Journal of Plant Physiology 314 (2025) 154615

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Salicylic acid and methyl jasmonate activate key genes of plant-defense pathways conferring partial protection to *Polystigma amygdalinum* in a susceptible almond cultivar

Núria Real [a], Gemma Pons-Solé [a], Jordi Luque [a], Mercè Llugany [b], Soledad Martos [b,*]

[a] IRTA, Sustainable Plant Protection, Centre de Cabrils, Cabrils, 08348, Spain
[b] Plant Physiology Laboratory, BABVE, Universitat Autònoma de Barcelona, Campus UAB, Bellaterra, 08193Spain

ARTICLE INFO

Keywords:
Almond
Defense genes
Hormone profiling
Lignin
Prunus amygdalus
Red leaf blotch

ABSTRACT

Red leaf blotch (RLB) of almond, caused by *Polystigma amygdalinum*, is an economically important foliar disease affecting almond crops. This study explored the hormonal responses of two almond cultivars, namely 'Tarraco' (highly susceptible) and 'Mardía' (highly tolerant), to *P. amygdalinum*. Hormonal profiling and gene expression analyses were conducted to examine the roles of salicylic acid (SA), jasmonic acid (JA), and 1-aminocyclopropane-1-carboxylic (ACC) acids, and methyl jasmonate (MeJA) in plant defense mechanisms. Results showed a significant accumulation of SA in symptomatic leaves of both cultivars, suggesting a SA-mediated defense response to the pathogen. However, no substantial changes in JA and ACC levels were observed. In 'Tarraco', expression of SA-responsive genes (*PR1* and *PR5*) and ET/JA-associated genes (*ACO* and *ERF1*) increased, but the cultivar remained susceptible. In contrast, symptomatic 'Mardía' leaves exhibited increased expression in *CAD*, linked to lignin biosynthesis, while other hormone-related genes (*ACO*, *ERF1*, *PR1*, and *PR5*) did not show significant changes. Thus, 'Mardía' could be following a different defense strategy against RLB. Exogenous applications of SA and MeJA significantly reduced RLB incidence and severity in young 'Tarraco' trees, with MeJA enhancing *ERF1* expression and SA increasing both *ERF1* and *CAD* expression. MeJA also inhibited plant growth. These findings reveal contrasting defense mechanisms between the two almond cultivars, suggesting a possible protection against RLB through lignin biosynthesis. Furthermore, the protective role of SA would be associated with *CAD*, indicating a connection between SA signaling and the phenylpropanoid pathway.

1. Background

Almond (*Prunus amygdalus*) is a widespread crop in the Mediterranean basin and the Middle East, which is traditionally grown in mostly dry areas with poor soil conditions (Gradziel et al., 2017). Red leaf blotch (RLB) of almond, caused by the ascomycete *Polystigma amygdalinum*, is an endemic foliar disease in this almond-growing area, which is considered to have significant economic impact (Banihashemi, 1990; Cannon, 1996; Saad and Masannat, 1997). *Polystigma amygdalinum* is a hemibiotrophic pathogen specific to almonds, though other *Polystigma* species are known to infect other *Prunus* species (Bundhun et al., 2019; Cannon, 1996; Suzuki et al., 2008). In Spain, where this study was conducted, RLB incidence has increased during the last years, and it is currently considered a re-emerging disease not only in old traditional plantations but also in most new ones (Ollero-Lara et al., 2016; Torguet et al., 2016). It has been hypothesized that increased incidence of RLB in Sain may be linked to the growing area of almond plantations in the last decades in this country, as well as the use of new late-flowering cultivars, particularly 'Guara', which are more susceptible to RLB than traditional cultivars (Miarnau et al., 2021; Ollero-Lara et al., 2019). First RLB symptoms appear in spring as pale green to yellowish spots on both leaf sides, later progressing to yellowish-orange and finally dark brown shades with age. Leaf spot size increases through the spring and summer, covering almost the entire leaf surface by late summer (Zúñiga et al., 2020). Occasionally, severe infections under hot and dry conditions can induce an early defoliation in summer, thus reducing photosynthetic activity of trees and their subsequent almond yield (López-López et al., 2016). Differential susceptibility to RLB is known to occur among almond cultivars (Heydarian and Moradi, 2005; Miarnau et al., 2021; Ollero-Lara et al., 2016, 2019). To date, no resistant cultivars to

* Corresponding author.
E-mail address: soledad.martos@uab.cat (S. Martos).

https://doi.org/10.1016/j.jplph.2025.154615
Received 10 April 2025; Received in revised form 15 September 2025; Accepted 16 September 2025
Available online 17 September 2025

P. amygdalinum have been identified, but extreme differences in tolerance to RLB have been reported (Miarnau et al., 2021; Ollero-Lara et al., 2016, 2019; Zúñiga et al., 2019). Among the most RLB-susceptible cultivars are 'Tarraco' and 'Guara', whereas 'Mardía' is recognized as one of the most tolerant cultivars used in Spain.

Plants have evolved sophisticated defense mechanisms to protect themselves, aiming to reduce the harm inflicted by natural enemies. These responses often converge on different phytohormone pathways with salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) being highlighted as classical key regulators (Loake and Grant, 2007; Lorenzo et al., 2003). Further advancements have revealed a more complex scenario where other hormones, including auxins, abscisic acid, cytokinins, gibberellins, and brassinosteroids, have emerged as important contributors in the interactions between plants and pathogens (Bari and Jones, 2009; Denancé et al., 2013; Naseem et al., 2015; Ren et al., 2020). The crucial role of plant hormones in the immune response is managed by an intricate network system. This system aims to achieve a finely-tuned regulation to mitigate the inherent fitness cost associated with plant defense (Aerts et al., 2021; Denancé et al., 2013; Vlot et al., 2021). It is widely accepted that SA signaling activates plant defense against biotrophic and hemibiotrophic pathogens, whereas a combination of JA and ET signaling modulates defense against necrotrophic pathogens and herbivores (Glazebrook, 2005). Research has confirmed the existence of SA-JA crosstalk in the regulation of plant immune responses, often in an antagonistic manner (Li et al., 2017, 2019; Pieterse et al., 2009). In general, increased SA resistance results in significant susceptibility against necrotrophs by suppression of the ET/JA signaling pathway (Spoel et al., 2007). Despite this general rule, there are several exceptions which suggest that SA, JA, and ET signaling pathways can induce resistance against both biotrophic and necrotrophic pathogens (Liu et al., 2016; Tsuda et al., 2009; Ullah et al., 2022). Mutations in key genes from plant hormone signaling pathways have revealed their functions within the immune response network. Likewise, the application of signaling molecules can demonstrate the role that a particular phytohormone plays in the response to a specific pathogen attack. Nonetheless, the manipulation of a plant hormone pathway can lead to adverse effects on growth and resistance to pathogens with different lifestyles (Holeski et al., 2012). Phytohormones and their signaling pathways interact with other defensive metabolites to orchestrate immune reactions in plants. Among these defensive compounds is the lignin, an aromatic heteropolymer predominantly deposited in secondary cell walls. Lignin is synthesized from three basic monomers (p-hydroxyphenyl, guaiacyl and syringyl monolignols) through the phenylpropanoid pathway. Its deposition is not only vital for the mechanical support and transportation through vascular tissues, but also plays a crucial role in plant protection, reinforcing cell walls to prevent pathogens from penetrating cells (Dong and Lin, 2021; Miedes et al., 2014). Moreover, substantial evidence indicates a connection between SA and lignification, thus highlighting the intricate interplay of these components in plant defense (Zhao and Dixon, 2011).

Interestingly, closely related plants can activate distinct mechanisms to combat a particular disease. Resistant or tolerant genotypes induce effective responses to control pathogens that may be absent, repressed, or only partially activated in susceptible plants, leading to severe symptoms. A study by Zúñiga et al. (2019) showed different response patterns to RLB in two almond cultivars by differentially expressing various defense-related genes. Briefly, 'Mardía' showed an early response to repress the RLB pathogen based on lignin accumulation and production of defensins in infected leaf tissues. In contrast, 'Tarraco' presented a later activation of lignin synthesis together with the production of anthocyanins and phenolic acids, which were inefficient to inhibit fungal growth (Zúñiga et al., 2019). Building on these findings, the current research aimed to expand the knowledge of the molecular strategies employed by almond trees to face RLB by focusing on the role of some hormone and plant-defense genes that can induce tolerance to RLB. Thus, the main objectives were 1) to explore the hormone and selected defense-genes profilings in tolerant and susceptible almond cultivars to *P. amygdalinum*, and 2) to study the effects of two hormone treatments on the expression of some defense genes and evaluate their protective effect against RLB infection in a susceptible cultivar.

2. Methods

2.1. Experimental field site

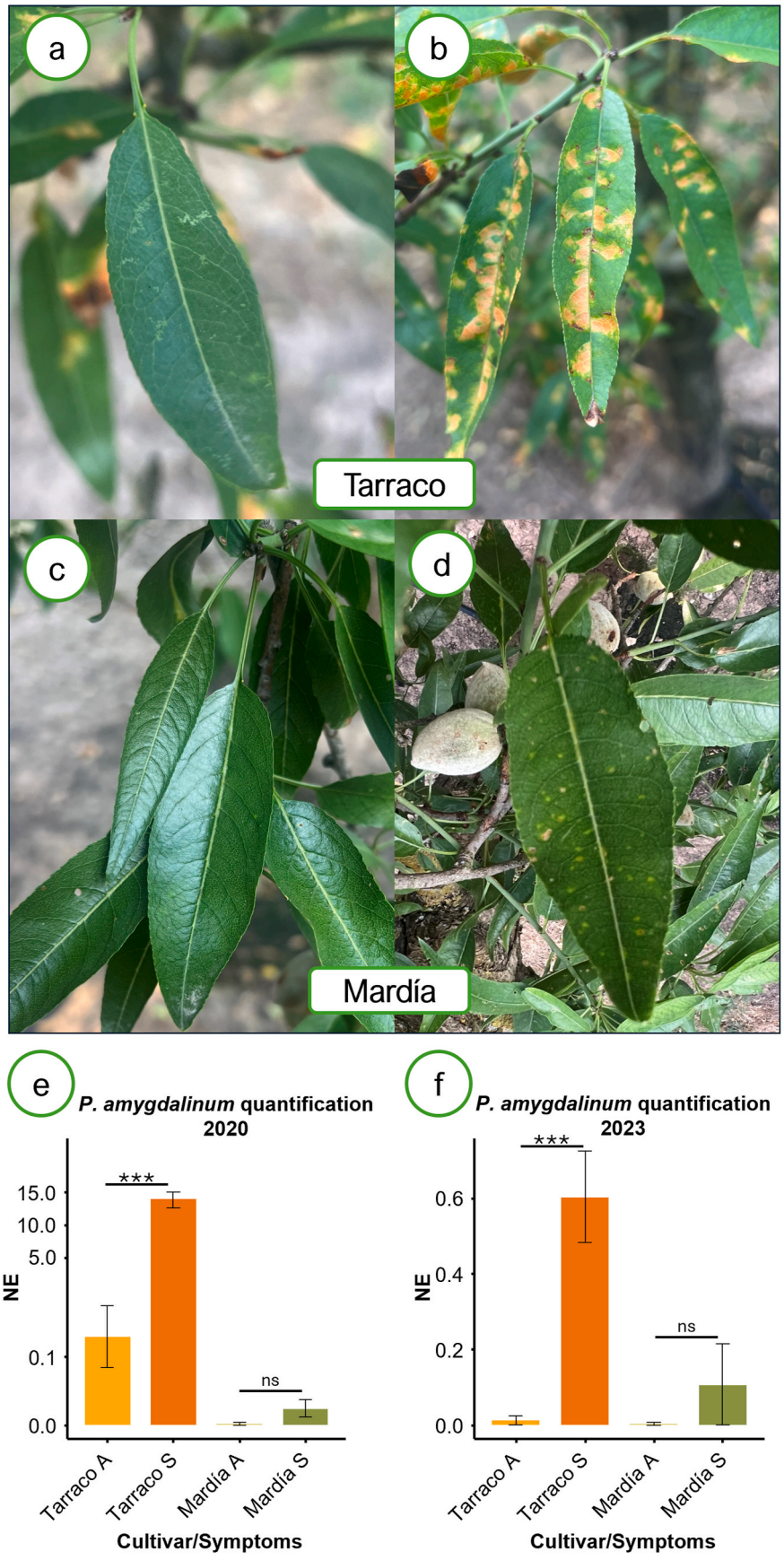
Two distinct experiments were conducted from 2020 to 2023 in an experimental almond orchard owned by IRTA and located in Les Borges Blanques, Catalonia, Spain (UTM 31 T, X = 320870, Y = 4597530). The orchard was planted in 2009 as bare root trees with 21 cultivars grafted onto 'INRA GF-677' rootstock, pruned as a central axis, and with a tree spacing of 4 × 2 m. The orchard was drip-irrigated, and pruning, soil management, and fertilization practices were based on the Spanish Integrated Production Management law (BOE, 2002). No fungicide treatments were applied during the experimental period to allow natural infection of trees. RLB occurs naturally in the area as known from previous studies (Miarnau et al., 2021). Cultivars used in this work were 'Mardía' (very tolerant to RLB) and 'Tarraco' (very susceptible).

2.2. Experiment 1. hormone and defense-related genes profiling in mature almond trees

This experiment aimed at exploring differences in hormones and defense gene profilings related to cultivar and RLB-infection status variables. Leaves from eight almond trees of each studied cultivar, 'Mardía' and 'Tarraco', were sampled separately for each RLB status (asymptomatic and symptomatic; see Fig. 1) in July of 2020 and 2023, resulting in total 32 samples each year. Five leaves per RLB status were collected from each tree at different heights (0.8–1.5 m) from the NE-facing side. Samples were processed for hormone profiling in 2020 and for gene expression analysis in 2020 and 2023. The endogenous plant hormones, 1-aminocyclopropane-1-carboxylic acid (ACC), salicylic acid (SA), methyl jasmonate (MeJA), and (±)-Jasmonic acid (JA) were quantified. Additionally, quantification of selected defense genes through qPCR was conducted in 2020 and 2023 (see below for details).

2.3. Experiment 2. defense-related genes profiling after hormonal treatments in young almond plants

A second experiment was conducted to investigate the gene expression of selected defense-related genes following periodic spraying of leaves with hormones (SA and MeJA) and simultaneous natural exposure to RLB infection in the field. One-year-old 'Tarraco' trees (N = 14 trees per treatment) were randomly placed between mature trees within rows in the experimental orchard. Plants were height measured before starting the hormonal treatment and then subjected to weekly applications of either SA or MeJA for a total of 9 weeks starting in mid-May until mid-July 2023 (period during which RLB infections mainly occur in the area), at the following concentrations: untreated control (2 % ethanol), salicylic acid (0.693 g L^{-1} SA in 2 % ethanol), or methyl jasmonate (1 g L^{-1} MeJA in 2 % ethanol). SA and MeJA were purchased from Sigma Aldrich (St Louis, MO, USA). At the end of the experimental period, plants were taken to IRTA facilities and processed for final height measurement and leaf collection for gene expression, that were conducted 10 and 13 days, respectively, after the last hormone treatment. For the gene expression analysis, eight bulk leaf samples (each consisting of five leaves) were randomly collected from four different trees and for each hormone treatment (N = 3) and RLB status (N = 2), resulting in a total of 48 samples for analysis. Finally, RLB incidence and severity among different hormone treatments were evaluated 10 days after the last hormone treatment using the methods described by Miarnau et al. (2021).



(caption on next page)

Fig. 1. Asymptomatic (a and c) and symptomatic (b and d) leaves of 'Tarraco' (a and b) and 'Mardía' (c and d) almond cultivars, and relative quantification of *Polystigma amygdalinum* in those leaf categories in 2020 (e) and 2023 (f). Asterisks (***) indicate significant differences ($P < 0.001$) and ns indicates 'non-significant differences' in the indicated pairwise comparison. Abbreviations: NE, normalized expression; A, asymptomatic leaves; S, RLB-symptomatic leaves. Error bars show the standard error of the mean (N = 8).

2.4. Plant material collection

Regardless of the experiment, collected leaves were wrapped in aluminum foil, appropriately labeled, immediately frozen in liquid nitrogen in the field, and taken to the laboratory. Frozen samples were finely ground in liquid nitrogen using a mortar and pestle and then stored at −80 °C until further processing.

2.5. Hormone quantification

The endogenous plant hormones, namely ACC, JA, MeJA and SA, were extracted and purified following a modified method based on Llugany et al. (2013). Briefly, 250 mg of fresh material were ground in an ice-cold mortar with 750 μL of the extraction solution, consisting of methanol (MeOH):2-Propanol (2-PrOH):acetic acid (HOAc) (20:79:1, v/v). Then, the supernatant was collected after centrifugation at 1000 g for 5 min at 4 °C. These steps were repeated two more times and pooled supernatants were lyophilized. Finally, samples were dissolved in 250 μL MeOH and filtered with a Spin-X centrifuge tube filter of 0.22 μm cellulose acetate (Costar, Corning Inc., Glendale, AZ, USA). Hormone quantification was done using a standard calibration curve with the respective hormone standard solutions ranging from 50 to 1000 μg kg^{-1}. The deuterated hormones 1-aminocyclopropane-2,2,3,3-d4-carboxylic acid (ACCd4), salicylic d6 acid (SAd6) at 30 μg kg^{-1} and jasmonic-2,4,4-d3-(acetyl-2,2-d2) acid (JAd5) at 300 μg kg^{-1} were used as internal standards in all the processed samples and standards. The analysis of plant hormones was conducted at the CCiT-UB (Centres Científics i Tecnològics) of the University of Barcelona, Barcelona, Spain. Plant hormones were analyzed by LC-ESI-MS/MS system in multiple reaction monitoring mode (MRM) as described by Segarra et al. (2006). First, phytohormones were separated using an HPLC Agilent 1100 (Waldbronn, Germany) on a Luna Omega C18 2.1 × 100 mm ID, 1.6 μm column (Phenomenex, Torrance, CA, USA) at 50 °C at a constant flow rate of 0.8 mL min^{-1} and 10 μL injected volume. The elution gradient was carried out with a binary solvent system consisting of 0.1 % of formic acid in methanol (solvent A) and 0.1 % of formic acid in Milli-Q water (solvent B) with the following proportions (v/v) of solvent A (t [min], % A): (0, 2) (0.2, 2), (1.6, 100), (2, 100), (2.1, 2) and (3, 2). MS/MS experiments were performed on an API 4000 triple quadrupole mass spectrometer (PerkinElmer Sciex, Concord, Ont, Canada). All the analysis were performed using the Turbo Ionspray source in negative ion mode for SA and JA, and positive ion mode for ACC and MeJA and the same mode for their respective deuterated forms. Hormone contents were finally expressed as ng·g^{-1} leaf fresh weight (FW) after proper calculations.

2.6. RNA extraction and cDNA synthesis

Total RNA was extracted from approximately 100 mg of frozen pulverized leaves using the Maxwell® RSC plant RNA kit (Promega Corporation, Madison, WI, USA) with the aid of a Maxwell robot (Promega Corp.) and following manufacturer's instructions. Extracted RNA was quantified and quality checked using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). One μg of RNA was used in a reverse-transcription reaction to obtain cDNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) and following manufacturer's instructions on a StepOnePlus™ Real-Time PCR System (Applied Biosystems™, Thermo Fisher Scientific). The resulting cDNA was used in a 1:20 dilution to quantify gene expression by qPCR.

2.7. RT-qPCR and gene expression analyzes

The pathogen-induced responses in mature (Experiment 1) and young (Experiment 2) almond trees were studied on genes involved in different signaling pathways. For the SA pathway, pathogenesis-related protein 1 (*PR1*) and pathogenesis-related protein 5 (*PR5*) were selected, while those selected for the ethylene signaling pathway were ethylene response factor 1 (*ERF1*) and 1-aminocyclopropane-1-carboxylate oxidase (*ACO*) Additionally, cinnamyl alcohol dehydrogenase (*CAD*) and hidroxycinamate quinole transferase (*HQT*) were included due to their reported importance in RLB tolerance, particularly within the phenylpropanoid pathway (Zúñiga et al., 2019) (Supplementary Table 1). The coding sequences to generate the primers for amplification were sourced from the Rosaceae database (https://www.rosaceae.org). Primer pairs were designed over the almond genome ALMONDv2 (Alioto et al., 2020) using the Primer-BLAST tool (Ye et al., 2012). To ensure reliable comparisons, the eukaryotic translation elongation factor 2 (*TEF2*) gene of almond was included as housekeeping gene (Tong et al., 2009). The RT-qPCR was conducted on a StepOnePlus™ Real-Time PCR System using TB Green® Premix Ex Taq™ (Takara, Saint-Germain-en-Laye, France) for amplification. The cycling conditions comprised an initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 20 s at 60–65 °C, and 72 °C for 20 s. A negative control without DNA template was also included in each reaction run. Three technical replicates were used in all reactions. A melting curve cycle from 65 to 95 °C was performed to assess amplicon specificity. Finally, gene expression was quantified using the methods described by Livak and Schmittgen (2001), with normalized expression (NE) against the housekeeping gene ($2^{-\Delta Ct}$) for all experiments. For young 'Tarraco' trees, the relative expression (RE) was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), with the RE ratio calculated as relative to the asymptomatic control leaves ($2^{-\Delta\Delta Ct}$). In contrast, for mature almond trees, including both 'Mardía' and 'Tarraco' (whether asymptomatic or symptomatic), no relativization to asymptomatic samples was performed as two distinct asymptomatic samples were available for comparison. Instead, the normalized expression ratio (NE) of each gene compared to the *TEF2* housekeeping gene was used directly for statistical analyses.

2.8. Pathogen quantification

DNA from about 100 mg of the pulverized leaf samples was extracted using the NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany) following manufacturer's instructions. Extracted DNA was quantified, and quality checked with a Nanodrop 2000 spectrophotometer. A specific pair of primers for quantification of *P. amygdalinum* was used: I2F4 [5′-GAAGTCCAATCAAGCCGTAG-3′] and I2R2 [5′-GTTTCACTACGCT CAGAGTC-3′] (forward and reverse, respectively) generating a 99 bp fragment (Zúñiga et al., 2018). Fungal quantification was normalized with the almond *TEF2* gene as reference (Tong et al., 2009). qPCR was performed on a StepOnePlus™ Real-Time PCR System using TB Green® Premix Ex Taq™ (Takara, Saint-Germain-en-Laye, France). The cycling conditions were as follows: denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s and then 60 °C for 30 s. A negative control without DNA template was also included in each reaction run. Three technical replicates were used in all reactions. To assess the amplification specificity, a melting curve analysis was performed at the end of each qPCR reaction, by monitoring the fluorescence from 75 to 95 °C, every 0.1 °C. The fungal amount was estimated indirectly by quantifying the normalized expression (NE) of the fungus against the housekeeping

gene *TEF2* in samples from four trees per cultivar and RLB status using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.9. Statistical analysis

Experimental data were analyzed using the R software v. 4.1.2 (R Core Team, 2023). Means of technical replications were calculated to reduce pseudo-replication and only means of biological replicates were further used in the analyses. All analyses were performed with linear modeling using the package *stats* v. 4.10 and a multifactorial design approach, except for the gene expression analyses in Experiment 1, and the RLB incidence data in Experiment 2. In the former case, experiment repetition in 2020 and 2023 was considered as a random factor and analyzed through linear mixed modeling (*lme4* v. 1.1–33; Bates et al., 2015). In the latter case, a generalized linear modeling approach (*glm* function, *stats* package) was used to analyze binomial data. When necessary, variables were transformed (*dplyr* v.1.0.7; Wickham et al., 2023) to ensure normality and homoscedasticity of data, and model assumptions were further assessed using *car* v. 3.1-2 (Fox and Weisberg, 2019) and *gvlma* v. 1.0.0.3 (Peña and Slate, 2006) for residual diagnostics, including test for normal distributions and independence of errors.

Linear models, which included cultivar (1 df) and RLB infection status (1 df) as fixed factors, and their interaction (1 df), were separately fitted for the analyses of hormone content and gene expression (dependent variables) of Experiment 1 data using *stats*. Based on the ANOVA results, we eventually refined the models for each dependent variable by progressively removing non-significant factors and interactions using the *anova* function with the LRT option (*stats*). As for the analyses of plant height, RLB severity, and gene expression data (dependent variables) of Experiment 2, the saturated model included hormone treatments (2 df) and infection status (1 df). Additionally, RLB severity scores (proportion) were arcsine-transformed (*dyplr*) to meet normality and homoscedasticity assumptions. After analyses, mean comparisons were made with the Tukey's HSD test at $\alpha = 0.05$ (*TukeyHSD* function, *stats*), and eventually with the Dunnett's test [*glht* function, *multcomp* v. 1.4–25 (Hothorn et al., 2008)] for specific comparisons between hormone treatments and the untreated control in the case of Experiment 2 data. Data visualization was performed using *ggplot2* v. 3.4.0 (Wickham, 2016).

3. Results

3.1. Experiment 1. hormone and defense-related genes profiling in mature almond trees

The presence of *P. amygdalinum* on leaves of both studied cultivars was initially determined by visual inspection of sampled leaves and later confirmed through qPCR. The mean relative quantity of *P. amygdalinum* in infected 'Tarraco' leaves was about 109-fold or 49-fold higher than in

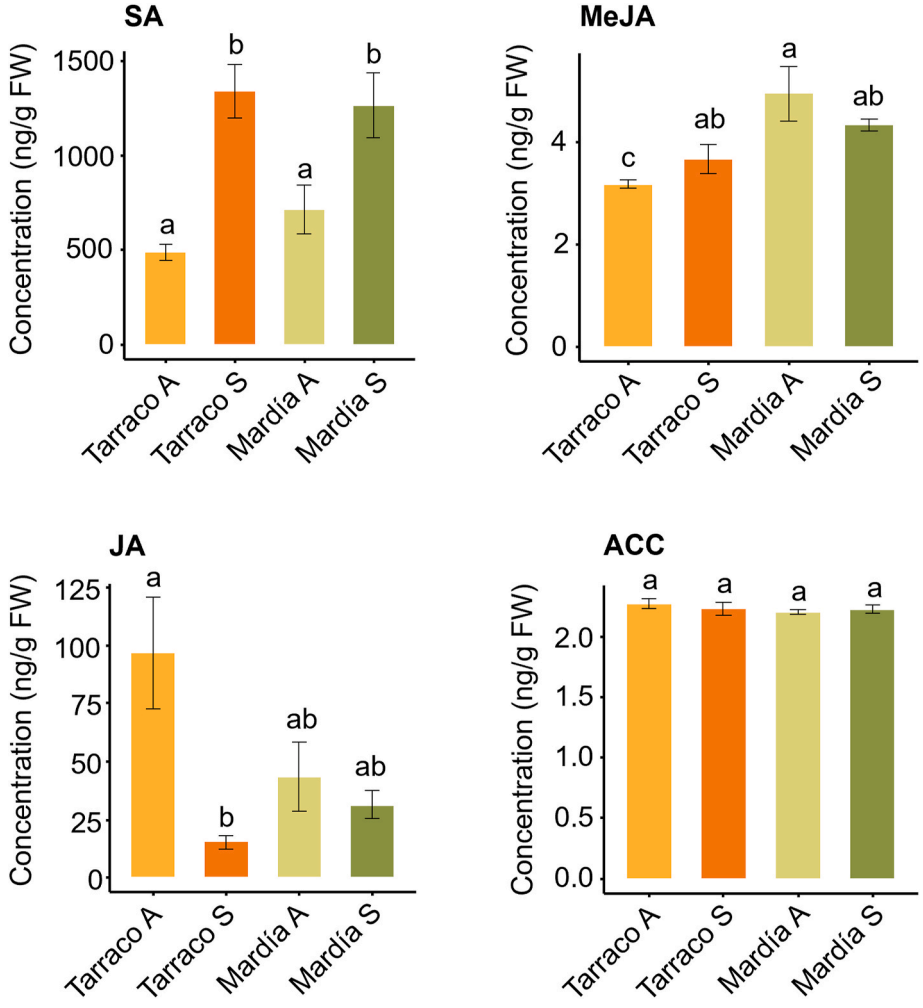


Fig. 2. Hormone contents in two almond cultivars ('Mardía' and 'Tarraco') showing differential susceptibility to *Polystigma amygdalinum*. Letters indicate significant differences between cultivar and disease status combinations according to Tukey's test ($P < 0.05$). Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; SA, salicylic acid; MeJA, methyl jasmonate; JA, jasmonic acid; A, asymptomatic leaves; S, symptomatic leaves. Error bars show the standard error of the mean (N = 8).

asymptomatic leaves for 2020 and 2023 respectively (Fig. 1). However, in symptomatic 'Mardía' leaves, the pathogen was either 565-fold or 5.5-fold lower than in infected 'Tarraco' leaves for 2020 and 2023 respectively.

Concentrations of phytohormones in mature almond trees revealed that SA levels increased significantly in symptomatic leaves compared to asymptomatic ones in both cultivars ($P < 0.001$) (Fig. 2), whereas no significant interaction between cultivar and RLB infection was detected. However, SA in 'Mardía' increased roughly 1.8-fold in infected leaves (up to 1262 ± 171 ng g^{-1} FW) (mean ± std err), whereas a 2.7-fold increase was observed in 'Tarraco' infected leaves. In contrast, mean MeJA contents were significantly lower ($P < 0.001$) in 'Tarraco' leaves (3.41 ± 0.15 ng g^{-1} FW) as compared to 'Mardía' (4.62 ± 0.26 ng g^{-1} FW), while they were independent on RLB infection (Fig. 2). As observed for SA, no significant interaction between cultivar and RLB infection status was detected for the leaf MeJA content. Regarding JA contents in leaves, a significant interaction ($P = 0.006$) between cultivar and RLB infection was detected, with 'Tarraco' showing a decrease in JA content in symptomatic leaves (about 6.3-fold lower) compared to asymptomatic ones, whereas 'Mardía' showed similar JA levels regardless of RLB infection (Fig. 2). Moreover, the factors cultivar and RLB infection status were statistically not significant in this later case. Finally, ANOVA outputs of the hormonal profiling analyses showed that ACC contents were not influenced by the factors: cultivar, RLB status, and their interaction (Fig. 2).

Normalized expression of defense-related genes indicated that all of them presented a significant interaction between cultivar and RLB infection status (all $P < 0.01$). Moreover, symptomatic 'Tarraco' leaves consistently exhibited the highest NE levels across all genes (Fig. 3). The NE of *PR1* and *PR5* was significantly affected by both cultivar and RLB infection status (all $P < 0.001$), while *ERF1* was only significantly affected by cultivar ($P < 0.001$). Regarding *PR1*, its NE in symptomatic 'Tarraco' leaves (8.11 ± 0.98) was 2.4-fold higher than in the asymptomatic ones (3.43 ± 0.59) and 5.5-fold higher than in symptomatic 'Mardía' leaves (1.48 ± 0.29). *PR1* expression in 'Mardía' leaves was similar regardless of the RLB status (Fig. 3). *ERF1* showed a similar pattern to *PR1*, with its NE in symptomatic 'Tarraco' leaves (0.04 ± 0.01) being 2.3-fold higher than in their asymptomatic counterparts and about 4.6 times higher than the mean expression in 'Mardía' leaves (0.01 ± 0.003). For *PR5*, its NE in symptomatic 'Tarraco' leaves (13.86 ± 0.93) was 5.2-fold higher than in the asymptomatic ones and similar to both types of 'Mardía' leaf status (Fig. 3). The NE of *ACO* and *CAD* was significantly affected by the RLB infection status besides its interaction with the cultivar factor. *CAD* expression was significantly higher in symptomatic leaves (up to 1.74 ± 0.21), regardless of cultivar, being 2.1-fold higher in symptomatic 'Tarraco' leaves and 1.5-fold higher in symptomatic 'Mardía' leaves, as compared to their respective asymptomatic counterparts (Fig. 3). For *ACO*, its NE in symptomatic 'Tarraco' leaves (58.42 ± 8.92) was 2.4-fold higher than in the asymptomatic ones. Interestingly, the opposite trend was observed in 'Mardía' leaves, where asymptomatic leaves (37.08 ± 7.04) presented a NE for *ACO* 1.4-fold higher than the symptomatic ones (Fig. 3). *HQT* was significantly affected only by the interaction between cultivar and RLB status ($P < 0.01$). Symptomatic 'Tarraco' leaves (0.02 ± 0.01) had NE 1.3-fold higher compared to their asymptomatic counterparts, whereas asymptomatic 'Mardía' leaves showed 2.2-fold higher NE compared to the symptomatic leaves (0.005 ± 0.001).

3.2. *Experiment 2. defense-related genes profiling after hormonal treatments in young almond plants*

After identifying differential hormone profiles and expression of key defense genes in mature almond trees, we investigated whether hormone treatment in young 'Tarraco' plants could result in biologically significant changes in their susceptibility to RLB, as well as in the expression of defense genes compared to untreated control plants. At the end of the experiment, control plants showed mean values of disease incidence and severity of 44.6 % and 23.8 %, respectively. Both

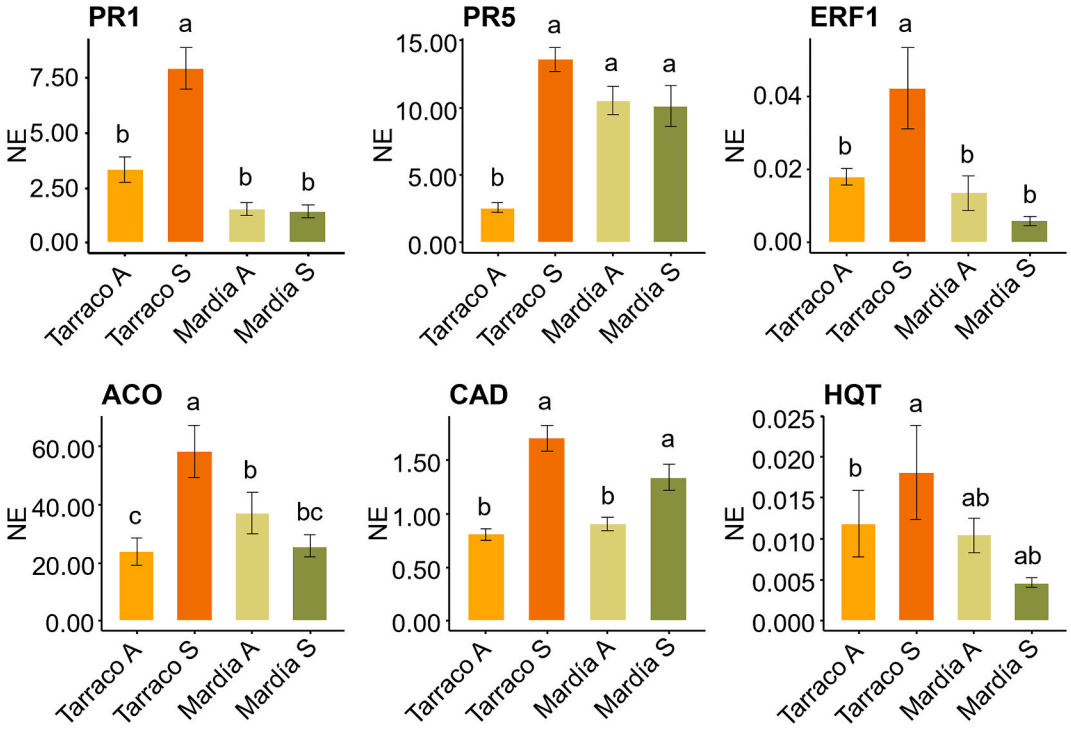


Fig. 3. Normalized expression of selected genes in leaves from two almond cultivars ('Mardía' and 'Tarraco') showing differential susceptibility to *Polystigma amygdalinum*. Mean values of two independent repetitions in 2020 and 2023. Letters indicate significant differences between cultivar and RLB status combinations according to Tukey's test ($P < 0.05$). Abbreviations: NE, normalized expression, A, asymptomatic leaves; S, symptomatic leaves. Error bars show the standard error of the mean (N = 16).

incidence and severity data were significantly reduced by MeJA and SA treatments (Fig. 4). Specifically, both MeJA and SA induced a 16 % reduction in RLB incidence (both with a $P = 0.023$). Similar figures for the reduction in RLB severity were 10 % and 11 % for MeJA and SA, respectively (both $P < 0.05$).

A profiling of defense-related genes in susceptible young 'Tarraco' plants was conducted to examine the hormone treatment effects on the relative expression (RE) of each gene (*PR1*, *PR5*, *ERF1*, *ACO*, *CAD*, and *HQT*) in both asymptomatic and symptomatic leaves. Results indicated that RLB infection significantly influenced RE of all genes (all $P < 0.05$), while hormone treatment additionally affected responses in *ERF1* and *CAD* ($P < 0.05$). *PR1* and *PR5* behaved similarly, with increased RE values in symptomatic leaves but without significant differences between the control and hormonal treatments (Fig. 5). Specifically, *PR5* was significantly increased in symptomatic leaves of both SA and MeJA treatments, whereas only *PR1* did in MeJA-treated plants. For those two defense genes, the relative ratios of RE values between symptomatic and asymptomatic leaves among the different hormone treatments ranged from 1.9 (*PR1* in SA) to 20.9 (*PR5* in MeJA). For both *ERF1* and *CAD*, hormone-treated plants with RLB symptoms showed significantly increased expression compared to the asymptomatic ones within the same hormone treatment (Fig. 5). The RE values for *ERF1* between symptomatic and asymptomatic leaves were 4.8 (for SA) and 5.2 (for MeJA) (Fig. 5), whereas *CAD* presented relative ratios of RE between symptomatic and asymptomatic leaves of 2.9 (for SA) and 1.5 (for MeJA). Regarding *ACO* and *HQT*, no significant differences in mean RE values were detected between symptomatic and asymptomatic leaves among all hormonal treatments including the untreated control (Fig. 5).

The height of young 'Tarraco' plants was similar across all hormone treatments at the experiment set up, with values ranging from 38.4 ± 0.9 to 41.9 ± 2.0 cm (mean ± std err) (Fig. 6). However, our results showed that periodic treatment with MeJA resulted in no growth in plant height during the experiment. Control plants showed an average height of 42.9 ± 1.9 cm at the end of the experiment, while height in MeJA-treated plants remained the same as the initial height. Interestingly, SA treatment did not result in reduced growth of sprayed plants, since they showed an average height of 47.2 ± 2.1 cm, not significantly different from control.

4. Discussion

In addition to their essential functions in plant growth, development, and reproduction, plant hormones are key mediators of stress perception and immune regulation (Bari and Jones, 2009; Pieterse et al., 2012). Our findings showed that almond leaves showing clear RLB symptoms accumulated higher levels of SA but did not show increased levels of components of the jasmonate family (JA and MeJA) or ACC (the ET precursor). This result indicates that there was a specific synthesis of SA in almond leaves in response to *P. amygdalinum* infection. Since *P. amygdalinum* is known as a hemibiotrophic pathogen (Cannon, 1996; Zúñiga et al., 2019), our findings confirm the classical linkage between pathogen lifestyle and associated plant hormones. However, the SA peak associated with the *P. amygdalinum* infection could not be linked to the establishment of local or systemic acquired resistance, as immunity to this pathogen has not been described in almond cultivars. Moreover, the endogenous increase of SA content was observed regardless of the cultivar's susceptibility to the pathogen. The susceptibility of 'Tarraco' despite the SA accumulation indicates that SA is not the sole or essential compound for effective defense against *P. amygdalinum*, or alternatively that 'Tarraco' may lack the downstream mechanisms activated by 'Mardía'. Another possible explanation is that 'Mardía' accumulates high levels of SA (for instance, stored in the vacuole as salicylic acid 2-O-β-glucoside), which may facilitate a rapid and early immune response to the pathogen in this cultivar, as described in other pathosystems (Zhang et al., 2025). Additionally, differences between cultivars were observed in the transduction of the SA signal and were potentially related to the pathogen-inducible genes *PR1* and *PR5*, whose activation is mediated through SA signaling (Thomma et al., 1998). The susceptible cultivar 'Tarraco' showed a highly enhanced expression of both SA-dependent genes. In contrast, 'Mardía' exhibited low levels of *PR1* and high expression of *PR5*, irrespective of RLB incidence. Similarly, resistant plum cultivars to *Monilinia fructicola* consistently expressed high foliar levels of *PR5* in symptomless leaves and only increased slightly after pathogen attack (El-kereamy et al., 2011). These results suggest an early role of this gene in brown rot resistance in plums. In our study, the natural production of *PR5* in 'Mardía' could make it effective against the fungus. This aligns with what has been observed in other pathosystems where resistance to pathogens is based on a combination of pre-existing and reprogrammed defense mechanisms (Bini et al., 2023; Lanubile et al., 2014). The complexity surrounding SA signaling warrants further investigation to clarify the true role of this hormone and its downstream effects in activating defense mechanisms against *P. amygdalinum*.

Polystigma amygdalinum has a long incubation period, typically spanning 30–35 days (Banihashemi, 1990; Saad and Masannat, 1997), resulting in imperceptible latent infections. This fungal characteristic

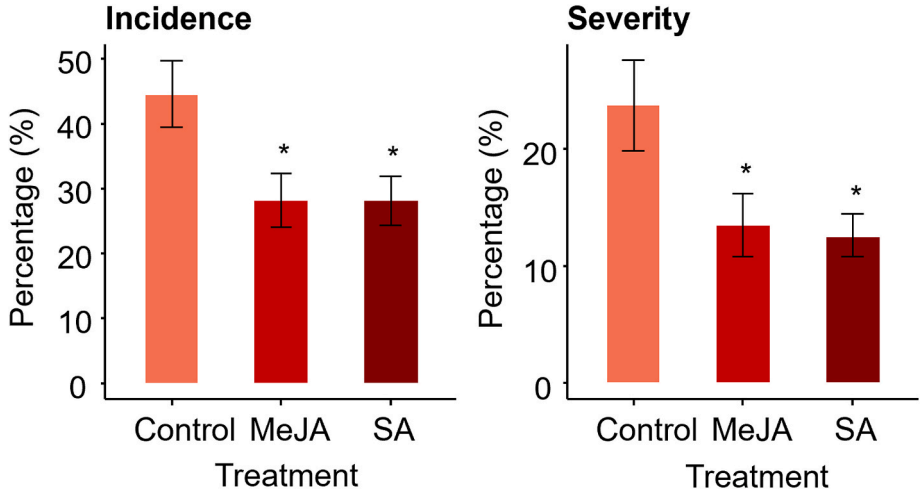


Fig. 4. Incidence and severity of almond red leaf blotch in young 'Tarraco' plants after periodic treatments with MeJA and SA. Asterisks (*) indicate significant differences between treatments compared to the untreated control according to Dunnett's test ($P < 0.05$). Abbreviations: SA, salicylic acid; MeJA, methyl jasmonate. Error bars show the standard error of the mean (N = 14).

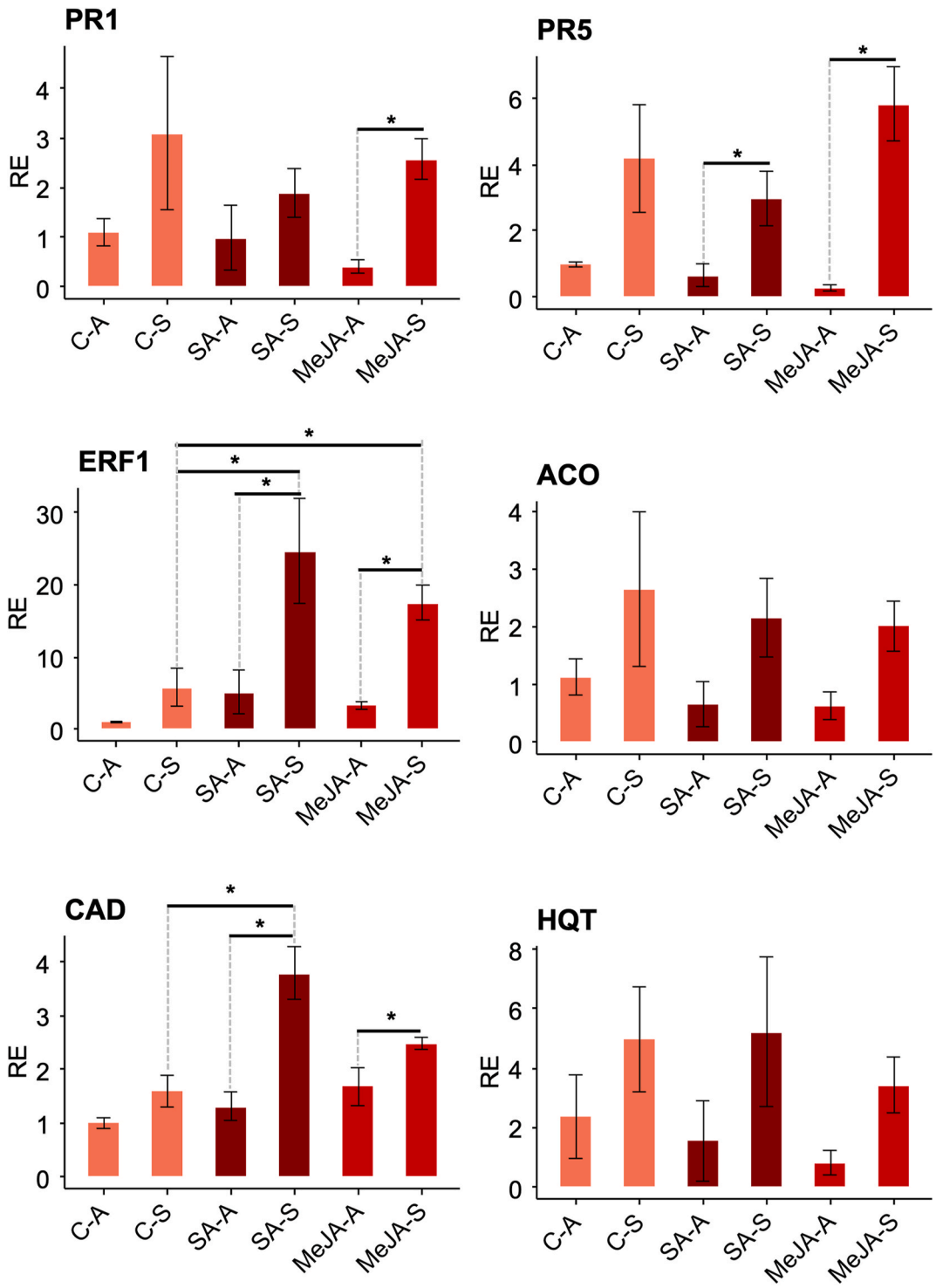


Fig. 5. Relative gene expression of defense-related (*PR1*, *PR5*, *ERF1* and *ACO*) and phenylpropanoid pathway (*CAD* and *HQT*) genes after MeJA and SA applications in young 'Tarraco' almond plants. Asterisks (*) indicate significant differences ($P < 0.05$) in the indicated pairwise comparison. Abbreviations: RE, relative expression; A, asymptomatic leaves; S, symptomatic leaves; SA, salicylic acid; MeJA, methyl jasmonate. Error bars show the standard error of the mean (N = 4).

compelled us to avoid using terms such as 'healthy' or 'diseased' leaves, opting instead for 'asymptomatic' or 'symptomatic'. Consequently, we cannot dismiss the possibility that the elevated JA levels in asymptomatic leaves may be due to latent infections. Nevertheless, the quantification of *P. amygdalinum* through qPCR indicated very low levels in asymptomatic 'Tarraco' leaves. On the other hand, our experiments were conducted in open field conditions. Considering that JA is a plant regulator which is also involved in environmental stress conditions (Wang et al., 2021), another possible explanation is that surveyed trees were being affected by a non-controlled abiotic stressor. Interestingly, cultivar 'Mardía' showed high content of MeJA in both types of leaves (symptomatic and asymptomatic). The role of this plant regulator and their biologically active forms (MeJA and JA-Ile [JA-isoleucine]) deserves further studies to decipher its potential impact on defense to RLB. Similarly, it would be interesting to investigate the role of other phytohormones, such as auxins, cytokinins, and gibberellins in the

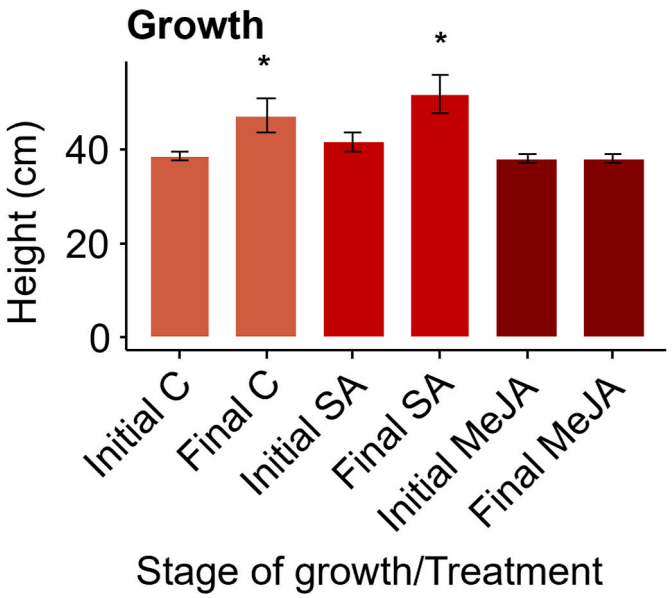


Fig. 6. Mean values of plant height in young 'Tarraco' almond plants after periodic hormone treatments at two timepoints measurements (mid-May the first measurement -Initial- and final-July the second one -Final-). Error bars show the standard error of the mean (N = 4). Asterisks (*) indicate significant differences ($P < 0.05$) between final and initial timepoints. Abbreviations: C, control treatment; SA, salicylic acid; MeJA, methyl jasmonate.

progression of RLB disease. In previous studies comparing susceptible and resistant genotypes of the genus *Prunus* (*P. domestica* and *P. salicina*) to the hemibiotrophic fungus *Apiosporina morbosa*, no differences in the quantification of SA and JA were observed between resistant and susceptible genotypes (Shinde et al., 2024). However, the interaction between auxins and cytokinins was found crucial in the disease progression in those genotypes (Shinde et al., 2023).

Additional cultivar differences were observed for genes *ERF1* and *ACO* in mature almond trees. As observed in the expression of *PR* genes, 'Tarraco' exhibited higher expression of *ERF1* and *ACO* in symptomatic leaves. In contrast, 'Mardía' reduced the expression of both latter genes in symptomatic leaves. Current knowledge indicates that ET and JA pathways converge in the transcriptional activation of *ERF1* being simultaneously required to activate it (Lorenzo et al., 2003) and confirmed its role in plant defense (Berrocal-Lobo et al., 2002). On the other hand, *ACO* is involved in the synthesis of ET by encoding the enzyme ACC-oxidase, that is required for the conversion of ACC to ET (Houben and Van de Poel, 2019). Thus, *ACO* is an upstream component in ET synthesis that, in combination with JA, activates *ERF1*. Interestingly, in our study the increased expression of *ACO* was not correlated with accumulation of ACC. This decoupling could be attributed to the close relationship between ET production and the homeostasis of S-adenosyl methionine (SAM), the initial substrate in the two-step reaction to produce ET (Pattyn et al., 2021). Additionally, these authors argue that ACC can also act as a signaling molecule independently of ET.

SA-dependent genes (*PR1* and *PR5*) and ET/JA associated genes (*ACO* and *ERF1*) were up-regulated in 'Tarraco' due to *P. amygdalinum* infection. The classical model of antagonistic strategies for JA and SA was not observed in our pathosystem. Our results indicate an activation of both hormone-dependent defense pathways (SA and ET/JA) in 'Tarraco', which appeared insufficient to face the disease. In contrast, 'Mardía' may not need to strongly induce these hormone pathways to cope with the disease, as it exhibits a relatively high level of signal transduction (as observed in the case of *PR5*). An alternative explanation would be that the effective defense in 'Mardía' may not solely rely on these plant hormones but could also be associated with an early lignin deposition, as observed previously by Zúñiga et al. (2019). These authors confirmed that an increased expression of *CAD*, followed by lignin deposition, is an effective strategy to control RLB at early stages in 'Mardía'. In their study, lignin deposition was also observed in 'Tarraco', although at later stages, and too late to be effective. Suppressively, these authors did not report an increase in *CAD* expression in 'Tarraco' at later stages, as we observed. However, *CAD* activation is essential for lignin deposition, and a time-point study, rather than a single-point sampling, would have captured its upregulation. Early symptoms of RLB manifest as diffuse, roundish discolored blotches (1–2 cm in diameter) on the leaves. In later stages, these lesions gradually expand and evolve into dark reddish to purplish necrotic areas as fungal stromata develop over the course of the season (Zúñiga et al., 2019). The expression of *HQT*, another gene from the phenylpropanoid pathway, was also analyzed in our pathosystem. *Polystigma amygdalinum* induced the activation of *HQT* on 'Tarraco' but was repressed in 'Mardía', as also seen in a previous study (Zúñiga et al., 2019). According to these authors, the increased activity of *HQT* would deviate the p-coumaric acid to the synthesis of chlorogenic acid instead of lignin, thus reducing its potential protective effects against the pathogen.

The exogenous application of SA and MeJA in young 'Tarraco' plants induced a reduction in both the incidence and severity of RLB. Numerous works reported higher protection against pathogens after treatments with SA and/or MeJA (Moreno-Pérez et al., 2024; Oliveira et al., 2015; Thomma et al., 2000; Yamchi et al., 2018). In some studies, the enhanced pathogen tolerance linked to these plant hormones has been attributed to the induction of defense markers, such as defense-related genes or pathogenesis-related proteins (Kępczyńska and Król, 2012; Loake and Grant, 2007). Specifically, regarding *Prunus* species, Svetaz et al. (2017) identified SA and pathogenesis-related proteins as the key defense mechanism for the resistance of a *P. persica* genotype against the fungal pathogen *Taphrina deformans*. Other research has connected the protective effect to morpho-anatomical changes (Moreno-Pérez et al., 2024), as well as physiological parameters (Awang et al., 2013).

In our study, the profiling of plant-defense genes in young plants was like that of mature trees, as *P. amygdalinum* induced an enhanced expression in most defense-related genes. Additionally, *ERF1* and *CAD* displayed up-regulation as a feasible consequence of plant hormones treatments, especially after SA application. Interestingly, the combination of the pathogen attack and SA treatment resulted in an additive effect over these two genes. The reduced incidence and severity of RLB in plants sprayed with hormones may be due to the activation of the ET/JA signaling pathway and the *CAD* gene, which is directly involved in lignin synthesis. As far as we know, this specific interaction has not been specifically described in literature for our almond/RLB pathosystem, but Venkatesh et al. (2024) suggested a similar defense mechanism in *Tectona grandis* affected by defoliators. These authors showed that lignin accumulation, along with other compounds and six stress-responsive genes, including *ERF1*, were increasingly enhanced in *T. grandis* plants subjected to defoliation induced by the moth *Hyblaea puera*. Similarly, hormonal crosstalk involving ET and JA was observed with early signaling, combined with lignin synthesis, in a resistant cultivar to the olive fungal pathogen *Venturia oleaginea* (=*Spilocaea oleaginea*), alongside other mechanisms (Marchese et al., 2023).

The accumulation of lignin is regarded as an active defense mechanism in plants against pathogens (Maher et al., 1994; Tronchet et al., 2010). To our knowledge, literature describing the connections between phytohormones and lignin deposition in plants under pathogen challenge is limited. However, some authors have reported that hormone treatments induce cell wall lignification, thereby enhancing resistance to pathogen attacks (Mandal, 2010; Yang et al., 2023; Zhang et al., 2019). Additionally, more research has been conducted on cell wall lignification following plant hormone treatments in response to abiotic stresses (Kobyletska et al., 2022; Li et al., 2017; Xue et al., 2008).

Although MeJA provides protection against *P. amygdalinum* in 'Tarraco', it additionally reduced the growth of treated plants compared to

those treated with SA or left untreated. Growth reduction has been previously revealed in other woody plants after MeJA applications (Heijari et al., 2005; Krokene et al., 2008). Literature relates this growth inhibition to physiological parameters such as lower rates of photosynthesis and induced stomatal closure (Shahzad et al., 2015). At the molecular level, Yang et al. (2012) indicated that angiosperms prioritize JA-mediated defense over growth by interfering with the gibberellin signaling cascade. To fully understand the mechanism behind the growth inhibition caused by MeJA application in 'Tarraco' young trees, additional experiments would be needed.

Our findings may help in contributing to a better knowledge of the hormone-mediated defense in almond. This knowledge might eventually lead to the building of new strategies for enhancing tolerance to *P. amygdalinum*.

5. Conclusions

This study provides significant insights into the differential defense responses of almond cultivars to *Polystigma amygdalinum* infection. The fungal infection triggers a rise in salicylic acid (SA) levels in both cultivars, but this increase does not seem to be correlated with effective protection. Thus, mature trees of the susceptible cultivar 'Tarraco' showed up-regulation of SA-dependent genes *PR1* and *PR5* and ethylene/jasmonate-associated genes *ACO* and *ERF1*, whereas the tolerant 'Mardía' maintained low or unchanged expression of those genes. Moreover, 'Mardía' upregulated *CAD* in symptomatic leaves instead, which is associated with lignin production, a critical component of plant structural defense. This study confirms that the application of SA or methyl jasmonate (MeJA) in young 'Tarraco' trees reduced disease incidence and severity by enhancing lignin synthesis and activating the ethylene/jasmonate pathway. However, the application of MeJA also led to reduced plant growth. Protection against this pathogen seems to be linked to lignin biosynthesis, and our results suggest that SA is likely involved in signaling this process. These findings contribute to expanding the knowledge on hormone-mediated defenses in almond and their interaction with lignin synthesis and may help in designing new potential strategies to enhance resistance to *P. amygdalinum*.

CRediT authorship contribution statement

Núria Real: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Gemma Pons-Solé:** Investigation, Data curation. **Jordi Luque:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Mercè Llugany:** Writing – review & editing, Resources, Methodology. **Soledad Martos:** Writing – review & editing, Writing – original draft, Conceptualization.

Data statement

The data that support the findings of this study are openly available in CORA (Catalan Open Research Area – Repositori de Dades de Recerca) at https://doi.org/10.34810/data1902.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Microsoft Copilot in order to improve language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding sources

This research was funded by INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Spain; grant RTA2017-00009-C04-01), and AEI (Agencia Española de Investigación, Spain; grant PID2020-114468RR-C31). Jordi Luque was supported by the CERCA Program, Generalitat de Catalunya, Spain. Núria Real is the recipient of an INVESTIGO 2023 postdoctoral contract (100049ID3) from the Generalitat de Catalunya. Gemma Pons-Solé was supported by a predoctoral grant (PRE2018-085207) from AEI, Spain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Isabel Cored and Sergi Borràs for their technical assistance in the field (SB, IC) and laboratory tasks (IC).

Glossary

ACC	1-aminocyclopropane-1-carboxylic acid
ACO	1-aminocyclopropane-1-carboxylate oxidase
CAD	cinnamyl alcohol dehydrogenase
ERF1	ethylene response factor 1
ET	ethylene
HQT	hidroxycinamate quinole transferase
JA	jasmonic acid
MeJA	methyl jasmonate
PR1	pathogenesis-related protein 1
PR5	pathogenesis-related protein 5
RLB	red leaf blotch
SA	salicylic acid

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jplph.2025.154615.

Data availability

The data of this study are available in CORA (Catalan Open Research Area) that will be available upon manuscript acceptance.

References

Aerts, N., Pereira Mendes, M., Van Wees, S.C.M., 2021. Multiple levels of crosstalk in hormone networks regulating plant defense. Plant J. 105 (2), 489–504.

Alioto, T., Alexiou, K.G., Bardil, A., Barteri, F., Castanera, R., Cruz, F., et al., 2020. Transposons played a major role in the diversification between the closely related almond and peach genomes: results from the almond genome sequence. Plant J. 1, 455–472.

Awang, N.A., Islam, M.R., Ismail, M.R., Zulkarami, B., Omar, D., 2013. Effectiveness of different elicitors in inducing resistance in chilli (*Capsicum annuum* L.) against pathogen infection. Sci. Hortic. 164, 461–465.

Banihashemi, Z., 1990. Biology and control of *Polystigma ochraceum*, the cause of almond red leaf blotch. Plant Pathol. 39 (2), 309–315.

Bari, R., Jones, J.D.G., 2009. Role of plant hormones in plant defence responses. Plant Mol. Biol. 69 (4), 473–488.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67 (1), 1–48.

Berrocal-Lobo, M., Molina, A., Solano, R., 2002. Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J. 29 (1), 23–32. https://doi.org/10.1046/j.1365-313x.2002.01191.x.

Bini, A.P., Rossi, G.D., Poeschl, Y., Serra, M.C.D., Camargo, L.E.A., Monteiro-Vitorello, C. B., Creste, S., 2023. Molecular, biochemical and metabolomics analyses reveal constitutive and pathogen-induced defense responses of two sugarcane contrasting genotypes against leaf scald disease. Plant Physiol. Biochem. 203, 108033.

BOE, 2002. Real Decreto 1201/2002, de 20 de noviembre, por el que se regula la producción integrada de productos agrícolas. Boletín Oficial del Estado. Número 287, páginas 42028-42040. https://www.boe.es/eli/es/rd/2002/11/20/1201.

Bundhun, D., Jeewon, R., Dayarathne, M.C., Bulgakov, T.S., 2019. A morpho-molecular re-appraisal of *Polystigma fulvum* and *P. rubrum* (*polystigma*, polystigmataceae). Phytotaxa 422 (3), 209–224.
Cannon, P.F., 1996. Systematics and diversity of the phyllachoraceae associated with rosaceae, with a monograph of *polystigma*. Mycol. Res. 100 (12), 1409–1427.
Denancé, N., Sánchez-Vallet, A., Goffner, D., Molina, A., 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front. Plant Sci. 4, 155.
Dong, N., Lin, H., 2021. Contribution of phenylpropanoid metabolism to plant development and plant-environment interactions. J. Integr. Plant Biol. 63 (1), 180–209.
El-kereamy, A., El-sharkawy, I., Ramamoorthy, R., Taheri, A., Errampalli, D., 2011. *Prunus domestica* pathogenesis-related Protein-5 activates the defense response pathway and enhances the resistance to fungal infection. PLoS One 6 (3), e17973.
Fox, J., Weisberg, S., 2019. An R Companion to Applied Regression, third ed. Sage.
Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227.
Gradziel, T.M., Curtis, R., Socias i Company, R., 2017. Production and growing regions. In: Socias i Company, R., Gradziel, T.M. (Eds.), Almonds: Botany, Production and Uses. CABI, pp. 70–86.
Heijari, J., Nerg, A., Kainulainen, P., Viiri, H., Vuorinen, M., Holopainen, J.K., 2005. Application of methyl jasmonate reduces growth but increases chemical defence and resistance against *Hylobius abietis* in Scots pine seedlings. Entomol. Exp. Appl. 115, 117–124. https://doi.org/10.1111/j.1570-7458.2005.00249.x.
Heydarian, A., Moradi, H., 2005. Relative resistance of selected almond cultivars to the causal agent of red leaf blotch disease, in chahar Mahal-V A-Bakhtiari province. Iran. J. Plant Pathol. 41, 57–169.
Holeski, L.M., Jander, G., Agrawal, A.A., 2012. Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol. Evol. 27, 618–626. https://doi.org/10.1016/j.tree.2012.07.011.
Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. Biom. J. 50, 346–363. https://doi.org/10.1002/bimj.200810425.
Houben, M., Van de Poel, B., 2019. 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO): the enzyme that makes the plant hormone ethylene. Front. Plant Sci. 10, 695. https://doi.org/10.3389/fpls.2019.00695.
Kępczyńska, E., Król, P., 2012. The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. J. Plant Interact. 7, 307–315. https://doi.org/10.1080/17429145.2011.633469.
Kobyletska, M., Kavulych, Y., Romanyuk, N., Korchynska, O., Terek, O., 2022. Exogenous salicylic acid modifies cell wall lignification, total phenolic content, PAL-activity in wheat (*Triticum aestivum* L.) and buckwheat (*Fagopyrum esculentum* moench) plants under cadmium chloride impact. Biointerface Res. Appl. Chem. 13, 117. https://doi.org/10.33263/BRIAC131.117.
Krokene, P., Nagy, N.E., Solheim, H., 2008. Methyl jasmonate and oxalic acid treatment of Norway spruce: Anatomically based defense responses and increased resistance against fungal infection. Tree Physiol. 28 (1), 29–35. https://doi.org/10.1093/treephys/28.1.29.
Lanubile, A., Ferrarini, A., Maschietto, V., Delledonne, M., Marocco, A., Bellin, D., 2014. Functional genomic analysis of constitutive and inducible defense responses to *Fusarium verticillioides* infection in maize genotypes with contrasting ear rot resistance. BMC Genom. 15, 710. https://doi.org/10.1186/1471-2164-15-710.
Li, N., Han, X., Feng, D., Yuan, D., Huang, L.-J., 2019. Signaling crosstalk between salicylic acid and ethylene/Jasmonate in plant defense: do We understand what they are whispering? Int. J. Mol. Sci. 20, 671. https://doi.org/10.3390/ijms20030671.
Li, H., Suo, J., Han, Y., Liang, C., Jin, M., Zhang, Z., Rao, J., 2017. The effect of 1-methylcyclopropene, methyl jasmonate and methyl salicylate on lignin accumulation and gene expression in postharvest 'xuxiang' kiwifruit during cold storage. Postharvest Biol. Technol. 124, 107–118. https://doi.org/10.1016/j.postharvbio.2016.10.003.
Liu, L., Sonbol, F.M., Huot, B., Gu, Y., Withers, J., Mwimba, M., Yao, J., He, S.Y., Dong, X., 2016. Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. Nat. Commun. 7, 13099. https://doi.org/10.1038/ncomms13099.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−(ΔΔCT) method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Llugany, M., Martin, S.R., Barceló, J., Poschenrieder, C., 2013. Endogenous jasmonic and salicylic acids levels in the cd-hyper-accumulator *noccaea* (*Thlaspi*) *praecox* exposed to fungal infection and/or mechanical stress. Plant Cell Rep. 32, 1243–1249. https://doi.org/10.1007/s00299-013-1442-2.
Loake, G., Grant, M., 2007. Salicylic acid in plant defence—the players and protagonists. Curr. Opin. Plant Biol. 10, 466–472. https://doi.org/10.1016/j.pbi.2007.08.008.
López-López, M., Calderón, R., González-Dugo, V., Zarco-Tejada, P.J., Fereres, E., 2016. Early detection and quantification of almond red leaf blotch using high-resolution hyperspectral and thermal imagery. Remote Sens. 8, 276. https://doi.org/10.3390/rs8040276.
Lorenzo, O., Piqueras, R., Sánchez-Serrano, J.J., Solano, R., 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15, 165–178. https://doi.org/10.1105/tpc.007468.
Maher, E.A., Bate, N.J., Ni, W., Elkind, Y., Dixon, R.A., Lamb, C.J., 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. Proc. Natl. Acad. Sci. U.S.A 91, 7802–7806. https://doi.org/10.1073/pnas.91.17.7802.
Mandal, S., 2010. Induction of phenolics, lignin and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. Afr. J. Biotechnol. 9, 8038–8047.
Marchese, A., Balan, B., Trippa, D.A., Bonanno, F., Caruso, T., Imperialem, V., Marra, F. P., Giovino, A., 2023. NGS transcriptomic analysis uncovers the possible resistance mechanisms of olive to *spilocea oleagina* leaf spot infection. Front. Plant Sci. 14, 1219580. https://doi.org/10.3389/fpls.2023.1219580.
Miarnau, X., Zazurca, L., Torguet, L., Zúñiga, E., Batlle, I., Alegre, S., Luque, J., 2021. Cultivar susceptibility and environmental parameters affecting symptom expression of red leaf blotch of almond in Spain. Plant Dis. 105, 940–947. https://doi.org/10.1094/PDIS-04-20-0872-RE.
Miedes, E., Vanholme, R., Boerjan, W., Molina, A., 2014. The role of the secondary cell wall in plant resistance to pathogens. Front. Plant Sci. 5, 358. https://doi.org/10.3389/fpls.2014.00358.
Moreno-Pérez, A., Martínez-Ferri, E., Van den Berg, N., Pliego, C., 2024. Effects of exogenous application of methyl jasmonate and salicylic acid on the physiological and molecular response of "dusa" avocado to *Rosellinia necatrix*. Plant Dis. 108, 2111–2121. https://doi.org/10.1094/PDIS-08-23-1597-RE.
Naseem, M., Kaltdorf, M., Dandekar, T., 2015. The nexus between growth and defence signalling: auxin and cytokinin modulate plant immune response pathways. J. Exp. Bot. 66, 4885–4896. https://doi.org/10.1093/jxb/erv297.
Oliveira, M.B., Junior, M.L., Grossi-de-Sá, M.F., Petrofeza, S., 2015. Exogenous application of methyl jasmonate induces a defense response and resistance against *Sclerotinia sclerotiorum* in dry bean plants. J. Plant Physiol. 182, 13–22. https://doi.org/10.1016/j.jplph.2015.04.005.
Ollero-Lara, A., Agustí-Brisach, C., Lovera, M., Roca, L.F., Arquero, O., Trapero, A., 2019. Field susceptibility of almond cultivars to the four most common aerial fungal diseases in southern Spain. Crop Prot. 121, 18–27. https://doi.org/10.1016/j.cropro.2019.03.012.
Ollero-Lara, A., Lovera, M., Roca, L.F., Arquero, O., Trapero, A., 2016. Susceptibilidad varietal del almendro a la mancha ocre en Andalucía. Vida Rural 412, 14–22.
Pattyn, J., Vaughan-Hirsch, J., Van de Poel, B., 2021. The regulation of ethylene biosynthesis: a complex multilevel control circuitry. New Phytol. 229, 770–782. https://doi.org/10.1111/nph.16873.
Peña, E.A., Slate, E.H., 2006. Global validation of linear model assumptions. J. Am. Stat. Assoc. 101, 341. https://doi.org/10.1198/016214505000000637.
Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S., Van Wees, S.C.M., 2009. Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. 5, 308–316. https://doi.org/10.1038/nchembio.164.
Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., Van Wees, S.C.M., 2012. Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055.
R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Ren, R., Yang, X., Song, A., Li, C., Yang, H., Kang, Y., 2020. Control of *Phytophthora melonis* damping-off treated with 24-epibrassinolide and a histological study of cucumber hypocotyl. Protoplasma 257, 1519–1529. https://doi.org/10.1007/s00709-020-01496-z.
Saad, A.T., Masannat, K., 1997. Economic importance and cycle of *Polystigma ochraceum*, causing red leaf blotch disease of almond, in Lebanon. EPPO Bull. 27, 481–485. https://doi.org/10.1111/j.1365-2338.1997.tb00676.x.
Segarra, G., Jáuregui, O., Casanova, E., Trillas, I., 2006. Simultaneous quantitative LC–ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. Phytochemistry 67, 395–401. https://doi.org/10.1016/j.phytochem.2005.11.028.
Shahzad, R., Waqas, M., Khan, A.L., Hamayun, M., Kang, S.M., Lee, I.J., 2015. Foliar application of methyl jasmonate induced physio-hormonal changes in *pisum sativum* under diverse temperature regimes. Plant Physiol. Biochem. 96, 406–416. https://doi.org/10.1016/j.plaphy.2015.08.019.
Shinde, R., Ayyanath, M.-M., Shukla, M., El Kayal, W., Saxena, P., Subramanian, J., 2023. Hormonal interplay leading to black knot disease establishment and progression in plums. Plants 12, 3638. https://doi.org/10.3390/plants12203638.
Shinde, R., Ayyanath, M.-M., Shukla, M., El Kayal, W., Saxena, P.K., Subramanian, J., 2024. Salicylic and Jasmonic acid synergism during black knot disease progression in plums. Plants 13, 292. https://doi.org/10.3390/plants13020292.
Spoel, S.H., Johnson, J.S., Dong, X., 2007. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc. Natl. Acad. Sci. U.S.A 104, 18842–18847. https://doi.org/10.1073/pnas.0708139104.
Suzuki, Y., Tanaka, K., Hatakeyama, S., Harada, Y., 2008. *Polystigma fulvum*, a red leaf blotch pathogen on leaves of *prunus* spp., has the *Polystigmina pallescens* anamorph/andromorph. Mycoscience 49, 395–398. https://doi.org/10.1007/S10267-008-0217-8.
Svetaz, L.A., Bustamante, C.A., Goldy, C., Rivero, N., Müller, G.L., Valentini, G.H., Fernie, A.R., Drincovich, M.F., Lara, M.V., 2017. Unravelling early events in the *taphrina deformans–prunus persica* interaction: an insight into the differential responses in resistant and susceptible genotypes. Plant Cell Environ. 40, 1456–1473. https://doi.org/10.1111/pce.12934.
Thomma, B.P.H.J., Eggermont, K., Broekaert, W.F., Cammue, B.P.A., 2000. Disease development of several fungi on arabidopsis can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem. 38, 421–427. https://doi.org/10.1016/S0981-9428(00)00766-4.
Thomma, B.P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A., Broekaert, W.F., 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in arabidopsis are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. U.S.A 95, 15107–15111. https://doi.org/10.1073/pnas.95.25.15107.

Tong, Z., Gao, Z., Wang, F., Zhou, J., Zhang, Z., 2009. Selection of reliable reference genes for gene expression studies in peach using real-time PCR. BMC Mol. Biol. 10, 71. https://doi.org/10.1186/1471-2199-10-71.

Torguet, L., Batlle, I., Alegre, S., Miarnau, X., 2016. Nuevas plagas y enfermedades emergentes, una amenaza para el cultivo del almendro en España. Rev. Frutic. 49, 152–165.

Tronchet, M., Balagué, C., Kroj, T., Jouanin, L., Roby, D., 2010. Cinnamyl alcohol dehydrogenases-C and D, key enzymes in lignin biosynthesis, play an essential role in disease resistance in arabidopsis. Mol. Plant Pathol. 11, 83–92. https://doi.org/10.1111/j.1364-3703.2009.00578.x.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., Katagiri, F., 2009. Network properties of robust immunity in plants. PLoS Genet. 5, e1000772. https://doi.org/10.1371/journal.pgen.1000772.

Ullah, C., Schmidt, A., Reichelt, M., Tsai, C., Gershenzon, J., 2022. Lack of antagonism between salicylic acid and jasmonate signalling pathways in poplar. New Phytol. 235, 701–717. https://doi.org/10.1111/nph.18121.

Venkatesh, Y.N., Ashajyothi, M., Rajarajan, K., Sahu, S., Chaturvedi, V., Prathima, P.T., Muthamilarasn, M., Handa, A.K., Arunachalam, A., 2024. Transcriptional and physio-chemical responses of *Tectona grandis* L. triggered by teak defoliator. Plant Stress 12, 100468. https://doi.org/10.1016/j.stress.2023.100468.

Vlot, A.C., Sales, J.H., Lenk, M., Bauer, K., Brambilla, A., Sommer, A., Chen, Y., Wenig, M., Nayem, S., 2021. Systemic propagation of immunity in plants. New Phytol. 229, 1234–1250. https://doi.org/10.1111/nph.16899.

Wang, Y., Mostafa, S., Zeng, W., Jin, B., 2021. Function and mechanism of Jasmonic acid in plant responses to abiotic and biotic stresses. Int. J. Mol. Sci. 22, 8568. https://doi.org/10.3390/ijms22168568.

Wickham, H., François, R., Henry, L., Müller, K., Vaughan, D., 2023. dplyr: a grammar of data manipulation. R package version 1.1.4 Available from: https://dplyr.tidyverse.org.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, ISBN 978-3-319-24277-4. Available from: https://ggplot2.tidyverse.org.

Xue, Y.J., Tao, L., Yang, Z.M., 2008. Aluminum-induced cell wall peroxidase activity and lignin synthesis are differentially regulated by jasmonate and nitric oxide. J. Agric. Food Chem. 56, 9676–9684. https://doi.org/10.1021/jf801365s.

Yamchi, A., Ben, C., Rossignol, M., Zareie, S.R., Mirlohi, A., Sayed-Tabatabaei, B.E., Pichereaux, C., Sarrafi, A., Rickauer, M., Gentzbittel, L., 2018. Proteomics analysis of *Medicago truncatula* response to infection by the phytopathogenic bacterium *Ralstonia solanacearum* points to jasmonate and salicylate defence pathways. Cell. Microbiol. 20, e12796. https://doi.org/10.1111/cmi.12796.

Yang, D.L., Yao, J., Mei, C.S., Tong, X.H., Zeng, L.J., Li, Q., Xiao, L.T., et al., 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. U.S.A 109, E1192–E1200. https://doi.org/10.1073/pnas.1201616109.

Yang, W., Guo, M., Zhang, W., Cheng, S., Chen, G., 2023. Methyl salicylate and methyl jasmonate induce resistance to *Alternaria tenuissima* by regulating the phenylpropane metabolism pathway of winter jujube. Postharvest Biol. Technol. 204, 112440. https://doi.org/10.1016/j.postharvbio.2023.112440.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., Madden, T.L., 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13, 1–11. https://doi.org/10.1186/1471-2105-13-134.

Zhang, X., Liu, J., Wu, L., Wang, Z., Zhang, S., 2019. *GbWRKY1*, a member of the WRKY transcription factor family identified from *Gossypium barbadense*, is involved in resistance to *verticillium* wilt. Biotechnol. Biotechnol. Equip. 33, 1354–1364. https://doi.org/10.1080/13102818.2019.1645313.

Zhang, C., Zhang, H., Lin, W., Chai, J., Shangguan, X., Zhao, T., 2025. ZmDREB1A controls plant immunity via regulating salicylic acid metabolism in maize. Plant J. 121 (2), e17226. https://doi.org/10.1111/tpj.17226.

Zhao, Q., Dixon, R.A., 2011. Transcriptional networks for lignin biosynthesis: more complex than we thought? Trends Plant Sci. 16, 227–233. https://doi.org/10.1016/j.tplants.2010.12.005.

Zúñiga, E., León, M., Berbegal, M., Armengol, J., Luque, J., 2018. A qPCR-based method for detection and quantification of *Polystigma amygdalinum*, the cause of red leaf blotch of almond. Phytopathol. Mediterr. 57, 257–268. https://doi.org/10.14601/Phytopathol_Mediterr-22746.

Zúñiga, E., Luque, J., Martos, S., 2019. Lignin biosynthesis as a key mechanism to repress *Polystigma amygdalinum*, the causal agent of the red leaf blotch disease in almond. J. Plant Physiol. 236, 96–104. https://doi.org/10.1016/j.jplph.2019.03.004.

Zúñiga, E., Romero, J., Ollero-Lara, A., Lovera, M., Arquero, O., Miarnau, X., Torguet, L., Trapero, A., Luque, J., 2020. Inoculum and infection dynamics of *Polystigma amygdalinum* in almond orchards in Spain. Plant Dis. 104, 1239–1246. https://doi.org/10.1094/PDIS-09-19-1942-RE.