Physiological and Molecular Plant Pathology 144 (2026) 103251

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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

Control of shoot blight in young apple trees of the cultivar 'WA 38' under field conditions using prohexadione-calcium and acibenzolar-*S*-methyl

Aina Baró [a,b,*], Sara Tianna DuPont [a,**]

[a] College of Agricultural, Human, and Natural Resource Sciences, Tree Fruit Research and Extension Center, Washington State University, Wenatchee, WA, 98801, USA
[b] Institute of AgriFood Research and Technology (IRTA), Sustainable Plant Protection Program, Centre de Cabrils, Cabrils, Catalonia, 08348, Spain

ARTICLE INFO

Keywords:
Erwinia amylovora
Integrated disease management
Plant defense inducer
Plant growth regulator
Shoot blight

ABSTRACT

Fire blight, caused by *Erwinia amylovora*, remains a major threat to apple production in Washington State. Although most infections occur during bloom, the post-bloom period also poses a high risk because actively growing shoots can be infected following injury caused by wind, rain, or hail. This study evaluated the efficacy of the systemic acquired resistance (SAR) inducer acibenzolar-*S*-methyl (ASM) and the plant growth regulator (PGR) prohexadione-calcium (ProCa) in managing shoot blight in young 'WA 38' apple trees. Field trials conducted in 2022, 2023, and 2024 assessed disease incidence and severity, and quantified the expression of six defense-related genes (*PR1*, *PR2*, *PR4*, *PR14*, *JAA*, and *NIMIN2*) to determine plant defense activation. ProCa consistently reduced shoot blight incidence and severity, and showed a strong and sustained upregulation of genes related to plant defense responses. The combined treatment, consisting of low-doses of ASM and ProCa, demonstrated additive effects, enhancing both the expression of genes related to defense pathways and disease control. These findings suggest that ProCa, alone or in combination with ASM, is a promising tool for integrated shoot blight management in 'WA 38' apple trees.

1. Introduction

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is one of the most important diseases affecting apple and pear crops. The disease has a widespread impact across many apple-producing regions of the world and is of particular concern in Washington State (WA), one of the leading apple-producing regions in the United States [1]. During warm and wet springs, conditions favorable to the pathogen, fire blight outbreaks can be severe, affecting 12–17% of WA apple acreage, as seen in 2017 and 2018 [2]. The economic consequences of fire blight in the United States are substantial, with estimated losses exceeding USD 100 million annually in years when disease outbreaks occur [3].

Current fire blight management strategies focus predominantly on the prevention of blossom infections through the application of antibiotics, biological control agents like *Aureobasidium pullulans*, and mineral compounds such as copper. These products function either by directly inhibiting the pathogen or by activating the plant's innate defense mechanisms [4–6]. While great progress has been made towards achieving alternative integrated management programs for controlling blossom infections [7,8], a critical management gap remains during the post-bloom period. This period is especially vulnerable, as actively growing shoots are at high risk of infection due to physical injury caused by wind, rain, or hail, and cankers and infected flowers serve as a source of *E. amylovora* inoculum that can be spread by insects, wind, or rain [9,10].

To protect new growth during this phase, growers commonly rely on repeated applications of antibiotics and copper-based products. However, these products do not confer full protection, and multiple repeat applications are not recommended in order to avoid resistance development. Some alternative organic products used during bloom, such as peracetic acid/peroxide formulations, have been considered for post-bloom application, but pose a risk of fruit russeting, limiting their use [11].

Considering these challenges, new research is focused on the potential of plant defense inducers and plant growth regulators (PGRs) to enhance the plant's own ability to resist infection. In particular, acibenzolar-*S*-methyl (ASM), a well-known inducer of systemic acquired resistance (SAR), and prohexadione-calcium (ProCa), a PGR with

* Corresponding author. Institute of AgriFood Research and Technology (IRTA), Sustainable Plant Protection Program, Centre de Cabrils, 08348 Cabrils, Catalonia, Spain.
** Corresponding author.
E-mail addresses: aina.baro@irta.cat (A. Baró), tianna.dupont@wsu.edu (S.T. DuPont).

https://doi.org/10.1016/j.pmpp.2026.103251
Received 3 February 2026; Received in revised form 2 May 2026; Accepted 2 May 2026
Available online 5 May 2026

reported fire blight suppression activity, have been studied for their efficacy in apple production systems [12–16]. While both compounds have shown promise in reducing disease incidence, further research is needed to optimize their use in order to identify the best rates, timings, and application strategies. Additionally, further exploring the combined application of both products is of interest, as it offers a more cost-effective alternative to ASM alone while mitigating the risk of growth suppression associated with higher ProCa rates [17,18].

Importantly, cultivar-specific responses to these treatments remain underexplored. Different apple genotypes may respond variably to chemical elicitors and PGRs due to underlying differences in their constitutive defense systems and growth habits. For example, disease suppression achieved through ASM application can depend not only on a cultivar's priming capacity, but also on whether induced defenses reach a threshold sufficient for effective protection [19]. Understanding both the basal defense level and the inducibility of plant defense responses is essential when evaluating the suitability of types and doses of plant defense inducers and PGRs for cultivar-specific fire blight management strategies. Combining these types of products with genotypes carrying multiple QTLs involved in distinct defense pathways may offer a particularly promising and sustainable approach to disease control [20].

This is especially important during the post-bloom period in young orchards, where tree growth is generally encouraged to promote canopy development, but this creates more new shoots and consequently increases tree susceptibility to fire blight. This developmental phase presents a physiological trade-off between promoting vegetative growth and enhancing resistance to pathogens [21]. Treatments like ASM can upregulate defense pathways but may come at a cost to growth, while ProCa may suppress growth to limit susceptibility but potentially inhibit canopy development [22]. These trade-offs are not only treatment-dependent, but also cultivar-dependent, as genetic background influences how trees allocate resources under stress [23,24].

Most commercial apple cultivars are susceptible to fire blight, despite the efforts to find resistance to this disease [3]. In 2017, the new apple cultivar ‘WA 38’, a high-quality and consumer-focused apple developed by the Washington State University breeding program, was released [25]. Since then, more than 20 million trees have been planted in WA, making it one of the major cultivars with a large percentage of young trees in the state [26]. ‘WA 38’, a hybrid of ‘Honeycrisp’ and ‘Enterprise’, combines traits from both moderately susceptible and highly resistant parents [27], resulting in a unique genetic profile that may affect its physiological and defense responses. Though initially classified as moderately resistant to shoot blight, subsequent field observations revealed variable performance, particularly under high disease pressure [28]. This variability highlights the importance of adapted disease management strategies that consider cultivar-specific responses in different environmental conditions.

The primary objective of this study was to evaluate the effectiveness of different ASM and ProCa treatments, comparing multiple rates and application methods, in reducing shoot blight incidence and severity in young ‘WA 38’ apple trees during the critical post-bloom period. Additionally, this work aimed to explore the activation of plant defense responses by quantifying the expression of six defense-related genes: *PR1*, *PR2*, *PR4*, *PR14*, *JAA*, and *NIMIN2*. These findings will contribute to the development of optimized, evidence-based fire blight management protocols for apple.

2. Materials and methods

2.1. Field trials

Field trials were conducted during 2022, 2023, and 2024 on apple trees of the cultivar ‘WA 38’ planted in 2021 at Columbia View orchard (48 Longview Rd., East Wenatchee, WA 98802-8283) at 2900 trees per ha. Experiments were arranged in a randomized complete block design with 3 biological replicate blocks. Each block contained 7 treatment plots, each one consisting of 5 trees. Treatments were randomly assigned within each block using a random-number generator to ensure an unbiased spatial distribution. To minimize interference among treatments, plots within each block were separated by at least 4 untreated buffer trees. All trees sampled met consistent criteria for vigor, canopy size, and absence of visible stress symptoms to reduce biological variability. Treatments were ASM (Actigard® 50WG) at 70.1 and 140.1 g/ha, ProCa (Kudos® 27.5 WDG) at 140.1 and 420.3 g/ha, and a combined treatment with ASM at 70.1 g/ha and ProCa at 140.1 g/ha. A water-treated control was applied as a negative control treatment. Products were applied to the whole tree according to manufacturer recommendations using a Stihl SR420 backpack sprayer previously calibrated to equal 935.4 L/ha. An additional drench treatment with ASM at 140.1 g/ha (equal to 0.05 g per tree) was included.

Gene expression analysis was conducted in 2022 and 2023, and pathogen inoculation experiments were performed in 2023 and 2024. In 2022, treatments were applied on May 13, May 20, May 27, and June 2. Leaf tissue was sampled 1 day prior to each spray, plus 1 and 2 weeks after the last spray (Fig. 1A). In 2023, treatments were applied on May 12, May 19, May 26, and June 2. Leaf tissue was sampled 1 day prior to each spray, and the inoculation of *E. amylovora* happened on May 23 (11 days after the first spray application) (Fig. 1B). In 2024, treatments were applied on May 2, May 10, May 18, and May 24, and inoculation of *E. amylovora* on May 13 (11 days after the first spray application) (Fig. 1C). Spraying and sample collection were conducted at the same time of the day (sprays between 6 a.m. and 10 a.m., sampling between 8 a.m. and 12 p.m.) to reduce variability due to environmental factors, and inoculation was carried out in the afternoon (between 4 and 7 p.m.) to reduce direct solar UV exposure of the bacterial suspension.

2.2. Gene expression analysis (in 2022 and 2023)

2.2.1. Sample collection in the field

Samples consisted of 2 shoot tips per tree from newly formed leaves from 5 trees per replicated treatment in 2022, and from 3 trees per replicated treatment (the ones not inoculated with the pathogen) in 2023. Sterile gloves and shears were used to cut the shoot tips and to place them inside bags, which were kept inside a cooler with dry ice while in the field. Once in the laboratory, samples were kept at −80 °C until processing.

2.2.2. Sample processing to obtain complementary DNA (cDNA)

Leaf samples from the orchard were ground in a cooled mortar and pestle using liquid nitrogen. Total RNA was extracted from ~200 mg of ground tissue using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's recommendations, with the addition of a centrifugation step (2 min at 15,000 rpm) prior to transferring the lysate to the QIAshredder spin column. RNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, U.S.A.). To remove any contaminant genomic DNA (gDNA), RNA was adjusted to 200 ng/µl and routinely subjected to DNase treatment using the Turbo DNA-free Kit (Invitrogen, Waltham, MA, U.S.A.). First-strand cDNA was generated from 0.96 µg RNA using SuperScript™ III Reverse Transcriptase (Invitrogen, Waltham, MA, U.S.A.) with Oligo (dT)20 primers according to the manufacturer's recommendations.

2.2.3. Quantitative real-time PCR (qPCR)

Quantitative Real-Time PCR analysis was performed in duplicate in a 20 µL reaction volume containing 2 µL of cDNA, 1 µL each of forward and reverse primers (10 µM), 6 µL nuclease-free water, and 10 µL of SYBR®Green PCR Master Mix (Applied Biosystems, Waltham, MA, U.S.A.) on a CFX Connect Real-Time PCR System (BioRad, Hercules, CA, U.S.A.). The relative quantification thermal cycling conditions were: denaturation at 95 °C for 5 min, followed by 41 cycles of 10 s denaturation at 95 °C, 30 s annealing at 60 °C, and 30 s extension at 72 °C. Melting curve

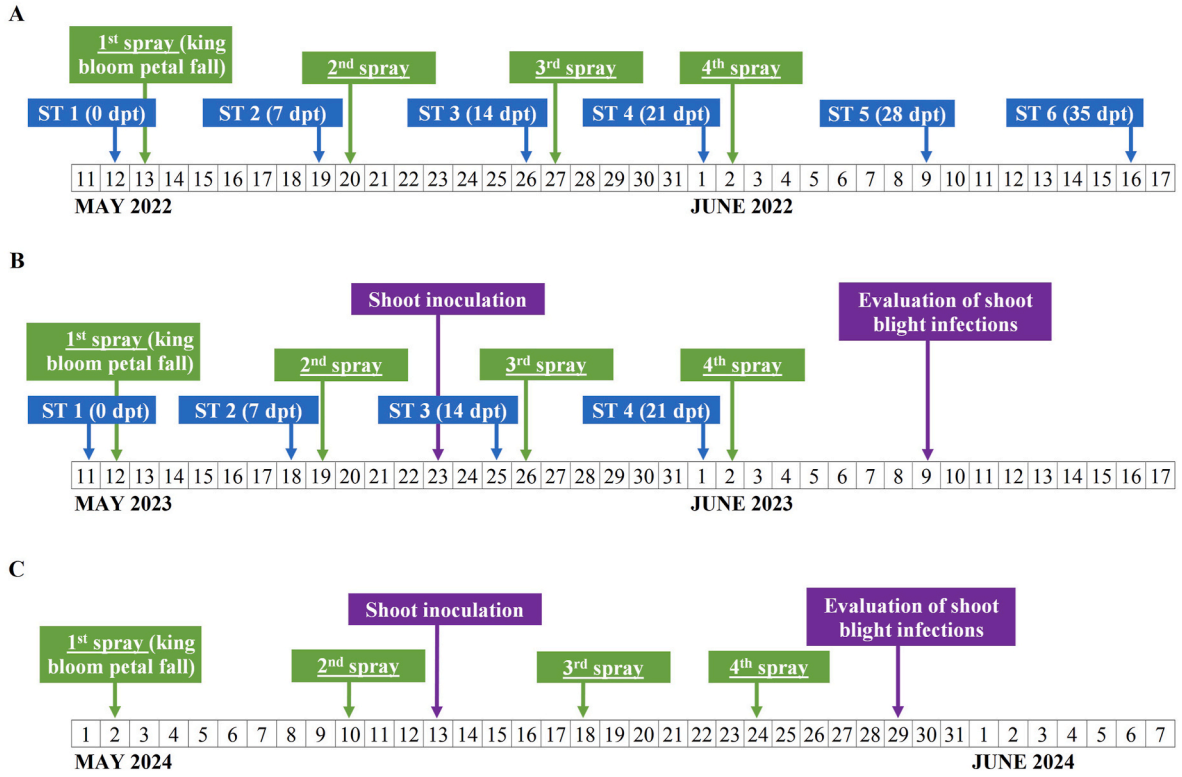

Fig. 1. Timeline of treatments (spray), sampling times (ST), and inoculation and evaluation dates for the field trials conducted on apple trees of the cultivar 'WA 38' in (A) 2022, (B) 2023, and (C) 2024. Note: dpt, days post-treatment.

analysis (from 65 °C to 95 °C with increments of 0.5 °C for 5 s) was performed after the final qPCR cycle to validate amplicon specificity. Non-template controls were also included in each qPCR run to assess the purity of the reagents.

The following genes implicated in plant defense mechanisms were analyzed: NIMI-interacting protein 2 (*NIMIN2*), Jasmonate-induced protein-like (*JAA*), Pathogenesis-related protein 1 (*PR1*), Pathogenesis-related protein 2 (*PR2*), Pathogenesis-related protein 4 (*PR4*), Pathogenesis-related protein 14 (*PR14*), and the constitutive β-actin (*β-actin*) as reference control. These genes were selected because they represent key markers of different branches of the plant defense response. PR1 is considered an indicator of the plant defense system, with multiple functions; one of them is to degrade endogenous substrates to generate elicitors, and thereby induce defense responses [29]. PR2 is a β-1,3-glucanase that has been proposed to degrade the cell walls of invading fungal pathogens [30] as well as, like PR1, generate elicitors by degradation of endogenous substrates to induce defense responses [31]. PR14 is a lipid transfer protein, which has been described to be involved in plant defense against biotic stresses, showing antifungal and antioxidant activity [32,33], antibacterial activity [34], and interaction with lipid-derived molecules to trigger long-distance signaling [35,36]. JAA is a jasmonate-induced like protein without clear function, but related to jasmonate, an essential phytohormone regulating plant growth, development, and defense.

Specific oligonucleotides (5′ to 3′) were used for the quantification of the target genes (Table 1), and for each gene system, the linearity within a range of number of copies of each gene was evaluated, and the efficiency for each curve was calculated as well (efficiencies ranging from 87.68 to 99.46%, $R^2 > 0.98$). Relative quantification of gene expression was done using the $\Delta\Delta C_T$ method [39], and the relative expression values were normalized against the *β-actin* gene as an internal control. Values of $\log_2$ (fold-change), being fold-change the difference of gene expression between the water-treated control and the treatment [40], are presented in the graphs.

Table 1
Genes and primers used for reverse transcription quantitative real-time PCR (RT-qPCR) analysis to quantify their expression levels in 'WA 38' apple trees.

Target gene	Gene description	Specific oligonucleotides (5′ to 3′)	Source
NIMIN2	NIMI-interacting protein 2	NIMIN2-q1: ACGGCGTTGTCTTGTCAGAT NIMIN2-q2: ACGTGACCTCCGTTACGTTC	[37]
JAA	Jasmonate-induced like protein	JAA-q1: GGGACCACAGTGGGCATATC JAA-q2: GTGGGTTTTGGTTCTTCGGC	[37]
PR1	Pathogenesis related protein 1	PR1-q1: CTTGACGTGGGATGACAATG PR1-q2: AGTGCTCATGGCAAGGTTTT	[38]
PR2	Pathogenesis related protein 2 (β 1,3-glucanase)	PR2-q1: ACACTGACCCTGCAAACCAA PR2-q2: GCAAGGTCTATGCTACCAGGG	This study
PR4	Pathogenesis related protein 4 (barwin domain chitinase)	PR4-q1: CATTGGGACAAAGTGCGACG PR4-q2: CAGTAGGCACTTACTGCCCG	This study
PR14	Pathogenesis related protein 14 (lipid transfer protein)	PR14-q1: GCTGCACAAAACACCACGAT PR14-q2: GGACAAGGAGAGCCACAGAC	This study
Actin	β-actin (reference gene)	Actin-q1: CTATGTTCCCTGGTATTGCAGACC Actin-q2: GCCACAACCTTGTTTTTCATGC	[38]

2.2.4. Data analysis

The statistical significance of the gene expression data was determined using the REST2009 Software, where differences in gene expression between control and treated samples were assessed by

randomization tests with a pair-wise reallocation [41]. Principal component analysis (PCA), performed using GraphPad Prism v 9, was used to evaluate the effect of the treatments on the expression of all of the genes. The analysis was performed with the $\log_2$ (fold-change) values of all 3 biological replicates for each treatment and time from both years (2022 and 2023). Statistical analysis to determine the effect of the treatments on the general expression of each gene individually was performed on the $\log_2$ (fold-change) of all biological replicates from both years using linear mixed models (MIXED) analysis of variance using SAS v 9.4. Time was considered as a repeated measure. Multiple means comparison was performed according to Tukey's honestly significant difference test at a P value of ≤ 0.05.

2.3. Control of shoot blight infections (in 2023 and 2024)

2.3.1. Inoculation of E. amylovora

For the inoculation, 11 days after the first treatment application, 2 shoot tips of 2 middle trees of the set of 5 were dip inoculated with *E. amylovora* strain Ea 153 at 1×10^8 CFU/ml [28]. Briefly, 2 actively growing shoots, generally greater than 15 cm in length, on independent branches were selected and marked prior to inoculation. To inoculate, the 2 youngest leaves at the shoot tip were bisected across the midrib using scissors and the cut was dipped in the *E. amylovora* bacterial suspension prepared in PBS and adjusted to 1×10^8 CFU/ml (verified at 5.2×10^7 CFU/ml in 2023 and at 1.5×10^8 CFU/ml in 2024). During inoculation, the stock inoculum suspension was stored on ice and kept out of direct sunlight; aliquots of inoculum were replenished between varieties and biological replicates. Sprinkler irrigation was used 12 to 36 h after the inoculation to increase the humidity in the tree canopy.

2.3.2. Evaluation of shoot blight infections

Shoot blight incidence and severity were evaluated for each treatment. Shoot blight incidence was calculated as the percentage obtained from dividing the number of blighted shoots by the total number of shoots inoculated. Shoot blight severity of each inoculated shoot was evaluated by measuring the total shoot length and the length of the necrotic lesion 16–17 days post-inoculation, and calculating it as follows:

$$\text{Shoot blight severity} = \frac{(\textit{length of necrotic lesion})}{(\textit{total shoot length})} \text{x } 100\ \%$$

The lesion was rapidly removed using sanitized loopers, leaving a 12 cm stub.

2.3.3. Data analysis

Two-way analysis of variance (ANOVA) was performed to determine the effect of the treatments and year on the incidence and severity of shoot blight using GraphPad Prism v 9. To assess differences between treatments and the water-treated control, we performed Dunnett's multiple comparison test at a P value of ≤ 0.05 for incidence and severity (severity data arcsin (sqrt (y/100)) transformed to meet normality and homoscedasticity).

3. Results

Similar degrees of shoot blight infection were observed in 2023 and 2024 in the water-treated control, with incidence around 85% and severity around 50% (Fig. 2). The two-way analysis of variance indicated a significant effect of treatment ($P = 0.0357$ for incidence; $P = 0.01$ for severity), with no significant effect of year ($P = 0.3123$ for incidence; $P = 0.1587$ for severity) or year by treatment interaction ($P = 0.9613$ for incidence; $P = 0.8658$ for severity). This indicated that treatments performed consistently across both years and that overall disease pressure did not differ meaningfully between years. For this reason, the data from 2023 to 2024 were pooled for analysis and visualization. The higher dose of ProCa (420.3 g/ha) provided significant reductions in both shoot blight incidence and severity compared to the water-treated control (Fig. 2). Shoot blight severity of the combined treatment was also significantly lower than the water-treated control. ASM treatments applied via spray or drench, as well as the lower dose of ProCa (140.1 g/ha), exhibited shoot blight severity and incidence not significantly different than the water-treated control (Fig. 2).

Analysis of relative gene expression in 'WA 38' apple trees during the 2022 and 2023 growing seasons showed a pronounced transcriptional response when prohexadione-calcium (ProCa) was applied (Figs. 3 and 4). Notably, in 2023, the combined treatment resulted in 4 genes significantly upregulated at 21 days post-treatment (dpt), while treatment ProCa at 140.1 g/ha and treatment ASM at 70.1 g/ha had 0 and 1 genes upregulated, respectively.

Application of ProCa at the higher dose (420.3 g/ha) led to consistent and significant upregulation of *JAA* and *PR1* across all time points, except at 7 dpt (Figs. 3 and 4). Additionally, *PR2* and *PR14* were also

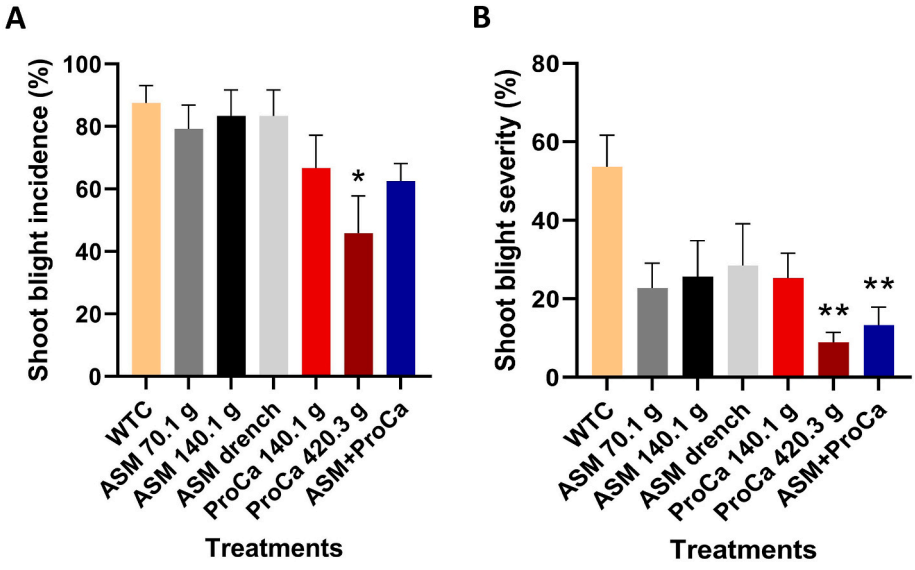

Fig. 2. The effect of acibenzolar-*S*-methyl (ASM) and prohexadione-calcium (ProCa) treatments compared to the water-treated control (WTC) on the incidence (A) and severity (B) of shoot blight infection in 'WA 38' in 2023 and 2024 (data collected 16–17 days post-inoculation). Asterisks indicate significant differences between the treatment and the WTC according to Dunnett's multiple comparison test at a P value of ≤ 0.05. Note: *, $P < 0.05$; **, $P < 0.01$.

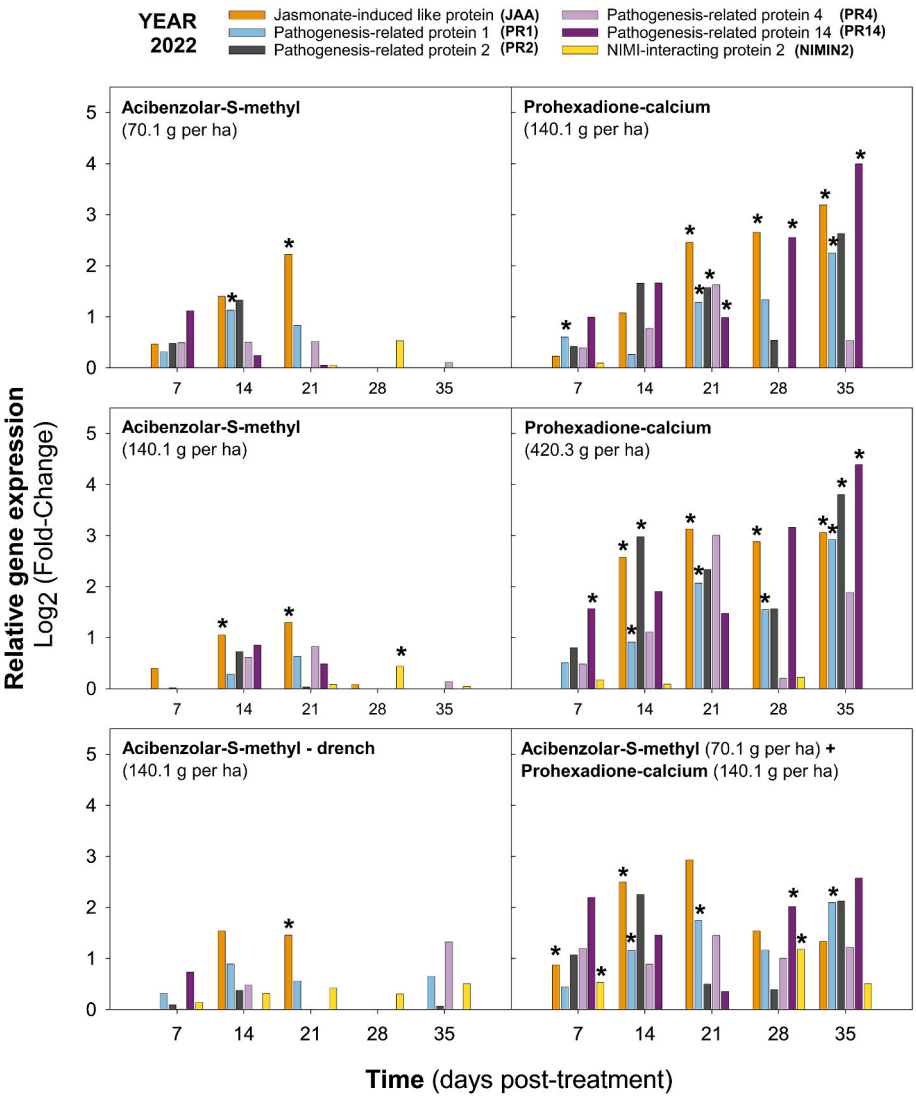

Fig. 3. Expression levels of defense-related genes (*JAA*, *PR1*, *PR2*, *PR4*, *PR14*, *NIMIN2*) in young 'WA 38' apple trees in 2022 in response to spray or drench treatments with either acibenzolar-*S*-methyl (ASM), prohexadione-calcium (ProCa), or both, using reverse transcription quantitative real-time PCR (RT-qPCR) analysis. The $\Delta\Delta C_T$ method was used for relative quantification, where each treatment was compared to its appropriate water-treated control. Asterisks indicate significant differences in gene expression between control and treated samples assessed by REST2009 Software [41]. β-actin was used for data normalization.

significantly upregulated, though not uniformly across all time points. In 2022, the peak transcriptional response was observed at 35 dpt, with 4 out of 6 target genes significantly upregulated, suggesting a time-dependent intensification of the plant's response to ProCa (Fig. 3).

In 2022, the lower dose of ProCa significantly induced the expression of *JAA* and *PR14* from 21 dpt to 35 dpt, and also upregulated *PR1* and *PR2*, though inconsistently across time points (Fig. 3). In contrast, in 2023, significant upregulation of *PR2* and *PR14* was only detected at 14 dpt (Fig. 4). Overall, there was a higher number of significantly upregulated genes at the higher dose compared to the lower dose of this PGR, indicating a correlation between dose and induction of plant defense responses in 'WA 38' apple trees.

In the case of ASM, no clear dose–response relationship was observed. Both doses led to significant but inconsistent upregulation of *JAA*, *PR1*, and *NIMIN2* primarily within the first 28 dpt. ASM applied via drench exhibited gene expression patterns similar to the foliar spray (Figs. 3 and 4).

The combined treatment also showed a sustained plant defense induction over time. In 2022, the number of upregulated genes remained consistent across time points (Fig. 3), while in 2023, 4 out of 6 genes were significantly upregulated at 21 dpt (Fig. 4). Even though the combined treatment occasionally induced equal or fewer genes compared to the low dose of ProCa alone, in 2022, *JAA* and *NIMIN2* were already significantly upregulated at 7 dpt (Fig. 3).

All biological replicates from both years (2022 and 2023) were used to perform a PCA (Fig. 5). The first two principal components accounted for 58.94 and 17.25% of the total variation in the dataset, respectively, making the two-dimensional scatter plot a good approximation, as it represents 76.19% of the total variation of the data. The first principal component is a measure of the overall expression of the pathogenesis-related (PR) genes and the *JAA*, as it shows approximately equal positive loadings for all of them. The second principal component has a high positive loading on the *NIMIN2* and small negative and positive loading on the *JAA* and *PR4*, respectively (Fig. 6).

The PCA illustrates that ProCa treatments, as well as the combined treatment, have a similar response in terms of inducing expression of the genes of interest. ProCa and the combined treatment clearly group at the positive side of the first principal component, which explains most of the variation. Some of the time points of these treatments are also at the negative side of the first principal component, but they mainly correspond to the first time point (7 dpt), when expression levels of the PR genes and the *JAA* were still relatively low for these treatments.

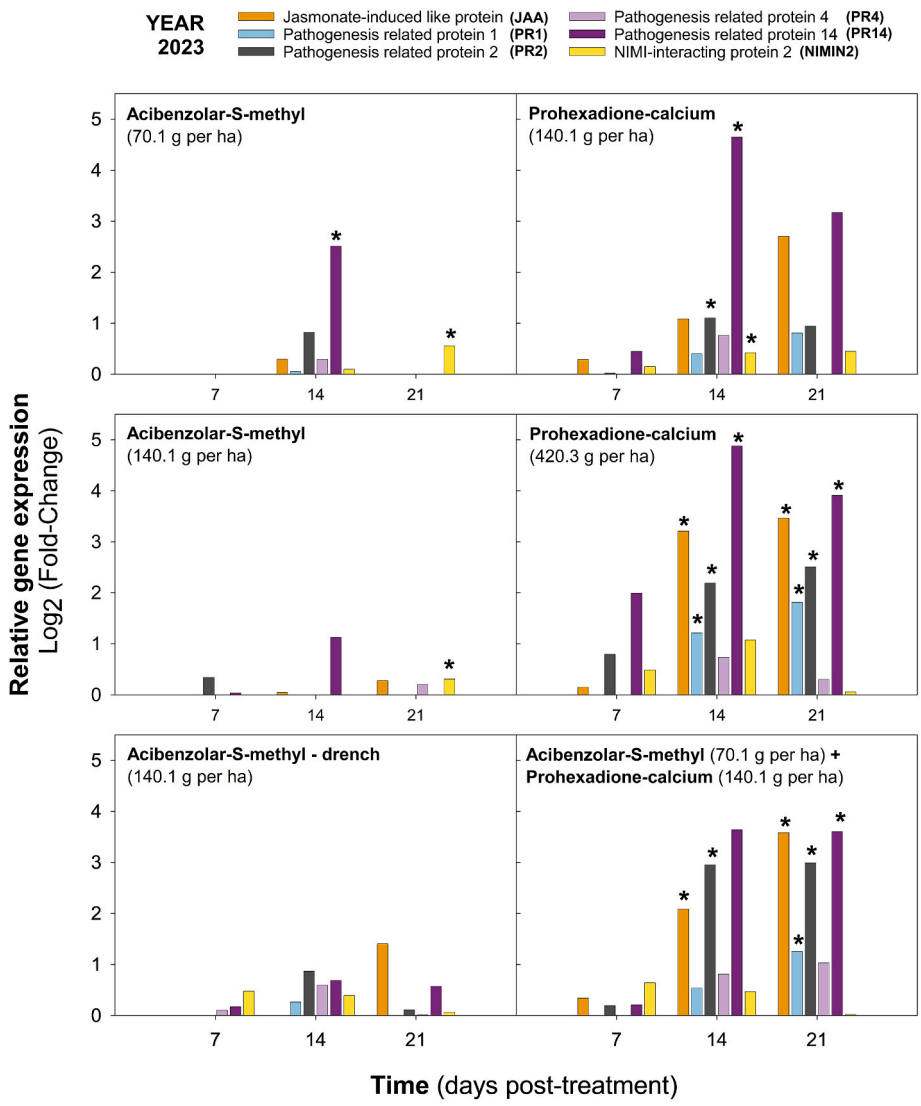

Fig. 4. Expression levels of defense-related genes (*JAA*, *PR1*, *PR2*, *PR4*, *PR14*, *NIMIN2*) in young 'WA 38' apple trees in 2023 in response to spray or drench treatments with either acibenzolar-*S*-methyl (ASM), prohexadione-calcium (ProCa), or both, using reverse transcription quantitative real-time PCR (RT-qPCR) analysis. The $\Delta\Delta C_T$ method was used for relative quantification, where each treatment was compared to its appropriate water-treated control. Asterisks indicate significant differences in gene expression between control and treated samples assessed by REST2009 Software [41]. β-actin was used for data normalization.

An analysis of variance including all biological replicates from both years was performed for each gene, while considering time as a repeated measure (Fig. 7). An interaction by treatment and gene was observed, but not by treatment and year ($P \geq 0.1$ for all of the genes). Results from both years and all sampling times confirmed the significant expression of all of the genes, except *NIMIN2*, for the ProCa treatments and the combined treatment when compared to the water-treated control. *JAA*, *PR2*, and *PR14* showed higher expression levels, followed by *PR1* and *PR4*. The dose–response relationship with the ProCa treatments was also reflected in these results. The ASM treatments were not significantly different from one another nor the water-treated control.

4. Discussion

This work demonstrates the ability of ASM and ProCa to mitigate fire blight infections in apple. SAR inducers and PGRs, such as ASM and ProCa, are increasingly used as part of an integrated disease management practice. Multiple studies show positive impacts of ASM and ProCa for fire blight management for the control of blossom and shoot blight [14–16,22,42–44]. ASM is widely known as an inducer of SAR, with the activation of pathogenesis-related proteins (PR-proteins) in intercellular spaces, mimicking the role of salicylic acid [45]. Additionally, the PGR ProCa induces thickening of the cell walls in the cortical parenchyma, creating a physical barrier capable of slowing infection and systemic spread of the pathogen [42,46]. In the present study, we assessed the potential of ASM and ProCa to control fire blight, specifically the prevention of shoot blight, in young apple trees of the cultivar 'WA 38' under field conditions. Unique to this investigation, gene expression quantification of six defense-related genes was conducted, providing insights into their capacity to induce plant defense responses.

For two consecutive growing seasons, the application of ProCa at a dose of 420.3 g/ha showed significant control of shoot blight in the field for the cultivar 'WA 38', with reduced incidence and disease severity (Fig. 2). In addition, the observed control correlated with a high number of significantly upregulated genes related to defense pathways: *JAA*, *PR1*, *PR2*, *PR4*, and *PR14* (Fig. 7). Some field studies have already demonstrated the control of fire blight using both pre-bloom and post-bloom applications of ProCa, but they associated it with the thickening of the cell walls in the cortical parenchyma, not with the induction of defense responses [16,42]. The capability of ProCa to upregulate defense-related genes has been demonstrated under controlled conditions (greenhouse, growth chamber), and this has been associated with

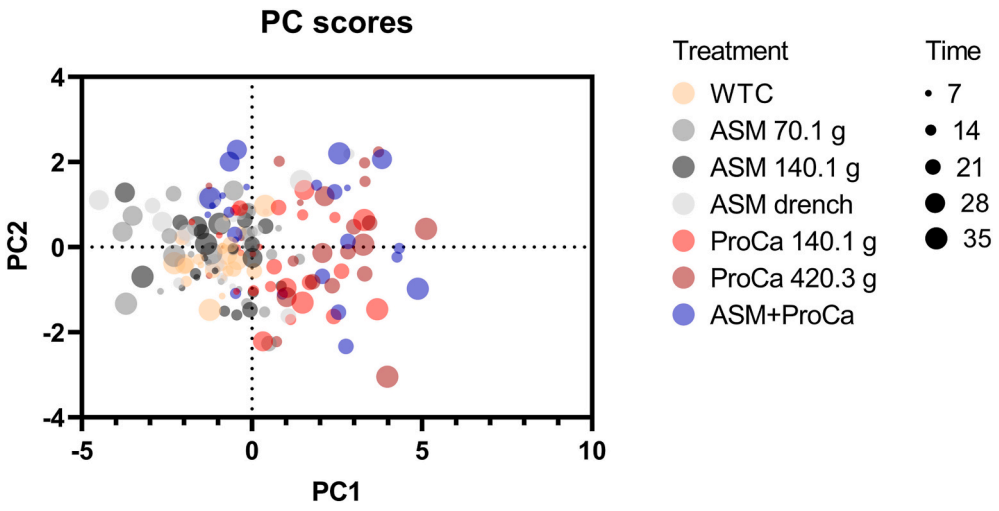

Fig. 5. Principal component analysis (PCA) score plot to evaluate the effect of acibenzolar-*S*-methyl (ASM) and prohexadione-calcium (ProCa) treatments on the expression levels of defense-related genes (*JAA*, *PR1*, *PR2*, *PR4*, *PR14*, *NIMIN2*) in young 'WA 38' apple trees in 2022 and 2023. Note: WTC, water-treated control; ASM at 70.1 g/ha (spray); ASM at 140.1 g/ha (spray); ASM drench, ASM at 140.1 g/ha (drench); ProCa at 140.1 g/ha (spray); ProCa at 420.3 g/ha (spray); ASM + ProCa, ASM at 70.1 g/ha and ProCa at 140.1 g/ha (spray).

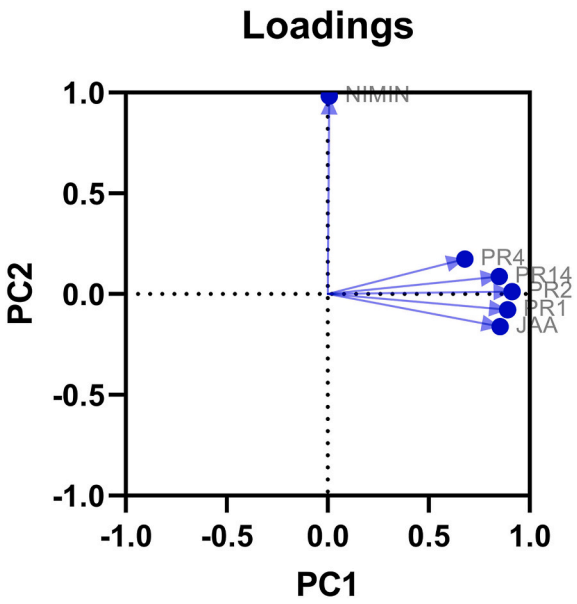

Fig. 6. Principal component analysis (PCA) loading plot showing the loadings of each variable on the first two principal components (PC1 and PC2). Arrows represent the contribution and direction of each variable, indicating their relationships with the principal components.

disease control observed in the field [37,47,48]. Our findings provide evidence that ProCa in-field fire blight control is also associated with induced plant defense responses. Additionally, the significant induction of defense-related genes already at 14 days post-treatment aligns with previous research saying that ProCa requires between 10 and 14 days to take effect [16].

Lower doses of ProCa (140.1 g/ha) did not significantly control shoot blight. Although the same genes were significantly upregulated compared to the high dose of this PGR, this induction was not as frequent across time points (Figs. 3 and 4), suggesting that temporal stability of the defense response may be critical for effective fire blight control. Other studies have also shown this dose–response relationship with ProCa, where lower doses demonstrated reduced shoot blight control compared to higher doses. For example, Aćimović et al. [43] reported that lower doses or minimal ProCa programs provided weaker shoot blight control under field conditions, particularly when rapid shoot growth diluted the protective effect and allowed pathogen progression. Slack et al. [49] further showed reduced cell wall thickening in cortical parenchyma tissues at lower ProCa doses. Together, these findings indicate that both the magnitude and persistence of ProCa induced responses are dose-dependent, and that suboptimal application rates may fail to provide adequate protection. These results reinforce the need to optimize application rates to achieve reliable disease control.

In already established orchards, the use of ProCa could serve as an integrated fire blight and crop management practice. ProCa is a PGR that reduces longitudinal shoot growth by inhibiting gibberellin biosynthesis, so in addition to providing shoot blight control, applications of this product during petal fall reduce vigor [3,50]. In the cultivar 'WA 38', excessive tree vigor is a potential contributor to the development of green spot, a disorder characterized by the presence of dark green halos on the apple's epidermis, along with necrotic, corky, and oxidized tissue beneath the affected areas [51]. Application of ProCa during petal fall in the cultivar 'WA 38' can minimize the amount of wood removed in winter pruning and contribute to the production of larger and more consistent fruit sizes [50].

It has been reported that the application of ASM upregulates defense-related enzymes such as β-1,3-glucanases [12], and induces the expression of the genes like *PR1* [14,15]. Even though the upregulation of *PR1*, *NIMIN2*, and *JAA* was observed in our study, it was not significant compared to the water-treated control in most of the times points studied. In both 2022 and 2023, no significant expression of *PR1*, *JAA*, or *NIMIN2* was observed at 7 dpt, though this agrees with the fact that multiple applications of the product are necessary to observe a significant effect in the field [52]. Genotype-specific variation in the induction of defense-related genes following ASM treatment could be another explanation for the lower expression levels observed in our study compared with previous reports [19]. The application method has also been shown to influence the activation of defense-related genes. ASM applied via trunk paint showed a better performance than drench application [15], and in another study, drench application showed higher *PR2* expression levels when compared to foliar spray [53].

No significant shoot blight control as either reduction in incidence or severity was observed when using ASM, regardless of dose or method of application (foliar spray or drench). As highlighted by Marolleau et al. [19], the efficacy of ASM in disease suppression may depend on whether the induced defense response exceeds a critical threshold, which is

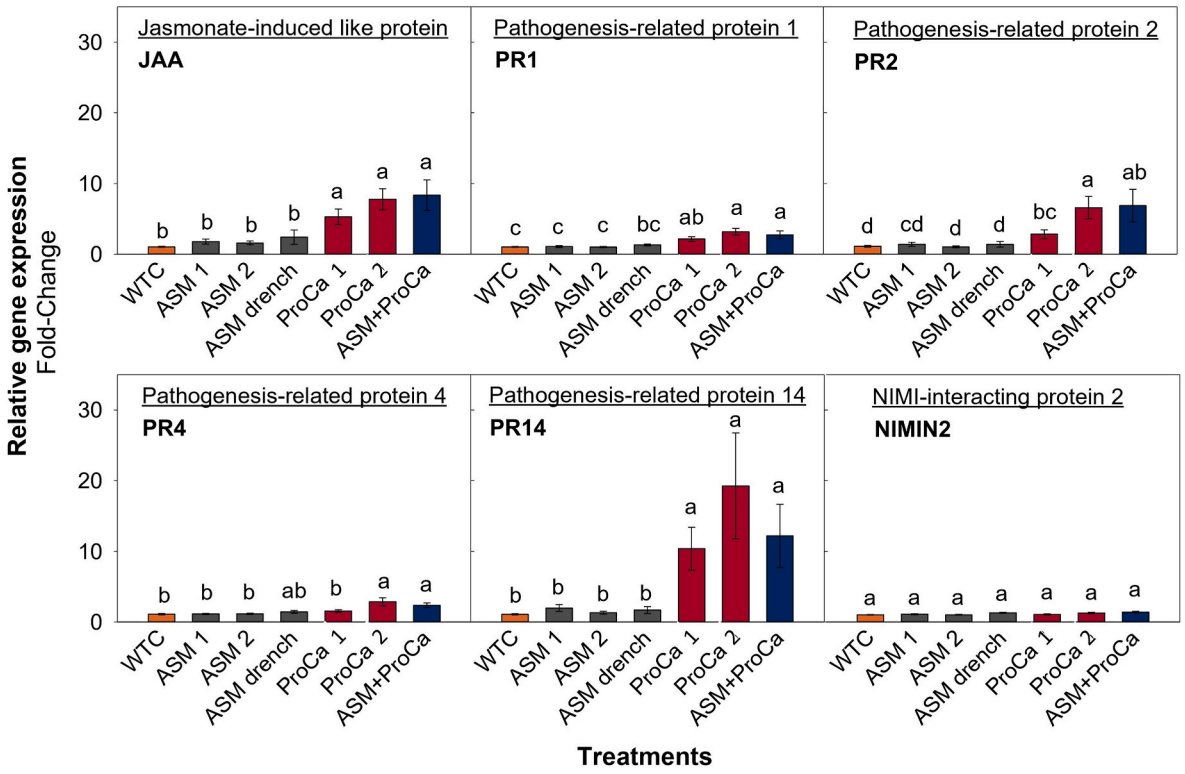

Fig. 7. Expression levels of defense-related genes (*JAA*, *PR1*, *PR2*, *PR4*, *PR14*, *NIMIN2*) in young 'WA 38' apple trees in 2022 and 2023 in response to spray or drench treatments with either acibenzolar-*S*-methyl (ASM), prohexadione-calcium (ProCa), or both, using reverse transcription quantitative real-time PCR (RT-qPCR) analysis. The $\Delta\Delta C_T$ method was used for relative quantification, where each treatment was compared to its appropriate water-treated control. Different letters within the same gene graph indicate significant differences between treatments according to Tukey's honestly significant difference test ($P \leq 0.05$). Note: ASM 1, 70.1 g/ha; ASM 2, 140.1 g/ha; ProCa 1, 140.1 g/ha; ProCa 2, 420.3 g/ha.

influenced by both the plant's responsiveness to treatment and its baseline level of constitutive defense. In our case, the application of ASM in young apple trees of the cultivar 'WA 38' did not result in sustained or strong induction of the selected defense-related genes over time, which may explain the lack of observable disease control under field conditions. Additionally, ASM performance under field conditions may be further constrained by environmental factors that influence salicylic acid–dependent signaling pathways. This is consistent with broader evidence that induced defense responses often perform inconsistently outside controlled environments, where efficacy is typically lower and more variable than in laboratory or greenhouse studies [54].

The combination of ProCa and ASM at low doses was also tested in our trials, showing a significant reduction of shoot blight severity compared to the water-treated control (Fig. 2). Because an equal or higher number of defense-related genes were significantly upregulated generally at all time points when compared to both products applied alone at the corresponding doses, the effect of the combined treatment on the induction of genes related to defense responses seems to be additive. This agrees with other studies using ProCa and plant inducers combined with ASM, which showed a synergistic effect on the expression of defense-related genes [19,49,55]. In our case, this synergistic effect positively impacted the control of shoot blight in the field, offering a more cost-effective alternative to using ASM alone. Even though reductions in severity were not different than ProCa applied alone at 420.3 g/ha, recent findings suggest that this combined treatment does not compromise tree growth [49] nor canopy development and orchard establishment [18], making it particularly beneficial in young high-density orchards. Additionally, ProCa applications have been associated with reduced return bloom and altered crop load dynamics in some cases [56,57], and combining it with ASM has been shown to mitigate these effects, improving crop load management [18].

As already mentioned, tree age and cultivar each influence constitutive defense levels, the magnitude of induced responses, and overall susceptibility to fire blight, potentially affecting the generalizability of our findings [28,58]. So, even though previous work has demonstrated fire blight suppression by these products in mature apple and pear orchards of various cultivars, the extent to which ASM or ProCa mediated defense activation varies across apple and pear genotypes remains unclear [54]. Future studies incorporating multiple cultivars and orchard ages will be essential to validate the broader applicability of these results and to refine management recommendations for diverse commercial production systems.

5. Conclusions

In summary, the present research demonstrates that ProCa, particularly at higher doses, is effective in controlling shoot blight in 'WA 38' apple trees under field conditions. This efficacy is associated with its capacity to induce plant defense pathways, with a sustained response over time. While ASM alone was not effective under our experimental conditions, its combination with ProCa enhanced the activation of plant defenses and improved shoot blight control, indicating a synergistic effect. Given the potential benefits of ProCa observed when there is a need for reducing excessive vigor (e.g., when using a top-working grafting approach), its use during the post-bloom period represents an integrated approach to fire blight and crop management in 'WA 38' and other vigorous apple production systems. The application of low doses of ProCa and ASM combined may offer a suitable alternative in newly established orchards, where rapid tree growth is encouraged.

Funding

This work was supported by the grant Comprehensive Fire Blight Management Systems for the United States (2020-51181-32158) funded

by the National Institute of Food and Agriculture (Specialty Crop Research Initiative).

CRediT authorship contribution statement

Aina Baró: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Sara Tianna DuPont:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing.

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.

Acknowledgments

We thank Gabriela Bolaños and Rebecca A. Schmidt for their technical contributions during the field and laboratory work. We are also grateful to the project collaborators for their support, especially Dr. Xiaochen Yuan for generating the primer sequences and making them available for this research. We thank Dr. George Sundin for leading this grant supported initiative.

Data availability

Data will be made available on request.

References

[1] C. Weber, S. Simnitt, H. Wakefield, S. Wechsler, United States department of agriculture, Economic Research Service (2024). https://www.ers.usda.gov/sites/default/files/_laserfiche/outlooks/110107/FTS-380.pdf?v=94276. (Accessed 7 July 2025).

[2] S.T. DuPont, T. Smith, K.B. Johnson, Y. Zhao, Washington State University, Pullman, WA, 2024. https://treefruit.wsu.edu/crop-protection/disease-management/fire-blight/. (Accessed 7 July 2025).

[3] J.L. Norelli, A.L. Jones, H.S. Aldwinckle, Fire blight management in the twenty-first century: using new technologies that enhance host resistance in apple, Plant Dis. 87 (2003) 756–765, https://doi.org/10.1094/PDIS.2003.87.7.756.

[4] K.B. Johnson, T.N. Temple, R.B. Elkins, T.J. Smith, Strategy for non-antibiotic fire blight control in U.S.-grown organic pome fruit, Acta Hortic. 1056 (2014) 93–100, https://doi.org/10.17660/ActaHortic.2014.1056.12.

[5] S.M. Slack, K.J. Walters, C.A. Outwater, G.W. Sundin, Effect of Kasugamycin, oxytetracycline, and Streptomycin on In-orchard population dynamics of *Erwinia amylovora* on apple flower stigmas, Plant Dis. 105 (6) (2021) 1843–1850, https://doi.org/10.1094/PDIS-07-20-1469-RE.

[6] Q. Zeng, K.B. Johnson, S. Mukhtar, S. Nason, R. Huntley, F. Millet, C.H. Yang, M. A. Hassani, N. Zuverza-Mena, G.W. Sundin, *Aureobasidium pullulans* from the fire blight biocontrol product, blossom protect, induces host resistance in apple flowers, Phytopathology 113 (7) (2023) 1192–1201, https://doi.org/10.1094/PHYTO-12-22-0452-R.

[7] K.B. Johnson, T.N. Temple, Evaluation of strategies for fire blight control in organic pome fruit without antibiotics, Plant Dis. 97 (2013) 402–409, https://doi.org/10.1094/PDIS-07-12-0638-RE.

[8] K.B. Johnson, T.N. Temple, A. Kc, R.B. Elkins, Refinement of nonantibiotic spray programs for fire blight control in organic pome fruit, Plant Dis. 106 (2022) 623–633, https://doi.org/10.1094/PDIS-07-21-1405-RE.

[9] S.M. Slack, Q. Zeng, C.A. Outwater, G.W. Sundin, Microbiological examination of *Erwinia amylovora* exopolysaccharide ooze, Phytopathology 107 (2017) 403–411, https://doi.org/10.1094/PHYTO-09-16-0352-R.

[10] M. Boucher, R. Collins, K. Cox, G. Loeb, Effects of exposure time and biological state on acquisition and accumulation of *Erwinia amylovora* by *Drosophila melanogaster*, Appl. Environ. Microbiol. 85 (2019), https://doi.org/10.1128/AEM.00726-19 e00726-19.

[11] S.T. DuPont, K. Cox, K.B. Johnson, K. Peter, T. Smith, M. Munir, A. Baró, Evaluation of biopesticides for the control of *Erwinia amylovora* in apple and pear, J. Plant Pathol. 106 (2024) 889–901, https://doi.org/10.1007/s42161-023-01372-7.

[12] M.-N. Brisset, S. Cesbron, S.V. Thomson, J.-P. Paulin, Acibenzolar-*S*-methyl induces the accumulation of defense-related enzymes in Apple and protects from fire blight, Eur. J. Plant Pathol. 106 (2000) 529–536, https://doi.org/10.1023/A:1008728119087.

[13] H.S. Aldwinckle, M.V. Bhaskara Reddy, J.L. Norelli, Evaluation of control of fire blight infection of apple blossoms and shoots with SAR inducers, biological agents, a growth regulator, copper compounds, and other materials, Acta Hortic. 590 (2002) 325–331, https://doi.org/10.17660/ActaHortic.2002.590.48.

[14] S.G. Aćimović, Q. Zeng, G.C. McGhee, G.W. Sundin, J.C. Wise, Control of fire blight (*Erwinia amylovora*) on apple trees with trunk-injected plant resistance inducers and antibiotics and assessment of induction of pathogenesis-related protein genes, Front. Plant Sci. 6 (2015) 16, https://doi.org/10.3389/fpls.2015.00016.

[15] K.B. Johnson, T.N. Temple, Comparison of methods of Acibenzolar-*S*-Methyl application for post-infection fire blight suppression in pear and Apple, Plant Dis. 100 (2016) 1125–1131, https://doi.org/10.1094/PDIS-09-15-1062-RE.

[16] A.E. Wallis, K.D. Cox, Management of fire blight using pre-bloom application of prohexadione-calcium, Plant Dis. 104 (2020) 1048–1054, https://doi.org/10.1094/PDIS-09-19-1948-RE.

[17] K. Maxson-Stein, S.-Y. He, R. Hammerschmidt, A.L. Jones, Effect of treating apple trees with Acibenzolar-*S*-methyl on fire blight and expression of pathogenesis-related protein genes, Plant Dis. 86 (2002) 785–790, https://doi.org/10.1094/PDIS.2002.86.7.785.

[18] A. Vogel, S. Villani, T.M. Kon, Prohexadione-calcium and Acibenzolar-*S*-methyl effects and interactions I: vegetative growth management in a young North Carolina apple orchard, Hortscience 60 (10) (2025) 1714–1724, https://doi.org/10.21273/HORTSCI18807-25.

[19] B. Marolleau, M. Gaucher, C. Heintz, A. Degrave, R. Warneys, G. Orain, A. Lemarquand, M.N. Brisset, When a plant resistance inducer leaves the lab for the field: integrating ASM into routine apple protection practices, Front. Plant Sci. 8 (2017) 1938, https://doi.org/10.3389/fpls.2017.01938.

[20] J. Bénéjam, E. Ravon, M. Gaucher, M.-N. Brisset, C.-E. Durel, L. Perchepied, Acibenzolar-*S*-methyl and resistance quantitative trait loci complement each other to control apple scab and fire blight, Plant Dis. 105 (2021) 1702–1710, https://doi.org/10.1094/PDIS-07-20-1439-RE.

[21] L. Faize, M. Faize, Functional analogues of salicylic acid and their use in crop protection, Agronomy 8 (1) (2018) 5, https://doi.org/10.3390/agronomy8010005.

[22] J.L. Norelli, S.S. Miller, Effect of prohexadione-calcium dose level on shoot growth and fire blight in young Apple trees, Plant Dis. 88 (2004) 1099–1106, https://doi.org/10.1094/PDIS.2004.88.10.1099.

[23] D.A. Herms, W.J. Mattson, The dilemma of plants: to grow or defend, Q. Rev. Biol. 67 (3) (1992) 283–335, https://doi.org/10.1086/417659.

[24] D.R. Walters, M. Heil, Costs and trade-offs associated with induced resistance, Physiol. Mol. Plant Pathol. 71 (1–3) (2007) 3–17, https://doi.org/10.1016/j.pmpp.2007.09.008.

[25] K.M. Evans, B.H. Barritt, B.D. Konishi, L.J. Brutcher, C.F. Ross, 'WA 38' apple, Hortscience 47 (2012) 1177–1179, https://doi.org/10.21273/HORTSCI.47.8.1177.

[26] R. Courtney, Good food grower. https://goodfruit.com/a-small-start-for-cosmic-crisp-expansion-overseas/, 2023. (Accessed 7 July 2025).

[27] S.A. Kostick, S.L. Teh, J.L. Norelli, S. Vanderzande, C. Peace, K.M. Evans, Fire blight QTL analysis in a multi-family apple population identifies a reduced-susceptibility allele in 'Honeycrisp', Hortic. Res. 8 (2021) 28, https://doi.org/10.1038/s41438-021-00466-6.

[28] S.A. Kostick, J.L. Norelli, K.M. Evans, Novel metrics to classify fire blight resistance of 94 apple cultivars, Plant Pathol. 68 (2019) 985–996, https://doi.org/10.1111/ppa.13012.

[29] J. Zhao, W. Bi, S. Zhao, J. Su, M. Li, L. Ma, X. Yu, X. Wang, Wheat apoplast-localized lipid transfer protein TaLTP3 enhances defense responses against Puccinia triticina, Front. Plant Sci. 12 (2021) 771806, https://doi.org/10.3389/fpls.2021.771806.

[30] G. Leubner-Metzger, F. Meins Jr., Functions and regulation of plant ß-1,3-glucanases (PR-2), in: S.K. Datta, S. Muthukrishnan (Eds.), Pathogenesis-Related Proteins in Plants, CRC Press LLC, Boca Raton, FL, 1999, pp. 49–76.

[31] L.C. van Loon, M. Rep, C.M. Pieterse, Significance of inducible defense-related proteins in infected plants, Annu. Rev. Phytopathol. 44 (2006) 135–162, https://doi.org/10.1146/annurev.phyto.44.070505.143425.

[32] J.E. McLaughlin, N.I. Darwish, J. Garcia-Sanchez, N. Tyagi, H.N. Trick, S. McCormick, R. Dill-Macky, N.E. Tumer, A lipid transfer protein has antifungal and antioxidant activity and suppresses fusarium head blight disease and DON accumulation in transgenic wheat, Phytopathology 111 (2021) 671–683, https://doi.org/10.1094/PHYTO-04-20-0153-R.

[33] J.-Y. Sun, D.A. Gaudet, Z.-X. Lu, M. Frick, B. Puchalski, A. Laroche, Characterization and antifungal properties of wheat nonspecific lipid transfer proteins, Mol. Plant Microbe Interact. 21 (2008) 346–360, https://doi.org/10.1094/MPMI-21-3-0346.

[34] F. Garcia-Olmedo, A. Molina, A. Segura, M. Moreno, The defensive role of nonspecific lipid-transfer proteins in plants, Trends Microbiol. 3 (1995) 72–74, https://doi.org/10.1016/s0966-842x(00)88879-4.

[35] A.M. Maldonado, P. Doerner, R.A. Dixon, C.J. Lamb, R.K. Cameron, A putative lipid transfer protein involved in systemic resistance signalling in arabidopsis, Nature 419 (2002) 399–403, https://doi.org/10.1038/nature00962.

[36] P.J. Seo, C.M. Park, MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in arabidopsis, New Phytol. 186 (2010) 471–483, https://doi.org/10.1111/j.1469-8137.2010.03183.x.

[37] X. Yuan, K. Gdanetz, C.A. Outwater, S.M. Slack, G.W. Sundin, Evaluation of plant defense inducers and plant growth regulators for fire blight management using transcriptome studies and field assessments, Phytopathology 113 (2023) 2152–2164, https://doi.org/10.1094/PHYTO-04-23-0147-KC.

[38] S. Schröpfer, C. Böttcher, T. Wöhner, K. Richter, J. Norelli, E.H.A. Rikkerink, M. V. Hanke, H. Flachowsky, A single effector protein, AvrRpt2EA, from *Erwinia amylovora* can cause fire blight disease symptoms and induces a salicylic acid-

dependent defense response, Mol. Plant Microbe Interact. 31 (11) (2018) 1179–1191.
[39] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[40] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (2008) 1101–1108, https://doi.org/10.1038/nprot.2008.73.
[41] M.W. Pfaffl, G.W. Horgan, L. Dempfle, Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR, Nucleic Acids Res. 30 (2002) e36, https://doi.org/10.1093/nar/30.9.e36.
[42] M.J. McGrath, J.M. Koczan, M.M. Kennelly, G.W. Sundin, Evidence that prohexadione-calcium induces structural resistance to fire blight infection, Phytopathology 99 (2009) 591–596, https://doi.org/10.1094/PHYTO-99-5-0591.
[43] S.G. Aćimović, C.L. Meredith, R.D. Santander, F. Khodadadi, Proof of concept for shoot blight and fire blight canker management with postinfection spray applications of prohexadione-calcium and Acibenzolar-*S*-Methyl in apple, Plant Dis. 105 (2021) 4095–4105, https://doi.org/10.1094/PDIS-08-20-1744-RE.
[44] V. Philion, V. Joubert, Use of a growth regulator (prohexadione-Ca) and summer pruning as post symptom rescue treatments following a fire blight infection during bloom, J. Plant Pathol. 103 (2021) 163–174, https://doi.org/10.1007/s42161-021-00805-5.
[45] D. Tripathi, G. Raikhy, D. Kumar, Chemical elicitors of systemic acquired resistance—Salicylic acid and its functional analogs, Curr. Plant Biol. 17 (2019) 48–59, https://doi.org/10.1016/j.cpb.2019.03.002.
[46] G. Costa, C. Andreotti, F. Bucchi, E. Sabatini, C. Bazzi, S. Malaguti, W. Rademacher, Prohexadione-Ca (Apogee®): growth regulation and reduced fire blight incidence in pear, Hortscience 36 (2001) 931–933, https://doi.org/10.21273/HORTSCI.36.5.931.
[47] F. Bini, A. Ragaini, C. Bazzi, Resistance responses induced by the plant growth retardant prohexadione-ca in apple against scab infections, Ann. Appl. Biol. 152 (2007) 19–27, https://doi.org/10.1111/j.1744-7348.2007.00188.x.
[48] T. Duge de Bernonville, B. Marolleau, J. Staub, M. Gaucher, M.N. Brisset, Using molecular tools to decipher the complex world of plant resistance inducers: an apple case study, J. Agric. Food Chem. 62 (2014) 11403–11411, https://doi.org/10.1021/jf504221x.
[49] S.M. Slack, X. Yuan, C.A. Outwater, G.W. Sundin, Identification of a reduced rate combination of a plant growth inhibitor with a plant defense inducer for the management of the shoot blight phase of fire blight, Plant Dis. 109 (5) (2025) 1148–1157, https://doi.org/10.1094/PDIS-09-24-2008-RE.
[50] S. Musacchi, R. Sheick, M.J. Mia, S. Serra, Studies on physiological and productive effects of multi-leader training systems and Prohexadione-Ca applications on apple cultivar 'WA 38, Sci. Hortic. 312 (2023) 111850, https://doi.org/10.1016/j.scienta.2023.111850.
[51] B. Sallato, M.D. Whiting, J. Munguia, Rootstock and nutrient imbalance leads to "Green Spot'' Development in 'WA 38' apples, Hortscience 56 (2021) 1542–1548, https://doi.org/10.21273/HORTSCI16213-2.
[52] R.J. Orpet, R.W. Cooper, E.H. Beers, L.B. Nottingham, Test of plant defense elicitors for arthropod pest suppression and *PR-1* gene induction in pear orchards, Entomol. Exp. Appl. 169 (2021) 1137–1146, https://doi.org/10.1111/eea.13110.
[53] M.I. Francis, A. Redondo, J.K. Burns, J.H. Graham, Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of citrus canker, Eur. J. Plant Pathol. 124 (2009) 283–292, https://doi.org/10.1007/s10658-008-9415-x.
[54] T. Reglinski, N. Havis, H.J. Rees, H. Jong, The practical role of induced resistance for crop protection, Phytopathology 113 (4) (2023) 719–731, https://doi.org/10.1094/PHYTO-10-22-0400-IA.
[55] M.A.E. Hassan, H. Buchenauer, Induction of resistance to fire blight in apple by Acibenzolar-*S*-Methyl and DL-3-aminobutyric acid, J. Plant Dis. Prot. 114 (4) (2007) 151–158, https://doi.org/10.1007/BF03356211.
[56] J. Lordan, P. Vilardell, E. Torres, S. Alegre, L. Asín, Use of root pruning, paclobutrazol, and prohexadione-Ca combination strategies to control growth and improve productivity on pear trees, Spanish J. Agric. Res. 17 (2) (2019) e0902, https://doi.org/10.5424/sjar/2019172-14579.
[57] W. Rademacher, K. Saarloos, J.A. Garuz Porte, F. Riera Forcades, Y. Senechal, C. Andreotti, F. Spinelli, E. Sabatini, G. Costa, Impact of Prohexadione-Ca on the vegetative and reproductive performance of apple and pear trees, Eur. J. Hortic. Sci. 69 (6) (2004) 221–228, https://doi.org/10.17660/eJHS.2004/25306.
[58] K.J.P. Silva, J. Singh, R. Bednarek, Z. Fei, A. Khan, Differential gene regulatory pathways and co-expression networks associated with fire blight infection in apple (*Malus* × *domestica*), Hortic. Res. 6 (2019) 35, https://doi.org/10.1038/s41438-019-0120-z.