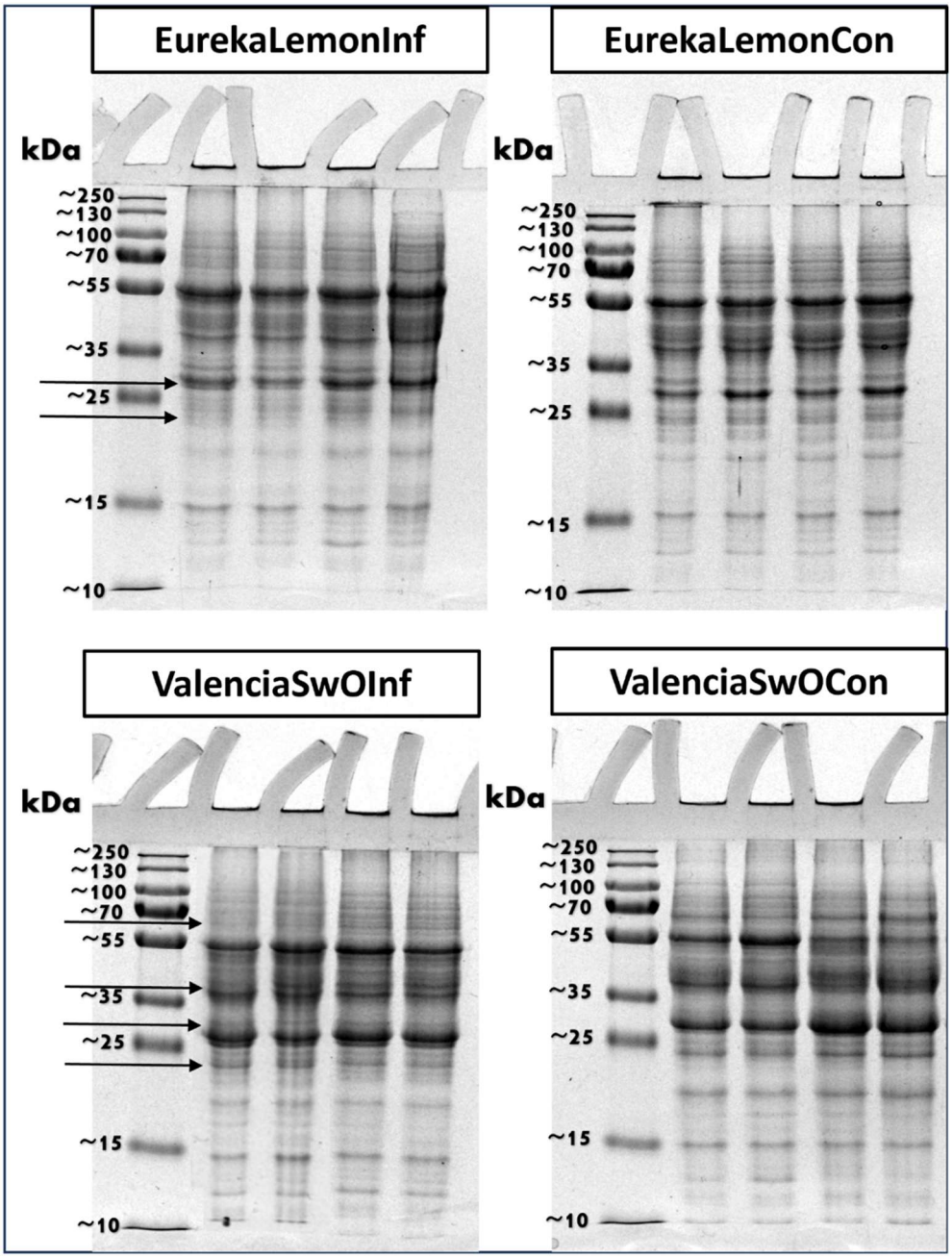

Figure S3.1 Protein profile of expressed proteins in the enriched vascular sap of lemon and orange plants in control and infested condition, analysed by SDS-Page (12%), one gel per condition. The gels named "EurekaLemonCon" and "EurekaLemonInf" represent 4 vascular samples of control and infested 'Eureka' lemon plants, respectively. The gels named "ValenciaSwOCon" and "ValenciaSwOInf" represents the 4 vascular samples of control and infested 'Valencia' sweet orange (SwO) plants, respectively. Thirty µg protein extracts were run in each well. Arrows highlight the areas with visible differences between infested and control profiles.

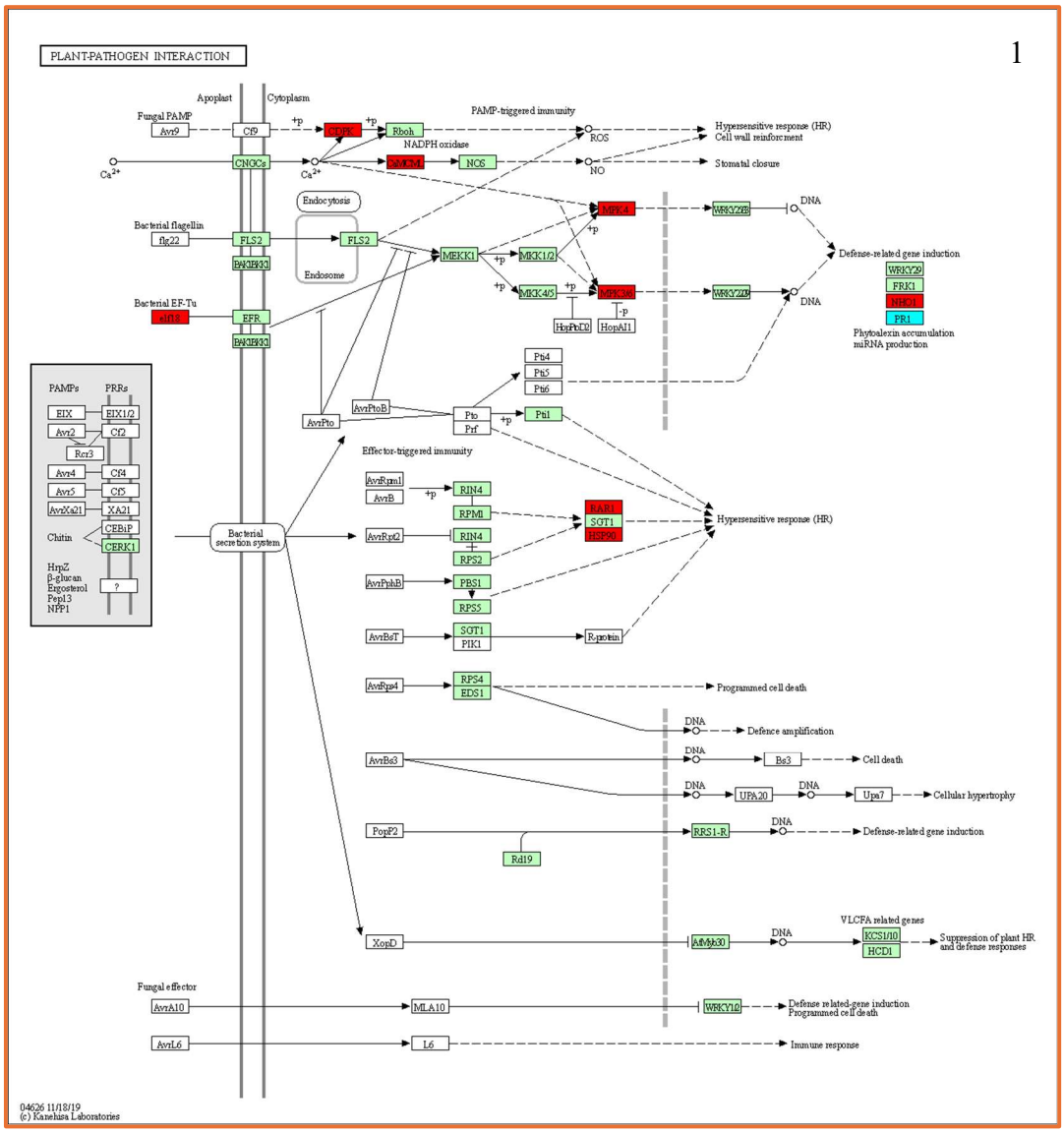

Figure S3.2 Representation of the enriched pathways that resulted from 'Valencia' sweet orange (SwO) in response to *Trioza erytreae*, (ValenciaSwOInf *vs* ValenciaSwOCon) comparison. Red highlighted forms represent the upregulated proteins, and blue highlighted proteins represent the downregulated proteins. 1: "Plant–pathogen interaction" pathway; 2: "Glyoxylate and dicarboxylate metabolism" pathway, with pink highlighted arrows representing the "photorespiration" module; 3: "Pyruvate metabolism" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module; 4: "Citrate cycle (TCA cycle)" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module.

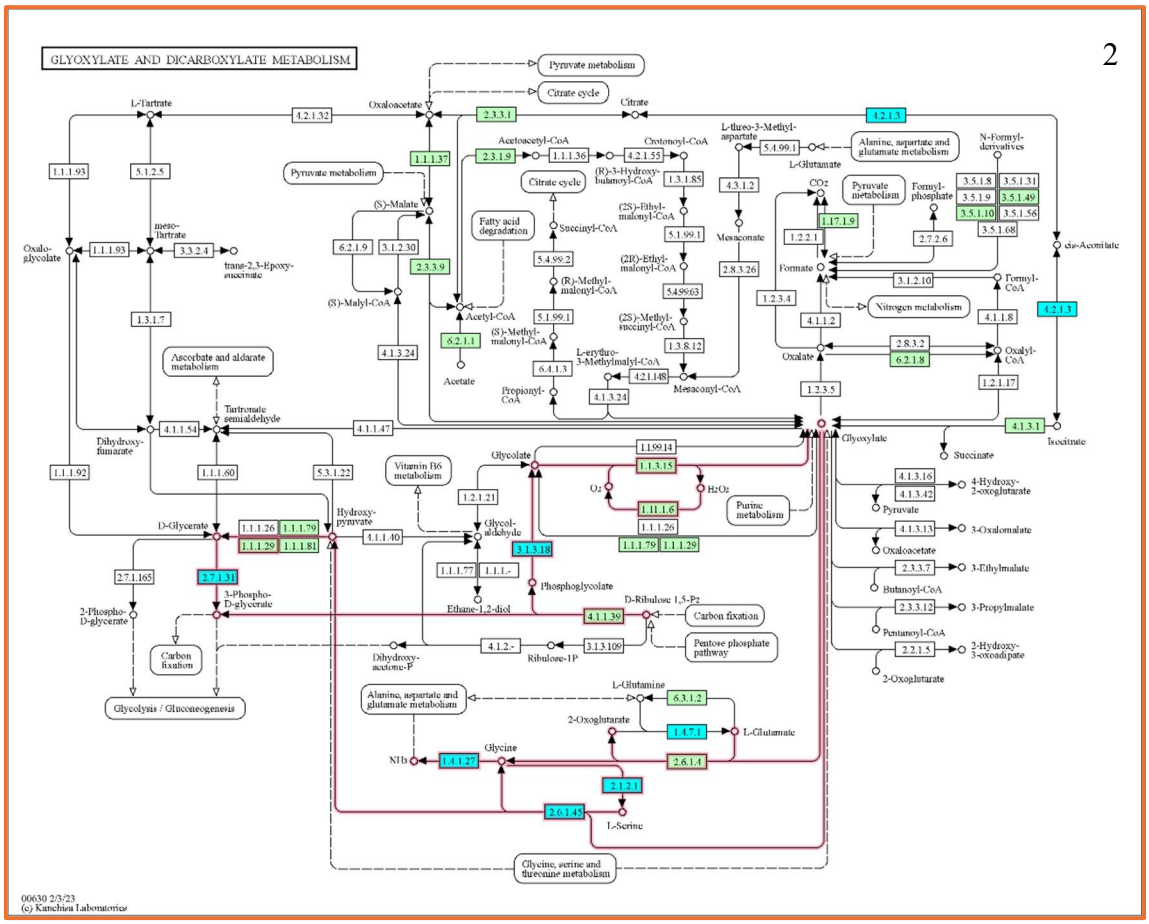

Figure S3.2 Representation of the enriched pathways that resulted from 'Valencia' sweet orange (SwO) in response to *Trioza erytreae*, (ValenciaSwOInf *vs* ValenciaSwOCon) comparison. Red highlighted forms represent the upregulated proteins, and blue highlighted proteins represent the downregulated proteins. 1: "Plant–pathogen interaction" pathway; 2: "Glyoxylate and dicarboxylate metabolism" pathway, with pink highlighted arrows representing the "photorespiration" module; 3: "Pyruvate metabolism" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module; 4: "Citrate cycle (TCA cycle)" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module.

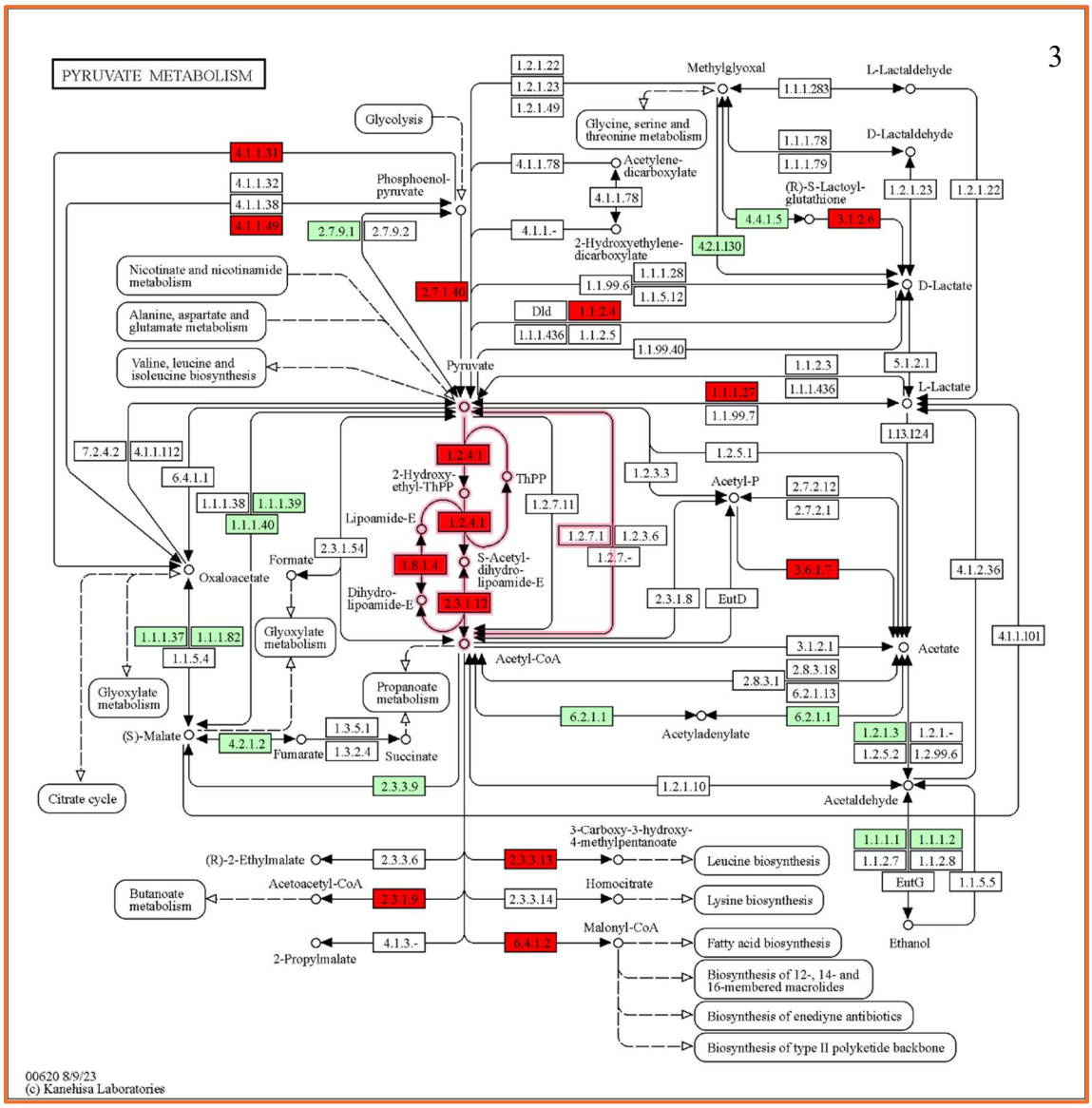

Figure S3.2 Representation of the enriched pathways that resulted from 'Valencia' sweet orange (SwO) in response to *Trioza erytreae*, (ValenciaSwOInf *vs* ValenciaSwOCon) comparison. Red highlighted forms represent the upregulated proteins, and blue highlighted proteins represent the downregulated proteins. 1: "Plant–pathogen interaction" pathway; 2: "Glyoxylate and dicarboxylate metabolism" pathway, with pink highlighted arrows representing the "photorespiration" module; 3: "Pyruvate metabolism" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module; 4: "Citrate cycle (TCA cycle)" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module.

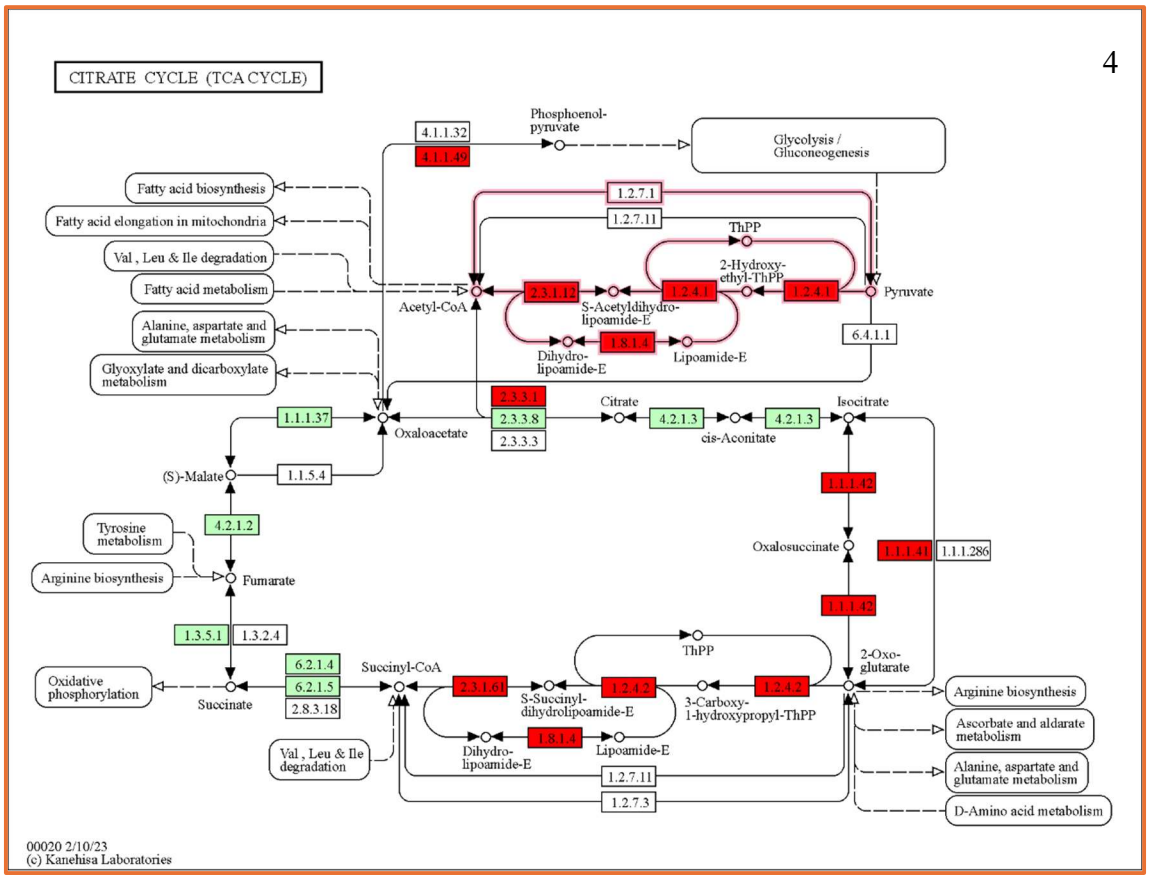

Figure S3.2 Representation of the enriched pathways that resulted from 'Valencia' sweet orange (SwO) in response to *Trioza erytreae*, (ValenciaSwOInf *vs* ValenciaSwOCon) comparison. Red highlighted forms represent the upregulated proteins, and blue highlighted proteins represent the downregulated proteins. 1: "Plant–pathogen interaction" pathway; 2: "Glyoxylate and dicarboxylate metabolism" pathway, with pink highlighted arrows representing the "photorespiration" module; 3: "Pyruvate metabolism" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module; 4: "Citrate cycle (TCA cycle)" pathway with the pink highlighted arrows representing the "pyruvate oxidation" module.

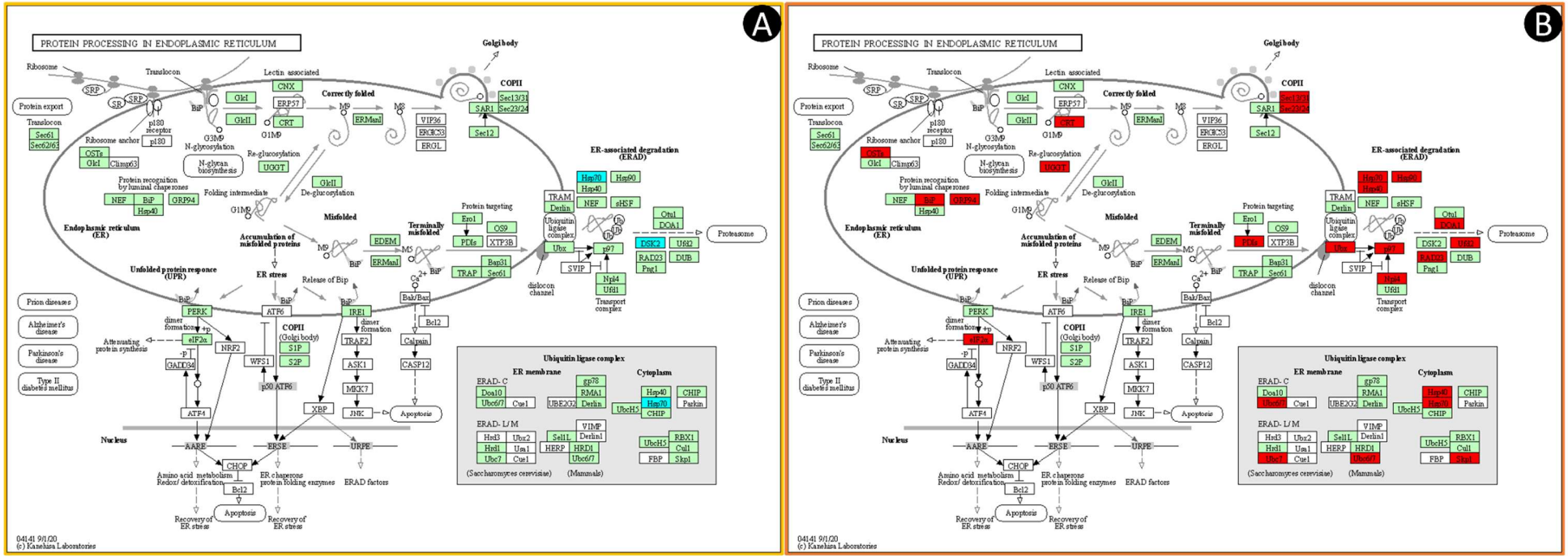

Figure S3.3 Representation of the up and downregulated proteins in the "Protein processing in endoplasmic reticulum" pathway in response to *Trioza erytreae*. Red highlighted forms represent the upregulated proteins, and blue highlighted proteins represent the downregulated proteins A - Represents the regulation in the 'Eureka' lemon plants (EurekaLemonInf *vs* EurekaLemonCon) comparison. B – Represents the regulation in the 'Valencia' sweet orange (SwO) (ValenciaSwOInf *vs* ValenciaSwoCon) comparison.

Table S3.1. Protein library resulting from the nano LC-MS/MS analysis of the EurekaLemonInf, EurekaLemonCon, ValenciaSwOInf and ValenciaSwOCon samples. Available online at: https://www.dropbox.com/scl/fi/qbmprvg1uieyv5j2evkv8/Table-S3_1-Protein-library-resulting-from-the-nano-LC-MSMS-analysis-of-the-EurekaLemonInf-EurekaLemonCon.xlsx?rlkey=xzssjha7ql50e2pd6b27iewtp&st=v6w5dxut&dl=0

Table S3.2. Differentially abundant proteins (DAPs) found in lemon and orange plants in response to *Trioza erytreae* infestation as compared to the respective controls. Available online at: https://www.dropbox.com/scl/fi/ab0kj7lngsgjks0s1k37x/Table-S3_2-Differentially-abundant-proteins-DAPs-found-in-lemon-and-orange-plants-in-response-to-Trioza-erytreae-infestatio.xlsx?rlkey=4uvg48lqcwj98mlu7fux21uta&st=h59lba6w&dl=0

Table S3.3. Loading values of the differentially abundant proteins (DAPs) related to the first component of the principal component analysis (PCA). Available online at: https://www.dropbox.com/scl/fi/852m7obcooaogae1vsb8y/Table-S3_3-Loading-values-of-the-differentially-abundant-proteins-DAPs-related-to-the-first-component-of-the-principal-com.xlsx?rlkey=w3i14727zx9a6lszr5vn29bqz&st=n9241eke&dl=0

Table S3.4. KEGG pathway enrichment analysis for the response of 'Eureka' lemon and 'Valencia' SwO to *Trioza erytreae*. Available online at: https://www.dropbox.com/scl/fi/cejma3b2s5r6rbdlut4ol/Table-S3_4-KEGG-pathway-enrichment-analysis-for-the-response-of-Eureka-lemon-and-Valencia-SwO-to-Trioza-erytreae.xlsx?rlkey=wk49q7s34kr6xqlolpyl4gv9h&st=gi0zmzhu&dl=0

Appendix Chapter 4

Table S4.1. Compound identification and quantitation. Available online at: https://www.dropbox.com/scl/fi/cb9zv6vji5cd6ggkwvj96/Table-S4_1-Compound-identification-and-quantitation.xlsx?rlkey=fk6p7iwrmjy9ycmd4yheaco6n&st=al1ujyvy&dl=0

Table S4.2. Differentially Abundant Metabolites Enriched KEGGs. Available online at: https://www.dropbox.com/scl/fi/wh9w31vzydpka8pt1eir6/Table-S4_2-Differentially-Abundant-Metabolites-Enriched-KEGGS.xlsx?rlkey=72fu55u2aymncprptzepl3vaa&st=46qoazws&dl=0

Table S4.3. Protein library resulting from the nano LC-MSMS analysis of the enriched vascular sap. Available online at: https://www.dropbox.com/scl/fi/bxt240jbv4v5gk7v7g17a/Table-S4_3-Protein-library-resulting-from-the-nano-LC-MSMS.xlsx?rlkey=aj9d81em5s8d82sjyg3yni2su&st=5cgvfad8&dl=0

Table S4.4. Differentially abundant proteins (DAPs) found in lemon and orange plants in response to *Trioza erytreae*. Available online at: https://www.dropbox.com/scl/fi/9ktoko68gjf4nv2bzrr35/Table-S4_4-Differentially-abundant-proteins-DAP-s.xlsx?rlkey=4t339hnnd89tfg5hekwpqo2tb&st=o9220g0t&dl=0

Table S4.5. Differentially abundant proteins (DAPs) enriched KEGGs. Available online at: https://www.dropbox.com/scl/fi/t8plawnz68v5v1t46pnwx/Table-S4_5-Differentially-abundant-proteins-DAP-s-enriched-KEGGs.xlsx?rlkey=ci3syzjqobp8330ynp7eei2k9&st=yia33u1c&dl=0

Appendix Chapter 5

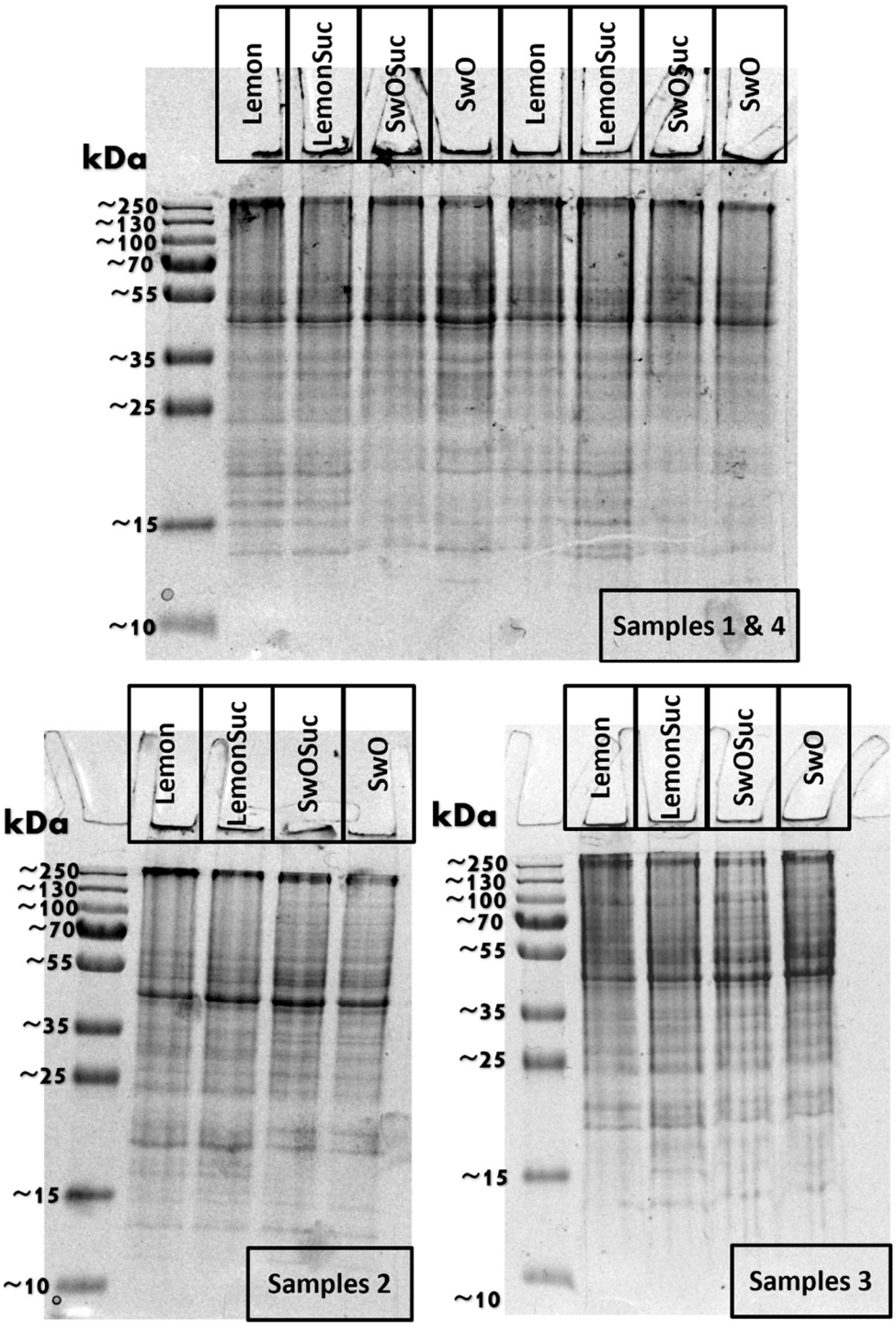

Figure S5.1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-Page) results of the *Trioza erytreae* nymphs' protein extraction from the Lemon, LemonSuc, SwO and SwOSuc samples. Stacking gel at 5% and separating gel at 12% acrylamide, 30 µg of protein of each sample was used. PageRuler Plus Prestained Protein Ladder 10 to 250 kDa (Thermo Scientific, Bremen, Germany) was used as the protein ladder. "Lemon": nymphs that developed exclusively on lemon plants. "LemonSuc": nymphs that developed on lemon plants and were then fed sucrose for24 h before sampling. "SwO": nymphs exclusively developed on SwO. "SwOSuc": nymphs that developed on SwO plants and were then fed sucrose for 24 h before sampling

Table S5.1. Protein library resulting from the nano LC-MSMS analysis of the *Trioza erytreae* nymphs. Available online at: https://www.dropbox.com/scl/fi/mkaf8dz21zak4wut5rwwo/Table-S5_1-Protein-library-resulting-from-the-nano-LC-MSMS.xlsx?rlkey=uj370ndaapi21w9lp6whd18ud&st=hxmvorhh&dl=0

Table S5.2. Differentially abundant proteins (DAPs) found in nymphs that developed on lemon and orange plants. Available online at: https://www.dropbox.com/scl/fi/c2s1q95k2q1hwwbmyg3o3/Table-S5_2-Differentially-abundant-proteins-DAPs.xlsx?rlkey=d0634cxriy85msbqbdeiwbjda&st=bf3epcem&dl=0

Appendix Chapter 6

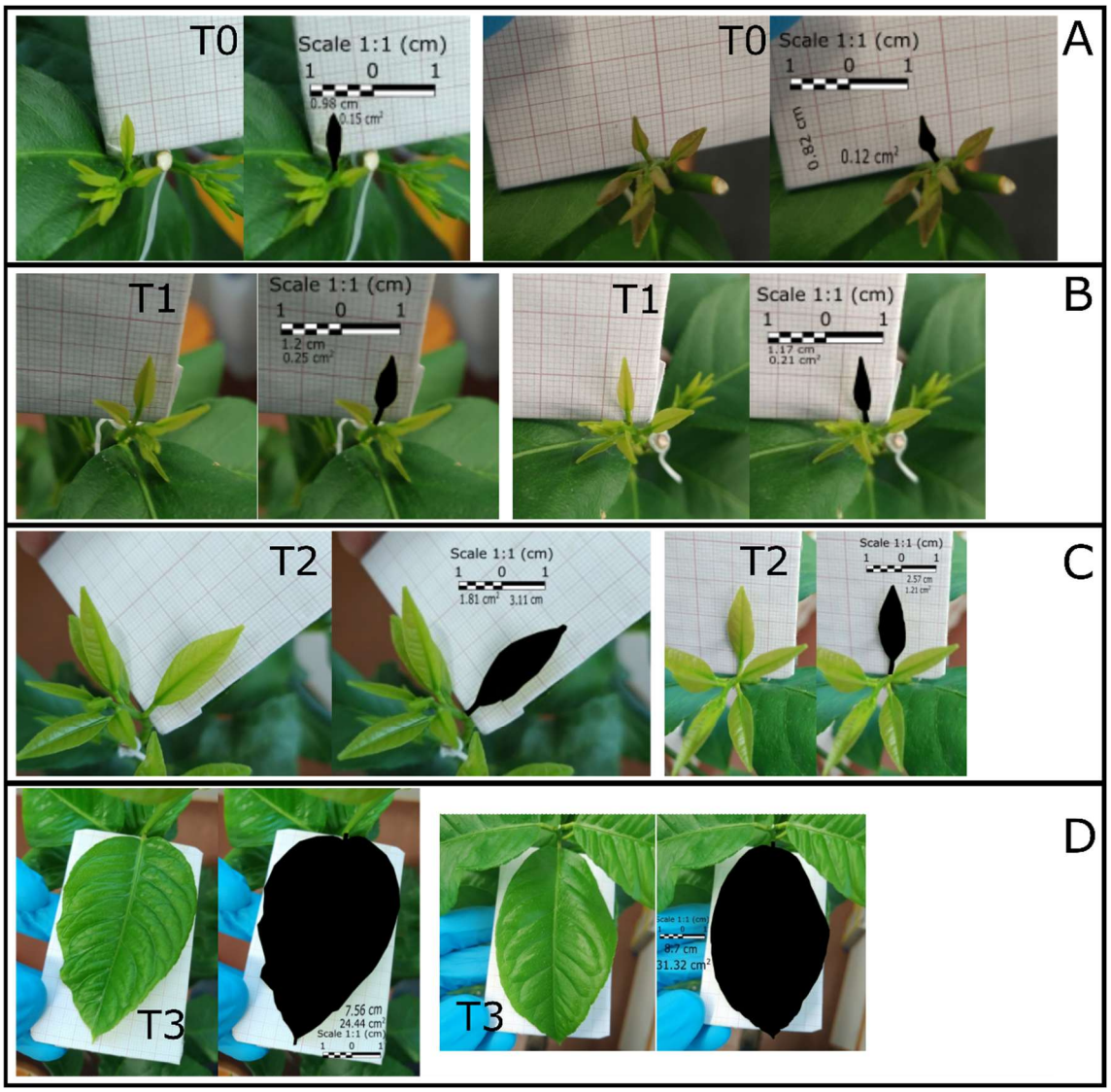

Figure S6.1. Examples of leaf areas measurement. The area was the measured in cm^2 through scaling and area measurement tools on Inkscape (Inkscape Project, 2020). A: Measurements from time point T0 B: Measurements from time point T1 C: Measurements from time point T2 D: Measurements from time point T3

Table S6.1. Most influential VOCs. Determined by a Random Forest (RF) analysis, conducted using the randomForest package (Breiman et al., 2024). The importance of each VOC was determined based on the Gini Importance Measure (GIM). Blue represents compounds with a GIM greater than 0.5. Red represents compounds with a GIM lower than 0.5.

Gini Importance Measure (GIM)	Compound
1.1079	(Z)-3-Hexenol acetate
1.0155	2-Hexanone
0.9096	Methyl benzoate
0.8863	2,2,4,4,6,8,8-Heptamethylnonane
0.8090	Clovene
0.8024	o-Cymene
0.7914	o-Xylene
0.7744	cis-Nerolidol
0.7733	alpha Muurolene
0.7587	Methyl salicylate
0.7570	Heptanal
0.7538	3-Methyl-4-methylenebicyclo[3.2.1]oct-2-ene
0.7265	beta Chamigrene
0.7199	Prehnitene
0.7030	4-methylene-2,8,8-trimethyl-2-vinyl-bicyclo[5.2.0]nonane
0.6881	(3E)-3-Hexenyl butyrate
0.6782	m-Xylene
0.6731	Nonadecane
0.6384	Indole
0.6248	alpha Bisabolene
0.6193	1,3,8-p-Menthatriene
0.6141	Caryophyllene oxide
0.6115	delta Cadinene
0.6103	alpha Phellandrene
0.6078	Phenol
0.6073	Octadecane
0.5909	trans-(.+-.)-2-methyl-2-(4-methyl-3-pentenyl)cyclopropanecarboxaldehyde
0.5908	2-Methyl-4-pentenal
0.5898	(Z)-3-Hexen-1-ol
0.5820	Hexadecane
0.5748	Epiglobulol
0.5670	3,3,7,11-tetramethyl-tricyclo[6.3.0.0(2,4)]undec-8-ene
0.5496	(E)-2-Hexenal
0.5155	delta Guaiene
0.5130	1-(2,6,6-Trimethylcyclohex-2-en-1-yl)pent-1-en-3-one
0.5090	(E)-2-Hexen-1-ol
0.4955	alpha Terpinene
0.4925	4-Methyl-3-(1-methylethylidene)-1-cyclohexene
0.4876	Butyl Acrylate

0.4821	2-Methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl propanoate
0.4743	Methyl ester octanoic acid
0.4703	Methyl geranate
0.4688	Dysoxylonene
0.4686	cis-3-Hexenyl-.alpha.-methylbutyrate
0.4618	(-)-Gamma-cadinene
0.4603	Isocarveol
0.4531	4,6-Dimethyldodecane
0.4507	Camphene
0.4350	6-Methyl-5-heptene-2-one
0.4314	Carveol
0.4229	alpha Bisabolol
0.4067	p-Propyltoluene
0.4067	(-)-Aristolene
0.4007	1-Dodecanol
0.4004	Isobornyl acetate
0.3962	Limonene 1,2-epoxide
0.3872	Levomenthol
0.3861	beta Phellandrene
0.3686	n-Butyl butanoate
0.3566	Humulen-(v1)
0.3556	2-Oxo-1,8-cineole
0.3516	Copaene
0.3503	Heptadecane
0.3399	4-Methylindane
0.3351	alpha Cubenene
0.3338	Nonanal
0.3325	(3E)-4-Ethyl-3-nonen-5-yne
0.3320	1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene
0.3189	trans-Sesquisabinene hydrate
0.3109	trans-3-Caren-2-ol
0.3096	Decanal
0.3080	Geranyl nitrile
0.3080	(-)-Carvone
0.2999	2-Ethyl-3-hydroxyhexyl 2-methylpropanoate
0.2950	2-Methyl-6-methyleneoctan-2-ol
0.2934	Cosmene
0.2933	Ethylbenzene
0.2877	alpha Terpineol
0.2852	Terpinen-4-ol
0.2784	1,2,4-trimethylbenzene
0.2772	Tetradecane
0.2618	Thujene
0.2561	Undecanal
0.2549	alpha Curcumene
0.2481	3-Carene
0.2471	cis-Geraniol

0.2406	beta Bisabolene
0.2319	Tridecane
0.2252	4-tert-Butylcyclohexyl acetate
0.2194	2-n-Butoxyethanol
0.1979	Neo-allo-Ocimene
0.1844	Decane
0.1843	Bicyclo[3.3.0]octan-2-one, 7-ethylidene-
0.1802	alpha Pinene
0.1683	beta Linalool
0.1676	4,5-Epoxycarene
0.1605	gamma Terpinene
0.1562	D-Limonene
0.1559	gamma Muurolene
0.1533	trans-Sabinene hydrate
0.1512	p-Diethylbenzene
0.1502	4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene
0.1485	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane
0.1472	trans-Geraniol
0.1454	alpha Bergamotene
0.1412	Allo-Ocimene
0.1335	trans beta Ocimene
0.1307	beta Curcumene
0.1208	trans alpha Bergamotene
0.1203	beta Farnesene
0.1187	Pentadecane
0.1172	Terpinolene
0.1136	beta Santalene
0.1109	beta Sesquiphellandrene
0.1089	Dodecane
0.1029	Citral
0.1000	Geranyl acetate
0.0965	cis beta Ocimene
0.0959	Geranial
0.0948	(R)-(+)-Citronellal
0.0947	beta Myrcene
0.0918	(-)-beta Pinene
0.0837	beta Citral
0.0831	Caryophyllene
0.0814	Verbenol
0.0760	beta Elemene
0.0669	Citronellyl propionate
0.0647	Nerol acetate
0.0593	Elixene
0.0589	beta Germacrene
0.0511	alpha Humulene
0.0231	Alloaromadendrene

Table S6.2. Volatile profile and time effect comparisons of lemon plants measured before spray (BS) (T0), one day after spray (DAS) (T1), five DAS (T2) and at 25 DAS (T3). Control- plants not sprayed with jasmonic acid (JA) and not infested by *Trioza erytreae*; JAsp- plants sprayed with JA and not infested; Psyll- plants not sprayed with JA and infested; JA_Psyll- plants sprayed with JA and infested. The results are expressed as nanograms per cm^2 leaf area (mean ± standard error, n =5). LRI =Linear Retention Index. ND = Not detected. NA = Not applicable. Comparisons with significant differences (p-value< 0.05) are highlighted in bold. One-way ANOVA was employed, followed by the Tukey's post-hoc multi-comparison test. When only two groups were compared, a Student's *t*-test was applied.

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
Thujene	977	**22.50±2.97a**	**10.45±1.93b**	**5.03±1.38bc**	**2.26±0.37c**	**6.44E-06**	10.26±3.70	6.85±1.84	1.97±0.16	0.09	27.79±9.38	3.62±0.82	0.06	5.56±0.91	8.84±2.36	0.32
alpha Pinene	982	**24.27±2.77a**	**14.12±1.73b**	**6.46±1.54c**	**3.75±0.51c**	**2.48E-06**	**16.11±4.47a**	**10.06±2.13ab**	**4.45±0.53b**	**0.04**	**30.04±7.89a**	**5.92±1.06b**	**0.03**	11.22±2.90	14.74±4.51	0.61
Camphene	994	**0.93±0.09a**	**NDc**	**0.26±0.04b**	**0.18±0.03bc**	**6.19E-09**	0.71±0.21	0.48±0.09	0.22±0.02	0.07	**1.27±0.28a**	**0.27±0.06b**	**0.03**	0.67±0.32	0.85±0.21	0.71
beta Phellandrene	1014	**59.32±8.56a**	**28.75±7.67b**	**16.62±5.58b**	**7.86±0.41b**	**1.93E-04**	**28.46±11.3a**	**18.24±3.55ab**	**NDb**	**0.04**	76.07±23.9	14.07±1.85	0.06	16.72±2.64	28.60±8.36	0.30
(-)-beta Pinene	1016	**166.1±25.6a**	**88.98±12.3b**	**50.86±13.4b**	**40.62±5.50b**	**1.63E-04**	128.9±44.0	76.33±15.8	50.54±5.97	0.16	180.5±47.0	60.09±9.18	0.05	81.62±15.8	149.9±46.4	0.27
beta Myrcene	1028	**149.7±31.7a**	**84.19±17.4ab**	**43.10±10.9b**	**20.41±3.18b**	**1.02E-03**	52.40±19.1	52.55±8.02	34.54±4.82	0.51	101.6±28.2	27.81±2.72	0.07	52.20±15.2	48.04±18.5	0.89
alpha Phellandrene	1050	**24.21±3.39a**	**NDb**	**4.05±0.89b**	**1.91±0.20b**	**9.70E-08**	**8.36±1.76a**	**6.90±1.26ab**	**3.16±0.30b**	**0.04**	19.38±5.73	6.80±0.98	0.06	9.58±3.47	12.12±3.60	0.70
3-Carene	1057	**113.8±18.8a**	**54.63±8.23b**	**15.34±4.02bc**	**6.95±0.66c**	**6.73E-06**	30.75±8.95	29.22±5.72	15.97±2.56	0.23	107.6±35.7	22.27±3.70	0.09	20.10±3.00	51.55±25.1	0.32
alpha Terpinene	1063	**9.19±1.39a**	**6.38±2.40ab**	**3.19±0.82b**	**0.88±0.14b**	**4.99E-03**	**4.76±1.15a**	**3.12±0.53ab**	**1.14±0.13b**	**0.02**	**9.99±2.95a**	**NAb**	**0.03**	3.17±1.05	8.40±1.95	0.07
o-Cymene	1068	ND	ND	ND	ND	NA	**6.12±0.80a**	**6.17±1.80a**	**NDb**	**3.05E-03**	**44.60±10.5a**	**5.55±1.38b**	**0.02**	**NDb**	**15.49±2.83a**	**5.42E-03**
D-Limonene	1078	**2740±483a**	**1724±256ab**	**804.9±161bc**	**207.4±19.5c**	**6.31E-05**	1025±344	1005±184	282.7±38.3	0.06	**2752±701a**	**534.3±57.6b**	**0.03**	916.5±225	868.6±256	0.92
trans beta Ocimene	1084	**81.75±22.0a**	**71.46±26.3ab**	**34.80±6.69ab**	**7.66±1.62b**	**0.03**	34.40±16.4	38.39±5.37	12.30±2.58	0.18	**54.82±13.6a**	**8.91±0.89b**	**0.03**	42.40±19.5	11.32±2.60	0.21
cis beta Ocimene	1093	**343.9±88.3a**	**348.3±106a**	**158.8±28.6ab**	**31.23±6.32b**	**0.01**	140.8±67.7	182.7±25.2	49.59±11.1	0.12	**227.0±53.2a**	**45.51±8.93b**	**0.04**	176.7±76.3	48.69±9.71	0.19
gamma Terpinene	1101	**22.13±3.08a**	**8.92±1.88b**	**5.79±1.92b**	**3.93±0.87b**	**5.48E-05**	11.47±4.95	6.81±1.96	2.54±0.27	0.17	20.56±6.36	4.00±0.57	0.07	8.92±1.77	8.28±1.80	0.83
1,3,8-p-Menthatriene	1118	**0.66±0.16a**	**0.42±0.06ab**	**0.27±0.08b**	**0.05±0.02b**	**2.62E-03**	**0.44±0.11a**	**0.35±0.04a**	**0.10±0.03b**	**9.47E-03**	**NDb**	**0.11±0.01a**	**1.78E-03**	**0.37±0.12a**	**NDb**	**0.04**
4-Methyl-3-(1-methylethylidene)-1-cyclohexene	1120	**NDc**	**NDc**	**0.18±0.02a**	**0.07±0.02b**	**4.17E-07**	**NDb**	**0.30±0.04a**	**0.18±0.04a**	**1.52E-04**	ND	ND	ND	0.47±0.13	0.98±0.53	0.44
Terpinolene	1125	**21.63±4.39a**	**8.81±1.86b**	**3.87±1.01b**	**1.86±0.29b**	**1.34E-04**	5.78±1.91	5.76±1.05	4.46±0.73	0.73	13.88±4.44	3.34±0.56	0.10	5.18±0.80	8.43±3.35	0.46

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
Cosmene	1167	**1.86±0.22 a**	**1.39±0.36 ab**	**0.61±0.16 bc**	**ND c**	**1.44E-04**	**ND c**	**0.85±0.08 a**	**0.21±0.03 b**	**1.73E-07**	**3.02±0.85 a**	**ND b**	**0.02**	1.67±0.73	ND	0.08
Allo-Ocimene	1174	**15.13±3.85 a**	**13.35±3.98 a**	**6.01±1.11 ab**	**1.44±0.30 b**	**0.01**	6.93±2.87	8.20±1.13	2.71±0.61	0.12	**15.65±3.74 a**	**2.25±0.25 b**	**0.03**	8.86±3.02	3.06±0.56	0.17
Neo-allo-Ocimene	1185	**12.34±3.07 a**	**11.24±2.77 a**	**4.69±0.82 ab**	**1.44±0.31 b**	**5.66E-03**	5.76±2.24	7.56±1.13	3.12±0.74	0.15	**14.30±3.24 a**	**2.14±0.31 b**	**0.03**	8.84±2.64	3.10±0.58	0.14
Verbenol	1206	**3.77±0.67 a**	**1.15±0.26 b**	**0.77±0.20 b**	**0.73±0.12 b**	**6.70E-05**	1.18±0.37	1.29±0.16	1.07±0.13	0.81	2.70±0.69	1.21±0.30	0.19	2.21±0.73	2.62±0.73	0.54
trans-Sabinene hydrate	1107	**2.42±0.48 a**	**0.79±0.25 b**	**0.59±0.15 b**	**0.20±0.03 b**	**2.14E-04**	0.92±0.29	0.69±0.09	0.32±0.06	0.10	2.49±1.03	0.44±0.10	0.13	0.77±0.11	0.83±0.21	0.81
beta Linalool	1146	**53.44±11.6 a**	**46.33±10.1 a**	**23.58±3.10 ab**	**4.25±0.44 b**	**1.59E-03**	**24.38±8.57 ab**	**32.60±6.31 a**	**6.99±1.47 b**	**0.04**	38.59±10.5	13.11±1.14	0.08	32.36±9.81	23.28±4.53	0.52
trans-3-Caren-2-ol	1156	**2.42±0.57 a**	**1.78±0.52 ab**	**0.44±0.17 bc**	**0.07±0.00 c**	**1.87E-03**	**0.41±0.09 ab**	**0.59±0.15 a**	**0.09±0.01 b**	**0.01**	2.25±0.63	0.78±0.15	0.05	0.52±0.13	1.00±0.21	0.10
Isocarveol	1177	**ND b**	**ND b**	**0.17±0.05 a**	**0.04±0.01 b**	**1.66E-04**	**ND b**	**0.11±0.03 a**	**0.05±0.00 b**	**9.36E-04**	**ND b**	**0.18±0.05 a**	**0.02**	0.22±0.05	0.33±0.07	0.24
Levomenthol	1212	**5.28±1.68 a**	**3.94±1.40 ab**	**0.85±0.36 ab**	**0.02±0.01 b**	**0.01**	**0.74±0.20 a**	**0.53±0.10 a**	**0.03±0.01 b**	**5.87E-03**	**7.36±2.61 a**	**1.97±0.74 b**	**0.05**	0.54±0.21	1.89±0.70	0.13
Terpinen-4-ol	1216	**1.17±0.12 a**	**1.14±0.27 a**	**0.24±0.05 b**	**0.10±0.01 b**	**7.38E-05**	**0.35±0.07 ab**	**0.58±0.15 a**	**0.07±0.01 b**	**9.15E-03**	1.22±0.15	1.74±1.16	0.67	1.38±0.56	0.38±0.10	0.18
Carveol	1217	**1.78±0.47 a**	**1.24±0.59 ab**	**0.16±0.03 b**	**0.29±0.08 ab**	**0.02**	**0.64±0.06 a**	**0.39±0.10 ab**	**0.19±0.04 b**	**3.41E-03**	**1.49±0.27 a**	**0.52±0.07 b**	**0.01**	4.55±1.95	1.61±0.64	0.23
alpha Terpineol	1227	**38.40±6.52 a**	**15.16±3.32 b**	**11.47±3.15 b**	**3.77±0.67 b**	**1.01E-04**	12.43±3.85	10.53±1.68	3.35±0.39	0.05	26.25±11.6	3.26±0.60	0.13	**15.04±3.31 a**	**5.43±0.97 b**	**0.04**
trans-Geraniol	1299	**24.45±7.21 a**	**10.26±3.15 ab**	**6.00±1.20 b**	**1.80±0.51 b**	**5.75E-03**	6.78±2.74	6.43±0.81	8.94±1.16	0.58	6.12±2.24	4.27±1.28	0.54	**4.81±0.97 a**	**1.91±0.27 b**	**0.03**
cis-Geraniol	1274	**29.97±9.13 a**	**11.69±3.14 ab**	**6.64±1.13 b**	**3.83±1.08 b**	**7.12E-03**	**6.67±2.48 b**	**8.18±1.05 ab**	**14.83±2.17 a**	**0.03**	5.66±3.46	6.38±1.51	0.88	**6.97±1.46 a**	**2.62±0.41 b**	**0.02**
Citral	1188	**9.86±1.68 a**	**2.64±0.56 b**	**1.80±0.51 b**	**1.54±0.26 b**	**2.16E-05**	3.07±0.96	3.57±0.49	2.05±0.26	0.27	4.07±1.31	2.44±0.61	0.42	4.59±1.53	5.89±1.67	0.42
(R)-(+)-Citronellal	1196	**5.35±1.16 a**	**1.84±0.35 b**	**1.29±0.53 b**	**0.72±0.15 b**	**6.35E-04**	1.18±0.39	1.06±0.14	1.18±0.28	0.94	2.20±0.71	1.89±0.51	0.79	2.10±0.58	2.88±0.84	0.42
beta Citral	1286	**206.6±34.5 a**	**59.76±15.1 b**	**43.88±12.7 b**	**45.83±8.46 b**	**6.53E-05**	62.14±20.2	68.52±8.57	62.40±8.43	0.93	96.88±40.8	76.18±20.9	0.73	131.9±43.1	161.2±47.7	0.46
Geranial	1313	**374.3±63.9 a**	**98.77±23.6 b**	**71.72±21.0 b**	**84.57±15.9 b**	**4.84E-05**	120.3±38.4	136.2±17.2	100.2±13.9	0.62	165.0±75.1	124.7±34.6	0.71	211.1±64.8	283.2±81.2	0.27
Methyl geranate	1371	**0.62±0.09 a**	**0.31±0.08 b**	**0.14±0.02 b**	**0.12±0.03 b**	**9.98E-05**	**ND b**	**0.19±0.04 a**	**0.23±0.03 a**	**3.39E-04**	0.86±0.29	0.20±0.03	0.10	0.17±0.03	0.49±0.22	0.21
Isobornyl acetate	1326	**0.66±0.08 a**	**0.51±0.10 a**	**0.11±0.02 b**	**ND b**	**3.23E-06**	ND	ND	ND	NA	**1.38±0.21 a**	**0.28±0.06 b**	**0.01**	0.29±0.19	0.58±0.25	0.45
Citronellyl propionate	1400	**0.47±0.10 a**	**0.36±0.11 ab**	**0.20±0.03 ab**	**0.07±0.01 b**	**9.82E-03**	0.15±0.03	0.15±0.02	0.12±0.02	0.57	0.27±0.07	0.15±0.02	0.22	0.24±0.08	0.29±0.11	0.76
Nerol acetate	1410	9.90±2.60	4.51±1.81	4.08±1.30	5.31±0.67	0.109	4.93±2.43	7.42±1.31	6.03±0.30	0.56	2.58±1.03	2.61±0.86	0.99	6.69±2.24	5.43±1.64	0.27
Geranyl acetate	1427	**23.45±5.70 a**	**8.07±3.41 b**	**6.81±2.21 b**	**4.55±0.81 b**	**6.19E-03**	10.76±4.01	14.71±2.65	7.36±1.09	0.23	6.99±3.74	3.30±0.94	0.44	9.49±3.05	11.27±2.82	0.25
4,5-Epoxycarene	1221	**7.45±1.28 a**	**2.22±0.50 b**	**1.46±0.43 b**	**1.37±0.21 b**	**4.63E-05**	2.44±0.75	2.78±0.40	2.11±0.27	0.67	3.39±1.37	2.33±0.52	0.58	5.05±1.33	6.26±1.22	0.42
Limonene 1,2-epoxide	1181	**0.40±0.03 a**	**0.20±0.02 b**	**0.08±0.02 c**	**ND c**	**4.64E-09**	**0.13±0.02 ab**	**0.23±0.06 a**	**0.08±0.01 b**	**0.04**	**0.40±0.08 a**	**0.10±0.03 b**	**0.02**	0.17±0.05	0.20±0.09	0.79

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
2-Oxo-1,8-cineole	1259	**0.73±0.14 a**	**0.51±0.16 ab**	**0.21±0.08 b**	**0.05±0.01 b**	**3.00E-03**	**ND b**	**0.17±0.04 a**	**0.07±0.02 ab**	**4.03E-03**	1.10±0.44	0.36±0.15	0.14	0.11±0.01	0.19±0.04	0.06
(-)-Carvone	1287	ND	ND	ND	ND	NA	ND	ND	ND	NA	**3.95±0.97 a**	**ND b**	**0.02**	ND	ND	NA
alpha Cubenene	1396	1.03±0.36	0.69±0.23	0.89±0.40	0.07±0.02	0.14	1.16±0.38 a	0.58±0.23 ab	0.13±0.02 b	**0.05**	ND	0.92±0.49	0.13	0.20±0.06	0.64±0.30	0.22
Copaene	1420	**0.13±0.01 a**	**ND c**	**0.07±0.01 b**	**0.06±0.02 b**	**1.45E-05**	**ND c**	**0.06±0.01 b**	**0.10±0.01 a**	**2.52E-05**	**ND b**	**0.07±0.01 a**	**8.54E-03**	0.11±0.03	0.36±0.17	0.25
beta Elemene	1435	0.33±0.08	0.30±0.07	0.21±0.05	0.12±0.02	0.08	0.22±0.10	0.22±0.02	0.19±0.02	0.95	**0.31±0.05 a**	**0.13±0.02 b**	**0.02**	0.42±0.13	0.26±0.11	0.49
4,11,11-Trimethyl-8-methylenebicyclo[7.2.0] undec-4-ene	1458	**0.89±0.25 a**	**0.66±0.21 ab**	**0.39±0.17 ab**	**0.06±0.01 b**	**0.03**	**0.21±0.06 ab**	**0.28±0.03 a**	**0.09±0.02 b**	**0.01**	0.67±0.07	0.45±0.14	0.10	0.51±0.22	0.31±0.01	0.42
alpha Bergamotene	1467	**3.87±0.92 a**	**2.40±1.01 ab**	**2.39±0.45 ab**	**0.63±0.10 b**	**0.04**	**0.86±0.36 b**	**2.01±0.22 a**	**1.04±0.09 b**	**0.01**	2.23±0.52	0.66±0.13	0.05	**1.93±0.22 a**	**0.73±0.17 b**	**0.02**
Caryophyllene	1471	52.80±13.8	41.13±17.8	46.17±8.30	17.45±2.17	0.22	39.87±21.2	45.72±7.93	28.28±6.48	0.66	40.69±8.07	18.47±3.21	0.07	73.12±23.9	22.27±5.32	0.09
trans alpha Bergamotene	1487	**58.82±13.8 a**	**37.76±15.6 ab**	**35.83±6.45 ab**	**9.22±1.34 b**	**0.04**	22.29±10.4	30.64±3.34	18.65±4.58	0.47	**36.59±8.50 a**	**10.21±2.00 b**	**0.05**	42.08±14.3	10.50±2.45	0.09
Alloaromadendrene	1489	0.85±0.20	0.48±0.13	0.45±0.17	0.32±0.05	0.10	0.37±0.11	0.37±0.08	0.47±0.04	0.65	0.67±0.14	0.44±0.10	0.09	0.34±0.05	0.71±0.30	0.33
delta Guaiene	1492	**0.32±0.02 a**	**0.13±0.05 b**	**0.14±0.01 b**	**0.10±0.02 b**	**4.36E-04**	**ND b**	**0.14±0.02 a**	**0.18±0.03 a**	**8.23E-05**	**ND b**	**0.07±0.01 a**	**7.75E-03**	0.16±0.04	0.12±0.05	0.60
Humulen-(v1)	1500	2.15±0.86	2.14±0.70	0.62±0.48	0.05±0.01	0.05	0.13±0.03	0.30±0.16	0.06±0.01	0.23	ND	ND	NA	0.50±0.18	0.29±0.12	0.38
alpha Humulene	1503	11.60±3.11	9.35±4.38	10.82±1.95	4.22±0.54	0.30	9.24±5.07	10.44±1.69	7.44±1.68	0.81	8.29±1.63	3.85±0.67	0.07	17.27±5.66	4.23±0.96	0.07
beta Farnesene	1506	3.25±0.79	2.38±1.19	2.21±0.41	0.36±0.05	0.08	1.47±0.56	1.71±0.17	0.68±0.20	0.15	**1.07±0.21 a**	**0.36±0.08 b**	**0.05**	1.80±0.62	0.73±0.31	0.22
gamma Muurolene	1524	**ND b**	**ND b**	**ND b**	**0.02±0.01 a**	**3.96E-04**	**ND b**	**ND b**	**0.04±0.00 a**	**1.33E-07**	ND	ND	NA	ND	ND	NA
beta Curcumene	1527	3.11±1.12	2.38±0.98	1.69±0.91	0.09±0.01	0.13	**1.25±0.33 ab**	**1.31±0.38 a**	**0.17±0.04 b**	**0.03**	2.07±0.71	2.43±1.08	0.68	0.57±0.14	1.00±0.31	0.29
alpha Curcumene	1529	**0.56±0.10 a**	**0.38±0.09 ab**	**0.23±0.04 b**	**0.10±0.01 b**	**2.44E-03**	0.23±0.07	0.28±0.03	0.22±0.04	0.60	**0.52±0.07 a**	**0.08±0.00 b**	**3.10E-03**	0.67±0.19	0.14±0.01	0.05
beta Sesquiphellandrene	1532	**2.43±0.59 a**	**1.63±0.69 ab**	**1.48±0.29 ab**	**0.30±0.04 b**	**0.04**	0.88±0.40	1.24±0.14	0.66±0.15	0.33	1.28±0.32	0.75±0.25	0.06	1.32±0.48	0.38±0.04	0.12
beta Chamigrene	1535	**ND b**	**0.44±0.14 a**	**0.07±0.02 b**	**0.02±0.00 b**	**1.58E-03**	**ND b**	**0.19±0.03 a**	**0.04±0.00 b**	**3.96E-06**	**ND b**	**0.17±0.04 a**	**0.01**	**0.04±0.01 a**	**ND b**	**2.98E-03**
Dysoxylonene	1537	**ND b**	**ND b**	**0.04±0.01 a**	**0.03±0.01 a**	**1.31E-05**	**ND b**	**ND b**	**0.06±0.01 a**	**2.03E-07**	**ND b**	**0.06±0.01 a**	**8.02E-03**	**0.05±0.01 a**	**ND b**	**9.06E-03**
beta Germacrene	1542	3.74±0.74	1.97±0.63	2.31±0.61	2.07±0.27	0.16	2.02±0.91	2.02±0.29	3.52±0.47	0.18	2.28±0.78	1.73±0.41	0.61	**3.22±0.78 a**	**1.36±0.32 b**	**0.02**
beta Bisabolene	1562	**41.16±9.48 a**	**34.43±13.5 ab**	**24.46±4.49 ab**	**4.43±0.49 b**	**0.04**	14.96±6.61	21.68±1.93	7.58±1.44	0.09	**17.65±3.68 a**	**5.22±0.91 b**	**0.05**	**21.64±6.06 a**	**5.07±0.48 b**	**0.05**

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
(-)-Gamma-cadinene	1567	**ND b**	**0.37 ± 0.11 a**	**0.20 ± 0.02 ab**	**0.10 ± 0.03 b**	**3.62E-03**	**ND b**	**0.17 ± 0.01 a**	**0.16 ± 0.02 a**	**1.49E-07**	ND	ND	NA	0.50 ± 0.19	ND	0.06
delta Cadinene	1576	**0.40 ± 0.11 ab**	**0.75 ± 0.13 a**	**0.25 ± 0.09 b**	**0.13 ± 0.03 b**	**2.17E-03**	0.27 ± 0.03	0.22 ± 0.03	0.20 ± 0.02	0.28	**0.33 ± 0.01 a**	**ND b**	**6.49E-06**	0.18 ± 0.04	0.17 ± 0.07	0.93
alpha Muurolene	1590	**0.16 ± 0.01 b**	**0.35 ± 0.08 a**	**ND c**	**0.02 ± 0.01 bc**	**3.82E-05**	**ND b**	**ND b**	**0.03 ± 0.00 a**	**2.86E-07**	**ND b**	**0.05 ± 0.01 a**	**6.13E-03**	ND	ND	NA
Elixene	1385	**0.88 ± 0.17 a**	**0.33 ± 0.09 b**	**0.49 ± 0.12 ab**	**0.46 ± 0.06 ab**	**0.02**	0.55 ± 0.19	0.48 ± 0.07	0.79 ± 0.10	0.24	0.83 ± 0.16	0.37 ± 0.10	0.13	0.65 ± 0.18	0.76 ± 0.33	0.81
beta Santalene	1509	**4.03 ± 0.93 a**	**2.56 ± 1.08 ab**	**2.40 ± 0.42 ab**	**0.63 ± 0.09 b**	**0.04**	1.71 ± 0.58	2.04 ± 0.20	1.30 ± 0.30	0.43	**2.64 ± 0.48 a**	**0.65 ± 0.12 b**	**0.02**	3.38 ± 0.91	1.45 ± 0.61	0.20
alpha Bisabolene	1556	4.82 ± 1.17	3.67 ± 1.55	2.95 ± 0.50	0.77 ± 0.10	0.07	**ND c**	**2.42 ± 0.25 a**	**1.47 ± 0.26 b**	**1.24E-05**	2.04 ± 0.52	1.61 ± 0.49	0.30	2.73 ± 0.84	0.75 ± 0.05	0.08
cis-Nerolidol	1613	**0.44 ± 0.08 b**	**1.13 ± 0.25 a**	**ND b**	**0.02 ± 0.00 b**	**4.22E-05**	**ND b**	**ND b**	**0.03 ± 0.01 a**	**2.74E-06**	**ND b**	**0.15 ± 0.03 a**	**7.97E-03**	ND	ND	NA
Epiglobulol	1668	**ND c**	**0.10 ± 0.03 a**	**0.08 ± 0.02 ab**	**0.02 ± 0.01 bc**	**1.18E-03**	**ND b**	**0.09 ± 0.01 a**	**0.02 ± 0.00 b**	**2.23E-07**	ND	0.05 ± 0.03	0.12	ND	ND	NA
alpha Bisabolol	1738	**ND b**	**ND b**	**ND b**	**0.01 ± 0.00 a**	**3.18E-06**	**ND b**	**ND b**	**0.01 ± 0.00 a**	**0.02**	ND	0.15 ± 0.07	0.11	ND	ND	NA
trans-Sesquisabinene hydrate	1585	**0.36 ± 0.04 a**	**0.27 ± 0.08 a**	**0.18 ± 0.06 ab**	**0.03 ± 0.01 b**	**4.58E-03**	**0.20 ± 0.01 a**	**0.13 ± 0.03 b**	**0.06 ± 0.01 c**	**1.85E-04**	0.22 ± 0.03	0.15 ± 0.05	0.08	0.15 ± 0.04	0.21 ± 0.09	0.62
3-Methyl-4-methylenebicyclo[3.2.1]oct-2-ene	1002	**0.98 ± 0.10 a**	**ND c**	**0.25 ± 0.04 b**	**0.07 ± 0.02 bc**	**4.30E-09**	**ND b**	**0.46 ± 0.08 a**	**0.18 ± 0.04 b**	**1.95E-04**	**ND b**	**0.25 ± 0.04 a**	**4.20E-03**	**0.46 ± 0.11 a**	**ND b**	**0.02**
Caryophyllene oxide	1632	**0.24 ± 0.02 a**	**ND c**	**0.05 ± 0.01 b**	**0.03 ± 0.00 bc**	**3.79E-10**	**0.10 ± 0.01 a**	**0.08 ± 0.02 a**	**0.03 ± 0.00 b**	**5.78E-03**	**ND b**	**0.07 ± 0.01 a**	**5.60E-03**	0.27 ± 0.16	0.11 ± 0.02	0.37
Clovene	1403	**ND b**	**ND b**	**0.49 ± 0.23 a**	**0.03 ± 0.00 b**	**1.84E-02**	**ND b**	**ND b**	**0.03 ± 0.01 a**	**7.06E-05**	ND	ND	NA	0.29 ± 0.12	0.32 ± 0.14	0.90
(-)-Aristolene	1424	**ND b**	**ND b**	**ND b**	**0.01 ± 0.00 a**	**3.63E-11**	**ND b**	**ND b**	**0.03 ± 0.00 a**	**2.03E-06**	ND	ND	NA	ND	ND	NA
1-(2,6,6-Trimethylcyclohex-2-en-1-yl)pent-1-en-3-one	1570	**0.55 ± 0.08 a**	**ND b**	**ND b**	**ND b**	**2.83E-08**	ND	ND	ND	NA	ND	0.32 ± 0.15	0.10	**0.27 ± 0.08 a**	**ND b**	**0.03**
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	1595	1.40 ± 0.33	1.08 ± 0.48	0.78 ± 0.13	0.20 ± 0.02	0.07	0.55 ± 0.19	0.67 ± 0.07	0.36 ± 0.06	0.23	**0.59 ± 0.09 a**	**0.13 ± 0.02 b**	**9.33E-03**	0.96 ± 0.22	0.34 ± 0.15	0.09
(Z)-3-Hexenol acetate	1055	**67.20 ± 10.0 a**	**61.46 ± 5.03 a**	**33.27 ± 6.67 b**	**ND c**	**5.97E-06**	**24.73 ± 4.89 a**	**3.54 ± 0.78 b**	**0.62 ± 0.17 b**	**1.16E-04**	20.31 ± 5.54	13.18 ± 2.78	0.09	**ND b**	**1.96 ± 0.22 a**	**8.27E-04**
4-tert-Butylcyclohexyl acetate	1333	**7.66 ± 1.83 a**	**5.91 ± 1.99 ab**	**1.45 ± 0.64 bc**	**0.04 ± 0.01 c**	**3.84E-03**	**0.93 ± 0.29 a**	**0.64 ± 0.15 ab**	**0.03 ± 0.01 b**	**0.02**	**12.89 ± 2.11 a**	**3.34 ± 1.16 b**	**2.52E-03**	1.87 ± 0.99	4.42 ± 1.20	0.17
2-n-Butoxyethanol	957	**13.56 ± 4.25 a**	**10.76 ± 3.90 ab**	**3.70 ± 1.68 ab**	**0.38 ± 0.05 b**	**0.02**	**4.92 ± 0.35 a**	**3.21 ± 0.71 a**	**0.52 ± 0.06 b**	**7.57E-05**	32.70 ± 16.85	10.69 ± 3.77	0.22	7.97 ± 3.14	7.24 ± 2.53	0.82
2-Methyl-6-methyleneoctan-2-ol	1111	**21.87 ± 5.25 a**	**17.07 ± 5.17 ab**	**3.11 ± 1.38 bc**	**0.15 ± 0.04 c**	**1.84E-03**	**2.64 ± 0.73 a**	**1.07 ± 0.21 ab**	**0.16 ± 0.04 b**	**5.77E-03**	**22.89 ± 4.55 a**	**7.72 ± 2.85 b**	**6.94E-03**	4.85 ± 1.96	9.30 ± 3.52	0.32

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
(E)-2-Hexen-1-ol	916	**5.07±0.45 b**	**18.20±4.00 a**	**3.43±0.27 b**	**0.44±0.03 b**	**6.13E-05**	**6.01±0.92 a**	**2.28±0.33 b**	**0.52±0.06 b**	**5.48E-05**	**ND b**	**4.10±1.18 a**	**0.03**	6.20±2.92	3.76±1.02	0.51
1-Dodecanol	1521	0.97±0.30	0.75±0.29	0.56±0.33	0.09±0.01	0.15	**0.40±0.10 ab**	**0.55±0.11 a**	**0.10±0.02 b**	**8.85E-03**	ND	0.59±0.22	0.05	0.20±0.04	0.24±0.02	0.44
(Z)-3-Hexen-1-ol	908	**13.17±1.68 a**	**17.76±2.98 a**	**3.64±0.42 b**	**0.04±0.01 b**	**5.10E-06**	**4.08±1.01 a**	**1.37±0.38 b**	**1.03±0.44 b**	**0.01**	11.37±6.69	2.52±0.66	0.27	1.93±0.39	1.28±0.27	0.28
(E)-2-Hexenal	906	**0.70±0.08 ab**	**1.05±0.31 a**	**0.24±0.04 bc**	**ND c**	**1.34E-03**	**ND b**	**0.25±0.03 a**	**ND b**	**5.96E-08**	1.22±0.42	0.05±0.02	0.05	ND	ND	NA
Heptanal	954	**0.28±0.06 a**	**0.35±0.02 a**	**0.11±0.01 b**	**0.02±0.00 b**	**6.17E-06**	**0.50±0.07 a**	**0.33±0.04 a**	**ND b**	**1.54E-05**	**1.02±0.20 a**	**0.35±0.06 b**	**0.01**	ND	0.37±0.15	0.07
Nonanal	1149	**7.47±1.08 a**	**6.24±1.84 a**	**1.50±0.46 b**	**0.12±0.02 b**	**4.14E-04**	**2.02±0.59 a**	**1.55±0.56 ab**	**0.14±0.02 b**	**0.04**	**15.11±3.91 a**	**1.69±0.47 b**	**0.02**	2.33±1.10	3.06±0.51	0.61
Decanal	1251	**3.24±0.61 a**	**2.50±0.79 ab**	**0.56±0.21 bc**	**0.08±0.02 c**	**1.07E-03**	**0.59±0.15 a**	**0.44±0.16 ab**	**0.06±0.01 b**	**0.03**	**3.88±1.00 a**	**0.84±0.23 b**	**0.02**	1.32±0.52	1.34±0.11	0.97
Undecanal	1355	**0.94±0.19 a**	**0.62±0.16 ab**	**0.36±0.16 ab**	**0.07±0.01 b**	**4.83E-03**	**0.27±0.05 a**	**0.31±0.06 a**	**0.04±0.01 b**	**1.69E-03**	**0.94±0.22 a**	**0.40±0.11 b**	**0.02**	0.24±0.06	0.32±0.04	0.32
Decane	1047	**4.08±1.29 a**	**3.47±0.56 a**	**1.25±0.40 ab**	**ND b**	**3.69E-03**	2.30±1.04	1.34±0.50	ND	0.09	6.11±1.93	1.74±0.51	0.08	3.14±1.11	4.19±0.94	0.57
Dodecane	1247	**2.11±0.30 a**	**0.86±0.14 b**	**0.40±0.06 b**	**0.46±0.11 b**	**1.19E-05**	**0.69±0.15 ab**	**0.93±0.14 a**	**0.34±0.06 b**	**0.02**	**1.48±0.31 a**	**0.54±0.08 b**	**0.04**	1.74±0.98	0.61±0.09	0.32
4,6-Dimethyldodecane	1322	**0.30±0.03 a**	**0.29±0.05 a**	**0.12±0.05 b**	**ND b**	**1.42E-04**	**0.20±0.07 a**	**0.10±0.02 ab**	**0.03±0.00 b**	**0.04**	**0.47±0.08 a**	**0.10±0.02 b**	**0.01**	0.17±0.10	0.13±0.03	0.75
Tridecane	1338	**0.35±0.03 a**	**0.26±0.04 ab**	**0.17±0.04 b**	**0.03±0.01 c**	**3.95E-05**	**0.25±0.04 a**	**0.15±0.04 ab**	**0.07±0.01 b**	**9.51E-03**	**0.70±0.13 a**	**0.20±0.06 b**	**7.78E-03**	0.12±0.03	0.19±0.08	0.52
2,2,4,4,6,8,8-Heptamethylnonane	1368	**2.53±0.31 a**	**2.65±0.35 a**	**0.29±0.04 b**	**0.08±0.02 b**	**2.68E-07**	**1.11±0.21 a**	**ND b**	**0.04±0.00 b**	**3.76E-05**	**5.88±1.39 a**	**1.03±0.35 b**	**0.02**	0.85±0.43	1.40±0.39	0.46
Tetradecane	1375	**0.56±0.09 a**	**0.46±0.13 a**	**0.23±0.09 ab**	**0.03±0.00 b**	**4.17E-03**	**0.30±0.01 a**	**0.20±0.03 b**	**0.05±0.01 c**	**4.61E-06**	**0.77±0.08 a**	**0.30±0.07 b**	**0.01**	0.22±0.14	0.18±0.05	0.84
Pentadecane	1450	**1.39±0.34 a**	**1.33±0.53 a**	**0.43±0.17 a**	**0.13±0.03 a**	**0.03**	0.58±0.11	0.39±0.13	0.19±0.02	0.06	**1.33±0.23 a**	**0.27±0.03 b**	**7.90E-03**	0.46±0.18	0.33±0.06	0.59
Hexadecane	1602	**0.57±0.11 ab**	**0.79±0.30 a**	**0.09±0.02 b**	**0.03±0.01 b**	**9.44E-03**	**ND b**	**0.13±0.02 a**	**0.01±0.00 b**	**4.52E-05**	**0.42±0.06 a**	**0.15±0.02 b**	**2.98E-03**	0.22±0.07	0.11±0.02	0.19
Heptadecane	1647	**3.24±0.82 ab**	**4.58±1.93 a**	**0.72±0.31 ab**	**0.05±0.01 b**	**0.03**	0.84±0.30	0.89±0.38	0.04±0.01	0.09	**1.90±0.20 a**	**0.39±0.05 b**	**1.03E-03**	0.55±0.26	0.58±0.13	0.92
Octadecane	1810	**0.48±0.10 ab**	**0.67±0.25 a**	**0.23±0.09 ab**	**0.02±0.00 b**	**0.03**	**ND b**	**0.17±0.02 a**	**0.01±0.00 b**	**7.68E-07**	**0.45±0.08 a**	**0.16±0.04 b**	**2.97E-03**	0.07±0.01	0.12±0.02	0.12
Nonadecane	1853	**1.47±0.33 ab**	**2.05±0.78 a**	**0.53±0.22 ab**	**0.02±0.00 b**	**0.02**	**0.22±0.05 a**	**0.32±0.07 a**	**0.01±0.00 b**	**2.26E-03**	**0.65±0.08 a**	**0.29±0.05 b**	**0.02**	0.18±0.07	0.31±0.04	0.25
p-Propyltoluene	1097	**ND b**	**ND b**	**ND b**	**0.09±0.02 a**	**1.76E-05**	**ND b**	**ND b**	**0.11±0.01 a**	**2.49E-07**	**2.03±0.59 a**	**ND b**	**0.03**	ND	ND	NA
Ethylbenzene	912	**ND b**	**ND b**	**ND b**	**0.09±0.01 a**	**1.18E-09**	ND	ND	ND	NA	ND	ND	NA	ND	ND	NA
o-Xylene	933	**ND b**	**ND b**	**ND b**	**0.10±0.01 a**	**2.67E-10**	**ND b**	**0.47±0.08 a**	**0.08±0.01 b**	**4.47E-05**	**3.43±0.47 a**	**0.70±0.19 b**	**5.18E-03**	1.09±0.60	0.70±0.23	0.61
1,2,4-trimethylbenzene	1009	**ND b**	**ND b**	**ND b**	**0.14±0.03 a**	**3.44E-06**	**ND b**	**ND b**	**0.18±0.02 a**	**2.00E-08**	**ND b**	**0.28±0.07 a**	**0.01**	ND	ND	NA
p-Diethylbenzene	1115	**0.56±0.10 a**	**0.18±0.02 b**	**0.12±0.05 b**	**0.14±0.05 b**	**3.32E-04**	0.27±0.06	0.25±0.03	0.23±0.07	0.90	0.64±0.15	0.32±0.04	0.14	0.41±0.18	0.93±0.39	0.35

		Control					JAsp				Psyll			JAsp_Psyll		
Compound	**LRI**	**T0**	**T1**	**T2**	**T3**	**p-value**	**T1**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**	**T2**	**T3**	**p-value**
4-Methylindane	1190	**ND b**	**ND b**	**ND b**	**0.06 ± 0.01 a**	**3.60E-05**	**ND b**	**ND b**	**0.12 ± 0.02 a**	**3.65E-06**	**ND b**	**0.28 ± 0.09 a**	**0.03**	ND	ND	NA
Prehnitene	1192	**ND b**	**ND b**	**ND b**	**0.09 ± 0.02 a**	**3.99E-05**	**ND b**	**ND b**	**0.18 ± 0.03 a**	**5.03E-06**	ND	ND	NA	ND	0.55 ± 0.21	0.06
m-Xylene	1121	**ND b**	**ND b**	**ND b**	**0.20 ± 0.05 a**	**6.33E-05**	**19.58 ± 7.96 a**	**ND b**	**0.38 ± 0.05 b**	**0.02**	**ND b**	**1.67 ± 0.37 a**	**0.01**	ND	ND	NA
Methyl salicylate	1230	**12.07 ± 3.35 b**	**25.97 ± 4.11 a**	**3.18 ± 1.14 bc**	**0.06 ± 0.01 c**	**2.00E-05**	**0.90 ± 0.26 a**	**0.40 ± 0.12 ab**	**ND b**	**8.11E-03**	2.52 ± 0.81	0.67 ± 0.07	0.09	1.42 ± 0.76	ND	0.13
Butyl Acrylate	935	ND	ND	ND	ND	NA	ND	ND	ND	NA	**8.03 ± 2.04 a**	**2.10 ± 1.16 b**	**0.03**	3.72 ± 2.76	1.30 ± 0.55	0.46
n-Butyl butanoate	1031	ND	ND	ND	ND	NA	ND	ND	ND	NA	1.01 ± 0.22	0.73 ± 0.38	0.50	ND	ND	NA
Methyl benzoate	1129	**30.58 ± 0.90 a**	**35.60 ± 2.95 a**	**8.31 ± 1.51 b**	**0.15 ± 0.02 c**	**1.49E-10**	**12.62 ± 1.32 a**	**3.23 ± 0.57 b**	**0.30 ± 0.07 b**	**5.88E-07**	**6.99 ± 1.83 a**	**ND b**	**0.02**	12.61 ± 7.54	ND	0.17
2-Ethyl-3-hydroxyhexyl 2-methylpropanoate	1417	1.37 ± 0.53	1.04 ± 0.33	0.87 ± 0.50	0.02 ± 0.00	0.15	1.17 ± 0.48	0.78 ± 0.28	0.10 ± 0.03	0.10	1.02 ± 0.28	1.21 ± 0.58	0.62	0.11 ± 0.03	0.54 ± 0.18	0.07
Methyl ester octanoic acid	1170	**0.85 ± 0.12 ab**	**1.47 ± 0.47 a**	**0.18 ± 0.04 b**	**ND b**	**2.17E-03**	**ND b**	**0.19 ± 0.03 a**	**ND b**	**1.10E-05**	ND	0.25 ± 0.09	0.06	0.53 ± 0.20	NA	0.05
(3E)-3-Hexenyl butyrate	1224	**ND b**	**1.27 ± 0.29 a**	**0.06 ± 0.01 b**	**ND b**	**1.98E-05**	**1.02 ± 0.33 a**	**ND b**	**0.05 ± 0.02 b**	**3.85E-03**	ND	ND	NA	ND	ND	NA
cis-3-Hexenyl-.alpha.-methylbutyrate	1278	**0.35 ± 0.06 b**	**0.65 ± 0.13 a**	**0.08 ± 0.01 bc**	**ND c**	**4.14E-05**	**0.26 ± 0.08 a**	**0.11 ± 0.02 ab**	**ND b**	**5.02E-03**	**ND b**	**0.08 ± 0.01 a**	**3.26E-03**	**0.15 ± 0.03 a**	**ND b**	**7.86E-03**
2-Methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl propanoate	1644	**1.57 ± 0.32 a**	**0.98 ± 0.24 ab**	**0.98 ± 0.45 ab**	**ND b**	**0.02**	**1.55 ± 0.37 a**	**0.85 ± 0.26 ab**	**0.15 ± 0.04 b**	**8.57E-03**	ND	1.41 ± 0.76	0.14	**ND b**	**0.47 ± 0.08 a**	**5.22E-03**
2-Hexanone	816	**5.96 ± 1.01 a**	**3.07 ± 0.84 b**	**0.40 ± 0.08 b**	**0.70 ± 0.18 b**	**6.72E-05**	**1.77 ± 0.28 a**	**ND b**	**0.28 ± 0.05 b**	**1.19E-05**	14.98 ± 3.72 a	4.03 ± 1.51 b	**0.03**	3.92 ± 1.68	5.85 ± 2.66	0.23
6-Methyl-5-heptene-2-one	1024	**3.14 ± 0.28 a**	**2.56 ± 0.59 a**	**0.40 ± 0.09 b**	**0.06 ± 0.01 b**	**6.81E-06**	0.98 ± 0.45	0.71 ± 0.24	0.07 ± 0.01	0.13	**8.38 ± 1.18 a**	**0.70 ± 0.11 b**	**2.68E-03**	2.05 ± 1.35	3.28 ± 1.51	0.59
Bicyclo[3.3.0]octan-2-one, 7-ethylidene-	1264	**4.04 ± 1.41 a**	**2.19 ± 0.80 ab**	**1.45 ± 0.64 ab**	**0.21 ± 0.04 b**	**0.05**	**2.25 ± 0.43 a**	**1.72 ± 0.61 ab**	**0.20 ± 0.02 b**	**0.01**	2.46 ± 0.75	0.85 ± 0.25	0.08	0.95 ± 0.31	1.94 ± 0.29	0.08
Indole	1331	**ND b**	**10.13 ± 1.70 a**	**1.39 ± 0.32 b**	**ND b**	**5.42E-07**	**4.55 ± 1.35 a**	**4.81 ± 1.05 a**	**0.02 ± 0.00 b**	**8.06E-03**	ND	ND	NA	**2.53 ± 0.88 a**	**ND b**	**0.05**
Geranyl nitrile	1162	**28.15 ± 12.3 ab**	**94.62 ± 31.9 a**	**26.17 ± 5.67 ab**	**1.33 ± 0.94 b**	**9.89E-03**	**2.95 ± 1.50 b**	**21.42 ± 6.14 a**	**0.43 ± 0.07 b**	**2.95E-03**	5.18 ± 1.57	3.61 ± 2.26	0.61	**20.42 ± 5.82 a**	**3.21 ± 1.04 b**	**0.03**
2-Methyl-4-pentenal	847	**3.98 ± 0.96 a**	**2.42 ± 0.45 ab**	**1.00 ± 0.08 bc**	**ND c**	**4.44E-04**	**1.61 ± 0.56 a**	**0.61 ± 0.07 ab**	**ND b**	**0.01**	**17.85 ± 4.56 a**	**0.85 ± 0.27 b**	**0.02**	**0.91 ± 0.20 a**	**ND b**	**0.01**

Compound	LRI	Control					JAsp				Psyll			JAsp_Psyll		
		T0	T1	T2	T3	p-value	T1	T2	T3	p-value	T2	T3	p-value	T2	T3	p-value
(3E)-4-Ethyl-3-nonen-5-yne	1159	**2.60 ± 0.41 a**	**0.79 ± 0.13 b**	**0.50 ± 0.20 b**	**0.38 ± 0.04 b**	**1.63E-05**	1.17 ± 0.30	1.03 ± 0.11	0.61 ± 0.04	0.13	2.44 ± 1.02	1.20 ± 0.16	0.33	1.86 ± 0.91	1.93 ± 0.38	0.95
trans-(.+-.)-2-methyl-2-(4-methyl-3-pentenyl)cyclopropanecarboxaldehyde	1267	**3.05 ± 1.05 a**	**1.85 ± 0.41 ab**	**0.25 ± 0.09 b**	**0.04 ± 0.01 b**	**5.06E-03**	**0.45 ± 0.13 a**	**ND b**	**ND b**	**1.37E-03**	**2.09 ± 0.18 a**	**0.17 ± 0.02 b**	**4.30E-04**	0.58 ± 0.33	1.39 ± 0.70	0.36
3,3,7,11-tetramethyl-tricyclo[6.3.0.0(2,4)]undec-8-ene	1439	**0.11 ± 0.02 a**	**ND c**	**0.05 ± 0.01 b**	**0.05 ± 0.01 b**	**2.75E-06**	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.01	0.12	**ND b**	**0.04 ± 0.01 a**	**2.86E-03**	**0.10 ± 0.02 a**	**ND b**	**4.11E-03**
4-methylene-2,8,8-trimethyl-2-vinyl-bicyclo[5.2.0]nonane	1462	**ND c**	**ND c**	**0.07 ± 0.01 a**	**0.04 ± 0.01 b**	**3.99E-08**	**ND c**	**0.10 ± 0.01 a**	**0.06 ± 0.01 b**	**4.06E-06**	ND	ND	NA	**0.20 ± 0.06 a**	**ND b**	**0.03**
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	1479	1.99 ± 0.52	1.51 ± 0.58	1.54 ± 0.23	0.49 ± 0.08	0.11	1.50 ± 0.73	1.94 ± 0.33	0.89 ± 0.20	0.33	**0.76 ± 0.14 a**	**0.40 ± 0.07 b**	**0.03**	1.81 ± 0.65	0.46 ± 0.10	0.09
Phenol	1022	**2.88 ± 0.08 a**	**1.65 ± 0.16 b**	**ND c**	**ND c**	**1.41E-13**	ND	ND	ND	NA	**12.20 ± 3.00 a**	**1.61 ± 0.51 b**	**0.02**	ND	3.48 ± 1.42	0.07