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Research paper

A plant-based biocontrol product confers protection against *Phytophthora infestans* and triggers tomato defense responses

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ABSTRACT

Phytophthora infestans, causal agent of late blight, is mainly controlled with fungicides, whose use is increasingly restricted in Europe, motivating the adoption of sustainable alternatives. Among these, plant-based biocontrol products, particularly those with elicitor and/or priming effects, offer promising perspectives. This study evaluated the protective action of a biocontrol formulated product (FV), derived from plant extracts, against tomato late blight. Greenhouse and growth chamber experiments were conducted to evaluate FV's preventive efficacy and to distinguish its direct effect from its indirect effect. FV provided protection against late blight as early as 2 hpt (hours post-treatment) and up to 8 dpt (days post-treatment). However, FV did not protect newly formed leaves at 8 dpt. FV application induced tomato defense responses at all time points, with a peak at 6 hpt, notably activating *PR2*, *PR5*, *POX*, and *ACCO* genes expressions. No priming effect on defense gene expression was observed following pathogen inoculation at 2 days post-inoculation (dpi). Additionally, FV's efficacy was reduced by washing leaf surface, suggesting the contact-dependent action mode. Overall, the results indicate that the product's efficacy is mainly attributable to a direct effect, accompanied by the activation of tomato defense responses prior inoculation, but without evidence of systemic activity.

Although the results are promising, further research is required to clarify the mode of action of this biocontrol product and to assess the potential contribution of induced tomato defense responses to its overall efficacy.

1. Introduction

Tomato (*Solanum lycopersicum*), the most widely produced fruit in the world, is susceptible to numerous pathogenic microorganisms (FAO, 2024; Blancard, 2012). Among the diseases affecting this crop, late blight, caused by the oomycete *Phytophthora infestans* (*P. infestans*), remains one of the most devastating (Nowicki et al., 2013). Despite being known since the 19th century and extensively studied, *P. infestans* continues to cause severe damages to tomato production worldwide (Kamoun et al., 2015; Ristaino, 1998). In the absence of protection, it can destroy an entire crop within **7–10 days** (Nowicki et al., 2012). In the United States, yield losses due to late blight have reached up to **56 million dollars in a single year** (Seidl Johnson et al., 2015). The success of *P. infestans* as a devastating pathogen is largely due to its **rapid and highly efficient asexual cycle** (Fry, 2008; Nowicki et al., 2013). Within infected lesions, sporangiophores produce sporangia that can either germinate directly to infect plant tissues or release motile zoospores capable of initiating new infections (Fry, 2008). Moreover, its **sexual reproduction, via oospore formation,** enhances long-term survival, further complicating *P. infestans* management (Andrivon, 1995; Yuen and Andersson, 2013).

For decades, synthetic fungicides have been the primary means of controlling *P. infestans* (Fry et al., 2015). However, the intensive use of pesticides, particularly phenylamide fungicides such as metalaxyl and mefenoxam, which inhibit ribosomal RNA biosynthesis, has led to the selection of resistant isolates (Childers et al., 2015; Fry et al., 2015). Recently, field effectiveness of several single-site fungicides has significantly declined. This is the case for fluazinam, for which strains exhibiting reduced sensitivity have been reported in the Netherlands (Schepers et al., 2018). Similarly, mandipropamid-resistant strains of *P. infestans* have been identified in Denmark (Abuley et al., 2023). Copper-based contact fungicides, widely used in organic farming, also

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show notable efficacy, but their use raises environmental concerns due to their soil toxicity and accumulation (Dorn et al., 2007; La Torre et al., 2019).

In this context and considering growing awareness of the environmental and health consequences of pesticide use, there is a strong demand for more sustainable and eco-friendly disease management strategies (van Lenteren et al., 2018). Among these, biocontrol products represent a promising alternative to reduce both the frequency and dosage of fungicide applications against late blight (Alexandersson et al., 2016; Hashemi et al., 2022; Walters et al., 2013). Biocontrol products based on microorganisms and/or natural substances have already shown efficacy against *P. infestans* under controlled conditions (Clinckemaillie et al., 2017; De Vrieze et al., 2020; Zheng et al., 2021). Their mode of action can be direct, indirect, or a combination of both (Köhl et al., 2019). Direct action involves a lethal or inhibitory effect on the pathogen, while indirect action occurs through the activation of plant immune responses via elicitation and/or priming (Conrath et al., 2002; Jones and Dangl, 2006; Köhl et al., 2019). Elicitation triggers a rapid activation of plant defenses following treatment with elicitors (PAMPs, MAMPs, DAMPs) in the absence of the pathogen, whereas priming enhances the plant's ability to mount a faster and stronger defense response upon subsequent stress exposure, such as pathogen infection (Conrath, 2011). Activation of plant immunity involves complex signaling pathways, including phytohormone-mediated regulation (e.g., salicylic acid, jasmonic acid, and ethylene), which modulate defense-gene expression and lead to reinforced physical and chemical barriers, including the production of pathogenesis-related proteins (PR), reactive oxygen species (ROS), antimicrobial compounds, and cell wall strengthening (Dodds et al., 2024; Duhan and Pasrija, 2025).

Several natural and synthetic substances have demonstrated proven effectiveness against late blight, including potassium phosphite, chitosan, COS-OGA, and various plant extracts and essential oils (Clinckemaillie et al., 2017; Deweer et al., 2023; La Torre et al., 2019; Machinandiarena et al., 2012; Zheng et al., 2021). Potassium phosphite has demonstrated a dual mechanism of action, combining a direct effect on *Phytophthora* spp., including *P. infestans*, with indirect elicitation and priming of plant defense responses (Burra et al., 2014; Eshraghi et al., 2011; Lim et al., 2013; Lobato et al., 2010; Machinandiarena et al., 2012; Smillie et al., 1989). Similarly, chitosan has been reported to exert both direct and indirect effects by inhibiting mycelial growth and sporangial germination of *P. infestans*, while also eliciting plant defense responses, notably through the accumulation of salicylic acid (SA) in potato plants (Zheng et al., 2021). In contrast, COS-OGA acts exclusively via an indirect mechanism involving elicitation and priming (Clinckemaillie et al., 2017). In addition, several plant extracts have shown promising potential both *in vitro* and *in planta* for controlling *P. infestans* (La Torre et al., 2019; Moushib et al., 2013; Najdabbasi et al., 2020; Stephan et al., 2005). For instance, Dorn et al. (2007), who screened more than 20 plant-based preparations, reported that some extracts, such as *Sophora flavescens* extract, were effective both against *P. infestans in vitro* and in infected tomato plants in controlled conditions. However, the same study also showed that several plant extract–based biocontrol products had limited efficacy under field conditions. This inconsistency may be due to fluctuating environmental conditions, plant genotype differences, and an incomplete understanding of their modes of action and the factors influencing efficacy (Dorn et al., 2007; Pressecq et al., 2025).

To improve the performance of biocontrol-based crop protection, it is therefore essential to elucidate their mechanisms, whether direct, elicitor-based, or priming-mediated, before moving on to field trials. For products acting via contact, detailed studies are needed to determine whether they directly affect key stages of the pathogen infection cycle, such as sporangia and zoospores, and associated processes including germination, development, and release (De Vrieze et al., 2018, 2020). Furthermore, in plant experiments, elicitor and priming effects should be assessed, and key parameters influencing efficacy including application timing, persistence, systemic activity, and wash-off resistance should be identified (Droby et al., 2016; Pressecq et al., 2025).

In this context, the present study aims to further elucidate the mode of action of a plant extract-based biocontrol product (FV) against *P. infestans* and to determine the factors influencing its efficacy *in planta*. Previous work demonstrated the direct impact of this product on *P. infestans*, particularly on sporangia and zoospores, which are infection structures (Penaud et al., 2025). Here, the preventive efficacy of FV was evaluated at varying intervals between treatment and inoculation to assess both early and late protection. The potential systemic activity of FV was also investigated by examining its capacity to protect newly growing leaves. Furthermore, the ability of FV to elicit and/or prime defense-related gene expression was evaluated using a targeted qRT-PCR approach (Brisset and Dugé de Bernonville, 2011). Finally, the persistence of FV on leaves was assessed through washing experiments to better characterize the relative contributions of its direct and indirect effects to its overall efficacy.

2. Materials and methods

2.1. Biocontrol product

The tested biocontrol product is a formulated version (FV) containing 2.5% plant extract combined with various co-formulants, developed and supplied by Biom InnoV (Saint-Malo, France). These co-formulants include a preservative to stabilize the extract, a sticker agent to improve adherence to plant surfaces, a surfactant to enhance application and penetration, and trace elements providing supplementary nutritional benefits. The exact composition of the plant extract and formulation remains confidential (Patent n° WO 2024/023446 A1).

2.2. Microorganism and growth conditions

The *Phytophthora infestans* 36_A2 strain was used in all bioassays (inov3PT, Achicourt, France). The strain was maintained in the dark at 15°C on agar plates supplemented with 10% (v/v) V8 tomato juice and 0.1% (w/v) calcium carbonate. Only actively growing mycelial cultures aged 10–14 days were used. To obtain sporangia, 4 mL of sterile distilled water were added to the culture surface, and the mycelium was gently scraped with a sterile rake. The resulting suspension was transferred to a Falcon tube, vigorously shaken to detach sporangia, and filtered through a 70 µm sieve. Sporangial concentration was determined using a Malassez cell and adjusted to 2×10^3 sporangia. mL^{-1}.

2.3. Plant material

Tomato (*Solanum lycopersicum*) cultivar 'Micro-Tom' (Baumaux Graines, France) seeds were sown on moist substrate (TS 3 fine, Klasmann-Deilmann) in miniature greenhouses equipped with transparent lids and maintained for seven days under optimal conditions, with the substrate temperature set at 19°C. After germination, lids were removed, and the plants were transferred to either greenhouses (Normand Serre) or a phytotron (fitrotron, Weiss Technik), where they were grown under the same controlled conditions (25°C day / 20°C night, 14 h light/10 h dark, 60% relative humidity) until day 18. Seedlings were then transplanted into individual pots filled with substrate (Substrate 5, Klasmann-Deilmann). Depending on the experiment, plants were grown for 15–20 days in greenhouses or 10–15 days in the phytotron before treatment. Plants were watered three times per week. In greenhouses, light conditions slightly varied due to natural daylight.

2.4. Effect of the biocontrol product on late blight infection (detached leaflet assay)

Plants were entirely sprayed on both adaxial and abaxial leaf surfaces until run-off with either a 0.1% FV solution or sterile distilled

water (control). After a defined post-treatment period (2- and 6-hours post-treatment, hpt; or 2- and 8-days post-treatment, dpt), 6 leaflets per plant were collected from 2 leaf ranks (typically ranks 3–5, where the first fully developed leaf is considered rank 1). In the experiments at 2–6 hpt and 8 dpt, 3 leaflets per rank (leaf ranks 4 and 5), totaling 6 leaflets per plant, were analyzed with 9 plants per treatment ($n = 9$). In the experiment at 2 dpt, 3 leaflets per rank (leaf ranks 3 and 4), totaling 6 leaflets per plant, were analyzed with 7 plants per treatment ($n = 7$). Leaflets were placed in Petri dishes on moistened filter paper with sterile distilled water and inoculated on the abaxial side with 20 µL of *P. infestans* sporangial suspension (2×10^3 sporangia. mL^{-1}). After 7 days post-inoculation in the dark at 20°C, disease severity was quantified using ImageJ. The symptomatic leaf area (necrotic and sporulation areas) was measured and expressed as the percentage of infected leaf area relative to the total leaf surface per plant. Only one experiment at 2 days post-treatment was performed in the phytotron; the second experiment (replicate) at the same time point, as well as all experiments at other post-treatment times (2–6 hpt and 8 dpt), were conducted in greenhouses. The experiment was performed twice.

2.5. Evaluation of protection of newly formed leaves

To determine whether FV could protect newly developed leaves, greenhouse-grown plants were sprayed either with FV (0.1%) or with water (control). At treatment time, leaves of ranks 7 and 8 were still developing, with rank 8 being less mature than rank 7. The spray was applied uniformly to the whole plant. However, because leaves of ranks 7 and 8 were still developing at treatment time, their actual coverage was naturally lower than that of the fully expanded rank 5 leaflets. After 8 dpt, when ranks 7 and 8 were sufficiently developed, nine leaflets per plant were sampled from ranks 5, 7 and 8 and inoculated with *P. infestans* sporangia as previously described. Disease severity was assessed 7 days after incubation at 20°C in the dark. Each treatment included nine plants (replicates), and the experiments were repeated twice.

2.6. Expression of 28 tomato defense-related genes

2.6.1. Effect of FV treatment

To evaluate the elicitor potential of FV, plants grown in a phytotron were treated with either FV (0.1%) or water (control). Samples were collected at 6, 24, and 48 hpt. For each treatment and time point, three composite samples (three plants each) were collected (three biological replicates). From each plant, leaflets from leaf ranks 3, 4 and 5 were harvested, frozen in liquid nitrogen, and stored at −80°C until RNA extraction. The experiment was repeated twice, yielding six biological replicates ($n = 6$).

After grounding samples in liquid nitrogen, RNA extraction was performed following Gaucher et al. (2022) using the Macherey-Nagel Nucleospin RNA Plant kit. Purity and concentration of the samples were assayed with a Nanodrop spectrophotometer (ThermoScientific). Reverse transcription was performed using M-MLV Reverse Transcriptase (Promega). PCR verification with elongation factor primers spanning an intron confirmed the absence of genomic DNA (Gilliland et al., 1990). Quantitative real-time PCR was conducted using a patented primer set targeting 28 defense-related genes and 3 reference genes (Brisset and Dugé de Bernonville, 2011). The analyzed genes included those encoding **pathogenesis-related (PR) proteins** (*PR1, PR2, PR4, PR5, PR8, PR10, PR14,* and *PR15*), **oxidative stress-related** proteins (*APOX, GST,* and *POX*), **secondary metabolism** proteins (*PAL, CHS, DFR, ANS, PPO, HMGR, FPPS,* and *TPS*), **cell wall-related proteins** (*CALS, CAD,* and *PECT*), and a **cysteine-related gene** (*CSL*). In addition, genes involved in **hormonal defense signaling pathways were assessed, including salicylic acid** (*EDS1* and *WRKY41*), **jasmonic acid** (*LOX* and *JAR*), and **ethylene** (*EIN3* and *WRKY30*). Reactions (15 µL) contained 0.15 µL of the cDNA, 3.75 µL of MESA BLUE qPCR MasterMix (Eurogentec), 1.5 µL of each primer, and 8.1 µL of water. Amplification was performed on a CFX96 Real-Time System (Bio-Rad) with the following conditions: 95°C for 5 min, followed by 40 cycles of 95°C for 3 s (denaturation) and 60°C for 60 s (primer hybridization and elongation). Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008) and normalized against three internal reference genes, *TubA*, *GAPDH*, and *Actin* (Vandesompele et al., 2002). The mean value of water-treated control samples collected at 6 hpt from both independent experiments was as the reference for $\log_2$ ratio calculations for each defense-related gene at each time point.

2.6.2. Effect of FV treatment followed by P. infestans inoculation

To assess the priming potential of FV, plants grown in phytotron were treated with either FV or water (control) and inoculated with *P. infestans,* 6 hpt, based on prior results. The leaflet collection at 6 hpt, which served as T0, was assessed using the same experimental design and protocol as the elicitation experiment. At 48 h post-inoculation (hpi), corresponding to the biotrophic infection stage (Zuluaga et al., 2016a, 2016b), infected leaflets were collected (composite samples of three plants per replicate), frozen in liquid nitrogen, and stored at −80°C. The experiment was repeated twice with three replicates per treatment and time point ($n = 6$ biological replicates). Gene expression analysis followed the same procedure as described for FV elicitation. Log_2 ratios were calculated relative to the mean value of control (6 hpt, water-treated) samples. The remaining 7 plants per treatment were used to assess protection at 7 days post-inoculation (dpi) by measuring disease severity, as performed in previous experiments. Infected leaves were left for an additional 3 days to allow symptoms to develop further, facilitating the quantification of FV's effect on sporulation. Sporangia were counted following Moushib et al. (2013), with minor modifications. At 10 dpi, twelve leaflets per plant were immersed in 10 mL of sterile distilled water in 50 mL Falcon tubes. Samples were agitated using a TopMix FB15024 vortex (Thermo Fisher Scientific) at 2000 rpm for 16 min, followed by a brief 4-second vortex. Sporangia were counted with a Malassez cell (two counts per sample were performed and averaged). The experiment was performed twice in phytotron, and the sporulation data were obtained from 14 plants.

2.7. Effect of wash-off on FV efficacy

To assess the contribution of direct and indirect effects of FV, a wash-off assay was conducted on plants grown in greenhouses. Bordeaux mixture RSR Disperss® (UPL, France; 500 mg. L^{-1}, 20% w/w metallic copper) served as a positive control, as it is expected to resist wash-off depending on the amount of water applied. One day post-treatment, half of the plants were sprayed with sterile distilled water on both leaf surfaces (100 spray bursts per side; ~ 150 mL per side, total of 300 mL). After drying (3–4 h), 10 leaflets per plant (ranks 4 and 5) were collected, inoculated on the abaxial side with 20 µL of *P. infestans* sporangial suspension (2×10^3 sporangia. mL^{-1}). After 7 days post-inoculation in the dark at 20°C, symptomatic leaf area was quantified using ImageJ. Each treatment included seven plants (replicates). The experiment was conducted twice.

2.8. Data analysis

All statistical analyses and figures were generated using RStudio (v2024.04.2; http://www.r-project.org/) and MetaboAnalyst 6.0 (https://www.metaboanalyst.ca/). Data normality (Shapiro-Wilk test) and homoscedasticity (Levenn or Fisher test) were verified prior to analysis. For comparisons between two groups, Student's t-test, Welch's *t*-test (for unequal variances), or the Mann-Whitney-Wilcoxon test was applied, depending on whether assumptions of normality and homogeneity were met ($p \leq 0.05$). For multiple group comparisons, Kruskal-Wallis tests were conducted, followed by pairwise Wilcoxon-Mann-Whitney tests with Bonferroni correction for multiple comparisons

($p \leq 0.05$).

For heatmap visualization of gene expression data, raw values were uploaded to the MetaboAnalyst platform (Pang et al., 2024), and data integrity was verified using the "SanityCheckData" function (Xia et al., 2009). No normalization or autoscaling was applied prior to visualization. Heatmaps were generated using the "PlotHeatMap" function with Euclidean distance, without clustering, and displaying the mean expression values for each treatment (Xia and Wishart, 2011). Partial Least Squares Discriminant Analysis (PLS-DA) was performed using component 1 (Comp. 1) and Variable Importance in Projection (VIP) scores greater than or equal to 1 were used to identify the most discriminant genes across treatments and time points (Pang et al., 2021). Principal Component Analysis (PCA) was carried out with the *Factoshiny* and *FactoMineR* packages in R (Husson et al., 2025), generating a two-dimensional (2D) scores plot with 95% confidence regions and corresponding Biplots.

3. Results

3.1. FV protects tomato against late blight infection

As previously reported by Penaud et al. (2025)), FV exhibits a direct effect on *P. infestans*, compromising infection structures such as sporangia and zoospores at 0.1%. To evaluate the preventive effect of FV against tomato late blight, assays were conducted on detached leaflets from plants treated with FV or water. Inoculations of *P. infestans* were performed at different time intervals following treatment (2 and 6 hpt; 2 and 8 dpt) to assess the influence of timing on protection (Fig. 1**A-B)**. Disease severity, expressed as a percentage of symptomatic leaf area, was evaluated seven days after inoculation. When inoculated 2 hpt, FV-treated plants showed only 4.7% disease compared to 82% in control plants. At 6 hpt, the symptomatic area in control plants reached 71%, whereas FV-treated plants exhibited only 18% symptoms on average. When inoculations were performed at two days post-treatment, FV still conferred significant protection, with 18% symptomatic area compared to 88% in the control, representing a 5-fold reduction **(**Fig. 1**A-B)**. When inoculated at 8 dpt, FV-treated leaflets displayed a 15% symptomatic area, compared to 61% in the control. These results demonstrate that FV provides effective preventive protection against *P. infestans* infection in tomato leaves, acting rapidly (as early as 2 hpt) and maintaining efficacy up to 8 dpt.

3.2. FV provides long-term protection but not of newly growing leaves

To investigate whether FV protects growing leaves, tomato plants, bearing five fully developed leaves (ranks 1–5) and two emerging leaves (ranks 7 and 8), were treated with water (control) or FV. Eight days post-treatment, when leaves at ranks 7 and 8 had expanded enough, leaflets were inoculated with *P. infestans*. Some variability in infection severity was observed among control plants, likely reflecting differences between independent experimental repetitions and natural biological variation in young leaves; however, overall trends between treatments were consistent. In control plants, disease severity was lower on younger leaves (ranks 7 and 8) than on older ones (rank 5; Fig. 2**)**. Specifically, rank 5 leaves showed 72% infected area, while ranks 7 and 8 exhibited 50%. In FV-treated plants, disease severity on rank 5 leaves was reduced to 14%, representing a 5-fold reduction relative to controls. However, no protection was observed on the younger leaves treated with FV compared to the controls (ranks 7 and 8). These results indicate that FV effectively protected leaves for up to 8 days post-treatment, but only when FV was applied to fully developed rather than developing leaves.

3.3. Effect of FV on tomato defense gene expression

3.3.1. Elicitation test

The ability of FV to modulate tomato defense gene expression was

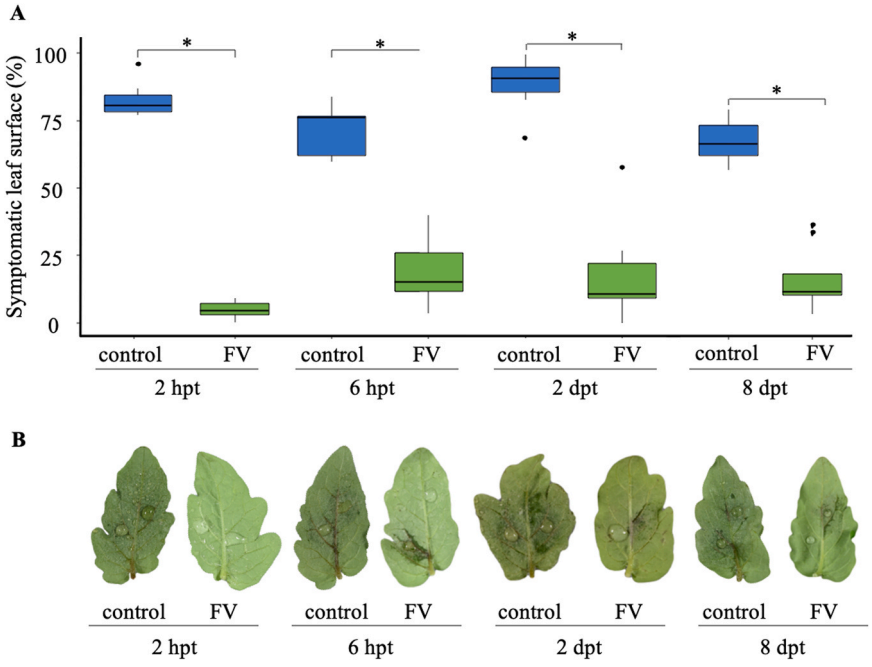

Fig. 1. Preventive effect of FV (formulated version) against *Phytophthora infestans* inoculated on detached leaflets, 2- and 6-hours post-treatment (hpt), 2- and 8-days post-treatment (dpt). **(A)** The boxplots show the percentage of infected leaf area per plant, including both sporulation and lesion zones of water-treated (control) and FV-treated plants. Percentage was obtained by relating the infected area to the total surface area of each leaflet. For each plant, the value corresponds to the average infected area measured on all inoculated leaflets. In the experiment at 2–6 hpt and 8 dpt, 3 leaflets per rank (leaf ranks 4 and 5), totaling 6 leaflets per plant, were analyzed with 9 plants per treatment ($n = 9$). In the experiment at 2 dpt, 3 leaflets per rank (leaf ranks 3 and 4), totaling 6 leaflets per plant, were analyzed with 7 plants per treatment ($n = 7$). Asterisks indicate significant differences in infected foliar surface between FV- and water-treated plants (Student's *t*-test, $*p < 0.0001$). Each experiment was repeated 2 times. **(B)** Representative pictures of control and infected leaflets at different time (2, 6 hpt and 2, 8 dpt) after 7 dpi.

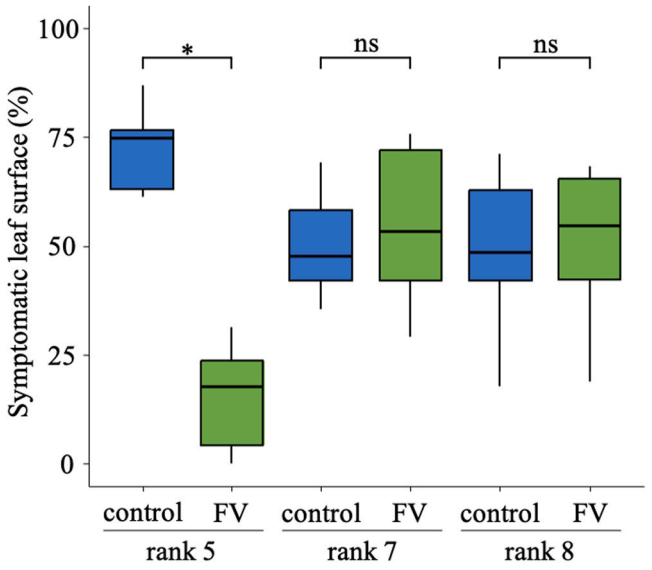

Fig. 2. Effect of FV (formulated version) on newly growing leaves against *Phytophthora infestans* 8 days post-treatment (dpt). The boxplots represent the percentage of infected leaf area per plant, including both sporulation and lesion zones of water-treated (control) and FV-treated plants. Percentage was obtained by relating the infected area to the total surface area of each leaflet. For each plant, the value corresponds to the average infected area measured on all inoculated leaflets. Rank 5 was fully developed when treated with FV, contrary to leaves from ranks 7 and 8, which were just emerging. Inoculation with *P. infestans* was done 8 days post-treatment to allow the development of leaves of ranks 7 and 8. Three leaflets per rank (leaf ranks 5, 7, and 8; totaling nine leaflets per plant) were analyzed with nine plants per treatment ($n = 9$). Experiment was repeated twice.

analyzed using a targeted biomolecular tool assessing 28 genes involved in various defense pathways (Brisset and Dugé de Bernonville, 2011). Gene expression was evaluated at 6, 24, and 48 hpt. To obtain an overview of gene expression patterns in relation to treatment and time, a **principal component analysis (PCA)** was performed, with gene expression as variables and samples as individuals. The first two principal components (Dim 1 and 2) explained **42.43% and 20.43% of total variability, respectively (**Fig. 3**A).** The score plots showed clear separation between control and FV treatments across time points, mainly along PC1, driven by genes such as ***ACCO, PR2, PR4, PR5*, and *GST*** (Fig. 3A-B). Furthermore, the treatment effect decreased over time, being more pronounced at **6 hpt** and less at **48 hpt**. PC2 primarily captured the effect of sampling time, influenced by ***PAL, CHS, FPPS*, and *PR15*** (Fig. 3A-B).

To visualize the differences in gene expression levels between the two treatments and across the three time points, a heatmap of gene expression was generated. Overall, the results showed that FV induced the expression of several genes compared to the control (water), with differences detected at one or more time points depending on the gene and classified according to their relative defense pathways **(**Fig. 3**C).** Among them, *PR* protein genes (*PR1, PR2*, *PR4*, *PR5*, and *PR15*) were the most strongly up-regulated following FV application. Genes related to oxidative stress (*POX* and *GST*) and hormone signaling pathways (*WRKY*, *LOX2*, and *ACCO*) were also up-regulated, associated with salicylic acid, jasmonic acid, and ethylene signaling. In contrast, genes related to cell wall modification were not up-regulated in response to FV treatment compared to control, nor were those involved in secondary metabolism pathways (phenylpropanoids and isoprenoids), except for *TPS*. Partial Least Squares Discriminant Analysis (PLS-DA) identified five genes, *PR2*, *TPS*, *PR5*, *ACCO* and POX, with a Variable Importance in Projection (VIP) score > 1, as the main discriminants distinguishing treatments and time points **(**Fig. 3**D).**

Compared to the control at each time point, expression levels of *PR2*, *PR5*, and *POX* were significantly higher in FV-treated plants **(**Fig. 4**).** *PR2*, *PR5*, and *POX* expression levels were up-regulated progressively over time, reaching approximately 2^4 times higher expression at 48 hpt for *PR2* and *PR5,* and 2^3 times for *POX* compared to the control at 6 hpt, although this difference was not statistically significant between FV treatment at different time points. *TPS* expression increased significantly at 24 hpt (2^4 times more expressed than control), whereas FV-treated plants showed a significant up-regulation of *ACCO* expression level earlier at 6 hpt, at a rate of 2^3 times more expressed compared to the control. Interestingly, some gene induction over time was also observed in water-treated controls.

An additional analysis of gene expression showing at least two-fold differential expression ($\log_2 > 1$ or < -1) relative to the control identified nine genes (*PR1, PR4, PR15, CHS, HMGR, GST, WRKY, LOX2,* and *JAR*; 5**)**. Compared to the control, FV treatment significantly increased *PR4, PR15, GST, WRKY,* and *LOX2* expression at 6 hpt, with an average of $2^{1,3}$ higher induction. Among them, *PR15* reached its maximum expression level at this early time point. In contrast, *CHS, HMGR*, and *JAR* were significantly down-regulated at 6 hpt by FV treatment compared to the control, with *CHS* expression level remaining repressed significantly at 24 hpt.

3.3.2. *Priming test*

The priming potential of FV was evaluated using the same biomolecular approach. Based on the protection and elicitation results, inoculation with *Phytophthora infestans* was performed at 6 hpt. Gene expression was then assessed at 6 hpt (before inoculation) and 48 h post-inoculation (hpi). PCA revealed that Dim 1 and Dim 2 explained 53.6% and 21.0% of total variability, respectively **(**Fig. 5**A).** The score plots indicated a clear treatment effect at 6 hpt, with initial separation between water- (ctrl_6) and FV-treated plants at 6 hpt (FV_6), and further separation between 6 hpt modalities (control and FV) and 48 hpi samples (control and FV), primarily driven by *PR5*, *PR2*, *PR4*, *POX*, and *ACCO* expression **(**Fig. 5**A-B).**

The heatmap revealed that FV induced up-regulation of several defense-related gene expressions at 6 hpt, consistent with the elicitation results **(**Fig. 3**C and 5C).** Most up-regulated genes encoded PR proteins, oxidative stress-related enzymes, or regulators of hormone signaling pathway **(**Fig. 5**C).** Many of these genes showed higher expression after inoculation (48 hpi) in both treatments. Partial Least Squares Discriminant Analysis (PLS-DA) identified eight discriminant genes between treatments and time points (*PR2, PR5, PPO, ACCO, PR1, CHS, PR4,* and *POX*) based on VIP > 1 **(**Fig. 5**D).**

At 6 hpt, FV treatment significantly induced *PR2* (2^6 times more expressed), *PR5* and *ACCO* (2^5 times more expressed) and to a lesser extent *PR1* and *POX* ($\sim 2^2$ times more expressed) relative to control **(**Fig. 6**).** While the expression levels increased after 48 h post-inoculation, no change > 1 $\log_2$ or $<$ -1 $\log_2$ were observed for these genes between the water- and FV-treated plants, both of which had been inoculated, except for *PR4*. Interestingly, *PR4* expression was the only gene showing significant regulation at both time points, being up-regulated by FV at 6 hpt and down-regulated at 48 hpi, compared to controls respectively **(**Fig. 6**).**

Genes showing also at least two-fold differential expression ($\log_2 > 1$ or < -1) compared to the control at 6 hpt included *PR8, PR15, GST, PECT, WRKY, LOX2,* and *JAR* **(**5**).** Expression of *PR8, PR15, GST,* and *WRKY* were significantly induced by FV at 6 hpt ($\sim 2^{1.5}$ times more expressed). In contrast, *JAR* and *PECT* were significantly repressed in FV-treated samples compared to the control at 6 hpt.

In parallel with gene expression analysis, protection was also verified by analyzing disease severity, including both foliar symptoms and sporangia quantification. Leaflets treated with FV 6 h prior to inoculation with *P. infestans*, exhibited significantly fewer disease symptoms with 30% infected area compared to 87% in the plants treated with water **(**Fig. 7**A).** Sporangia production was also reduced by 3.2-fold,

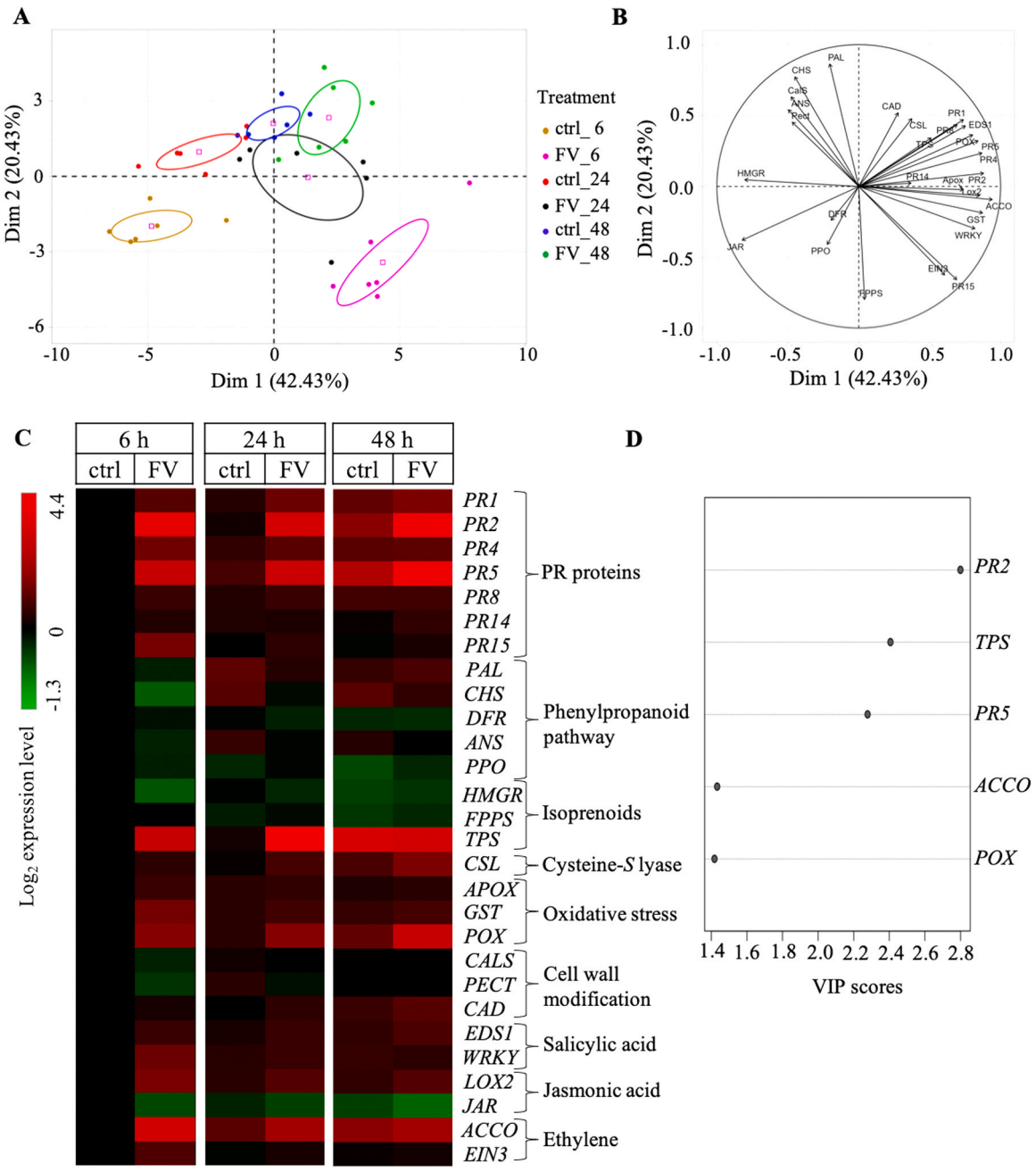

Fig. 3. Elicitation effect of the formulated biocontrol product on 28 tomato defense gene expression. Defense gene expression was obtained after treatment of the formulated version (FV) and water-treated plants (ctrl) using leaves (leaf ranks 3, 4, and 5) collected at three time points post-treatment (6, 24, and 48 hours). Data were obtained from two independent experiments, each including three biological replicates per treatment per time point ($n = 3$ per experiment), where each biological replicate consisted of a composite sample from three plants. Since data from both experiments were pooled, final sample size was $n = 6$ per treatment per time point. The mean value of water-treated leaves (ctrl) at 6 hpt was used for the calculation of the $\log_2$ ratio of each defense gene. Principal Component Analysis (PCA) was performed, with a score plot **(A)** and biplot **(B)** representing all treatments at the three time points. Each treatment is depicted in a specific color in the score plot. The percentage of variance explained by each principal component is indicated on the respective axes (PC1 and PC2). Arrows in the biplots indicate the effect and direction of each factor's contribution to treatment dispersion. **(C)** Heatmap profiling of the average expression levels of 28 defense-related genes in tomato across all experimental conditions; treatment (FV or ctrl) and time points (6, 24 and 48 hours). The $\log_2$ expression levels above or below 0 represent an induction (in red) or a repression (in green), respectively. **(D)** Analysis of discriminant genes across treatment and three time points using the Variable Importance in Projection (VIP) score plot, derived from Partial Least Squares-Discriminant Analysis (PLS-DA). Data represent genes with VIP score ≥ 1. *ACCO* = 1-aminocyclopropane-1-carboxylic acid oxidase; *ANS* = anthocyanidin synthase; *APOX* = ascorbate peroxidase; *CALS* = callose synthase; *CAD* = cinnamyl alcohol dehydrogenase; *CHS* = chalcone synthase; *CSL* = cysteine sulfoxide lyase; *DFR* = dihydroflavonol reductase; *EDS1* = enhanced disease susceptibility 1; *EIN3* = ethylene-insensitive 3; *FPPS* = Farnesyl pyrophosphate; *GST* = glutathione-*S*-transferase; *HMGR* = hydroxymethyl glutarate-CoA reductase; *JAR* = jasmonate resistant 1; *LOX* = 13-lipoxygenase 2; *PAL* = phenylalanine ammonia-lyase; *PECT* = pectin methyl esterase; *POX* = peroxidase; *PPO* = polyphenol oxidase; *PR1*, *PR2*, *PR4*, *PR5*, *PR8*, *PR10*, *PR14*, and *PR15* = pathogenesis-related protein; *TPS* = terpene synthase; and *WRKY* = WRKY DNA-binding protein 41.

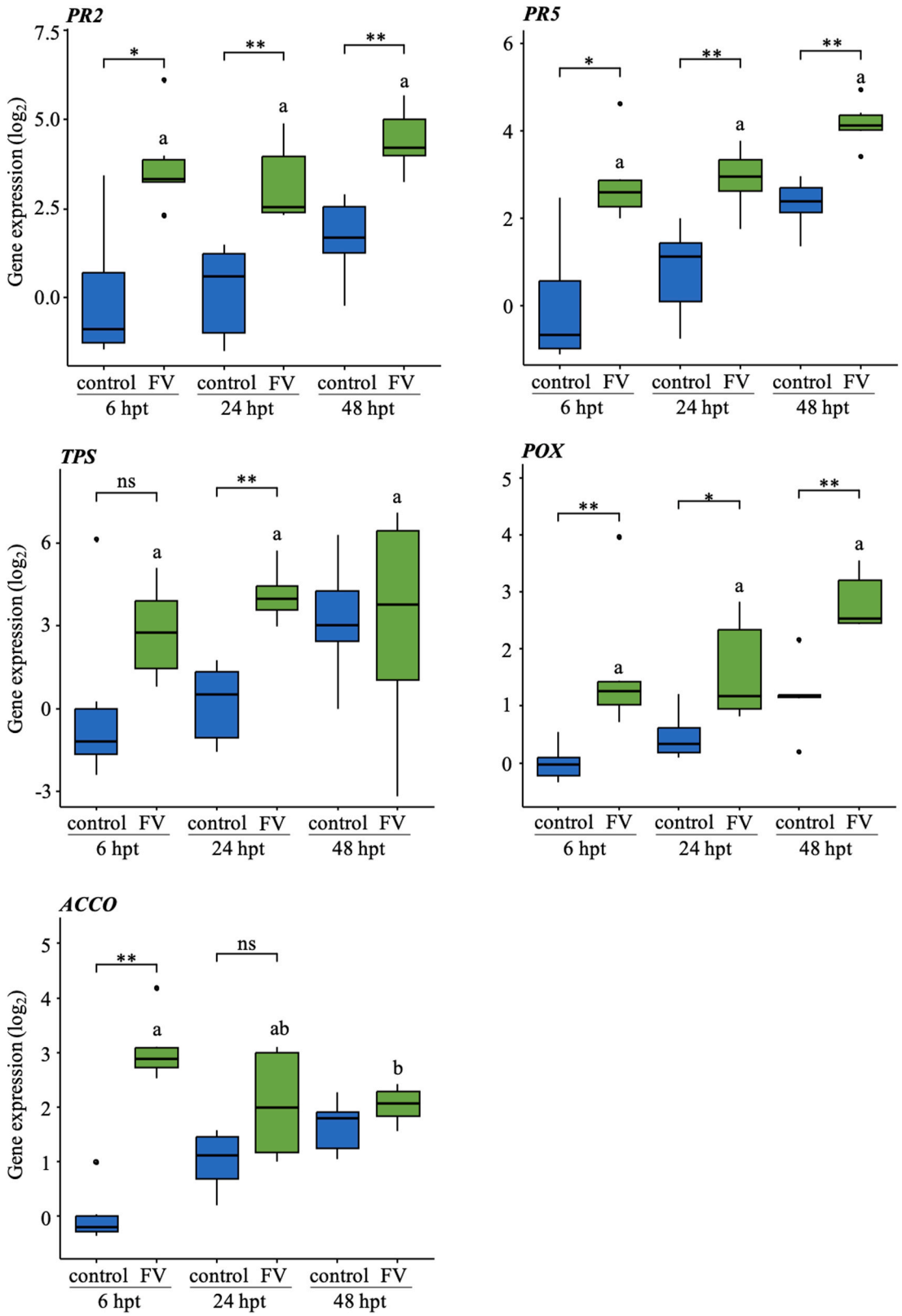

Fig. 4. Expression levels of the most discriminant genes (*PR2, PR5, TPS, POX,* and *ACCO*), selected based on the VIP score across treatments (FV and control) and time points (6, 24, and 48 hpt). Gene expression levels are represented as $\log_2$ ratios, calculated using the mean expression value of water-treated leaves at 6 hpt as the calibrator. Gene expression data were obtained by the expression of genes from leaves of rank 3, 4 and 5 treatments and by time from the 2 independent experiments, $n = 6$. To compare the expression levels of gene between FV and control treatment at each time point, statistical tests were conducted when gene expression levels showed an induction (> 1 $\log_2$) or a repression (< -1 $\log_2$) compared to the average control value at each time point. To compare the expression levels of FV between the 3 time points, statistical tests were conducted when gene expression levels showed an induction (> 1 $\log_2$) or a repression (< -1 $\log_2$) compared with at least one treatment. Stars indicate significant differences between FV and water treatments (Wilcoxon-Mann-Whitney test: * $p < 0.05$; ** $p < 0.01$).

Letters indicate significant differences between FV treatment and control across time points (Kruskal-Wallis test followed by Wilcoxon rank-sum test for multiple comparisons, $p < 0.05$).

averaging 2.2×10^4 sporangia.mL^{-1} in the FV-treated samples compared to 7.0×10^4 sporangia.mL^{-1} in controls **(**Fig. 7**B)**.

3.4. Wash-off effect on FV protection against late blight

To assess FV's persistence and evaluate the contribution of direct effects, a wash-off assay was performed after treatment with FV or water **(**Fig. 8**)**. Bordeaux mixture was used as a reference because it is known to be susceptible to leaching, with its persistence depending on the applied dose and the amount of water (Gessler et al., 2011; Pérez-Rodríguez et al., 2016). At 7 dpi, all control plants, whether washed or not, showed infection with 44% symptomatic leaflet. Washing had no impact on infection in the controls. FV-treated plants showed significantly reduced infection, with 6% symptomatic area in non-washed and 21% in washed plants. However, washing significantly reduced FV efficacy compared to non-washed FV-treated plants, resulting in a 3.5-fold increase in disease severity and a drop in protection from 86% to 52%. The Bordeaux mixture demonstrated high efficacy, with 2% and 0.75% symptomatic area in non-washed and washed plants, respectively. In this experiment, Bordeaux mixture was not significantly affected by wash-off and provided protection comparable to that of FV under non-washed conditions. In conclusion, although FV is partially sensitive to wash-off, it still maintains substantial protection relative to water-treated plants.

4. Discussion

4.1. Protector effect of the biocontrol product against late blight

Plant extracts represent a promising alternative for managing late blight, although their efficacy *in planta* can be variable (La Torre et al., 2019; Stephan et al., 2005). In this study, different approaches were applied to investigate the efficacy conditions and mode of action of a plant extract-based biocontrol product (FV). A significant protective effect of FV was observed following inoculation from 2 hpt up to 8 dpt, with high protection levels ranging from 75% to 94%. Generally, a 24–48 h interval between treatment and inoculation is used to assess the protective effect of biocontrol products, assuming that protection primarily results from induced plant resistance (Bengtsson et al., 2014; Dorn et al., 2007; Gauthier et al., 2014; La Torre et al., 2019; Stephan et al., 2005).

The strong protection observed at 2 hpt likely reflects a direct contact effect of FV, since treatment droplets were still present on leaf surfaces during inoculation. Similarly, protection observed at 6 hpt suggests a rapid mode of action, possibly through contact activity. Although no direct evidence was obtained for FV acting on sporangia inoculated *in planta*, except for reduced sporangial production, previous *in vitro* studies demonstrated that FV directly affects *P. infestans* infection structures (Penaud et al., 2025). Comparable findings have been reported for other biocontrol compounds, such as sugar beet extract and the cyclic lipopeptide massetolide A, which reduce both lesions development and sporangial production (Moushib et al., 2013; Tran et al., 2007). Rapid preventive protection, as early as 2 hpt, has also been observed with oxathiapiprolin (OXPT) on tomato and potassium phosphite on potato (Burra et al., 2014; Cohen et al., 2018). These products are known to have multiple modes of action, including a direct effect on Oomycetes (Machinandiarena et al., 2012; Smillie et al., 1989). OXPT targets the oxysterol-binding protein (OSBP) of Oomycetes, disrupting pathogen development, and exhibits translaminar and acropetally systemic movement, protecting both treated and newly emerging leaves (Cohen et al., 2018; Pasteris et al., 2016). Several studies suggest that phosphites inhibit phosphorylation in oomycete metabolism and key enzymes essential for their development. Indirectly, they induce defenses via the SA pathway and exhibit systemic activity (Burra et al., 2014; Havlin and Schlegel, 2021; Machinandiarena et al., 2012).

To assess whether FV could protect young, growing leaves, both fully developed and newly formed leaves at the time of treatment were inoculated 8 days post-treatment. Fully developed leaves were significantly protected by FV, whereas young leaves showed disease levels like the control. The protection of mature leaves indicates a long-term effect of FV, likely resulting mainly from its contact activity due to complete coverage of leaf surface, which might be possibly reinforced by localized and persistent defense induction in treated leaves. Comparable persistence (≥7 dpt) has been reported against *P. infestans* following foliar application of potassium phosphite and acibenzolar-*S*-methyl (ASM) on Micro-Tom tomato (Dallagnol et al., 2023), and even longer durations for β-aminobutyric acid (BABA, up to 12 days) and chitooligosaccharides associated with oligogalacturonides (COS-OGA) up to 21 days (Cohen et al., 1994; Cohen, 2002; van Aubel et al., 2018). The lack of protection on young growing leaves suggests limited systemic activity of FV, consistent with previous observations for many fungicides (Cohen et al., 2018). Indeed, studies with OXPT and partner fungicides showed that while treated mature leaves were well protected, newly emerged leaves often received little to no protection unless systemic compounds were applied, highlighting that limited systemic activity is a common trait among many protective treatments (Cohen et al., 2018). Our results are also comparable to copper-based treatments, which provide only contact protection and similarly fail to protect newly developing leaves (La Torre et al., 2018).

The 8-day interval between treatment and inoculation may have exceeded the potential duration of systemic protection, making it impossible to draw a definitive conclusion about FV's systemic activity. These results underscore the importance of adjusting treatment frequency to plant growth dynamics to ensure continuous protection. Further experiments assessing shorter intervals between application and inoculation or comparing treated and untreated leaf ranks, as done for potassium phosphite and ASM, would help clarify this point (Burra et al., 2014; Dallagnol et al., 2023). The inclusion of a known systemic inducer (positive controls) such as ASM or BABA would also strengthen the interpretation (Cohen and Gisi, 1994; Dallagnol et al., 2023).

4.2. Elicitor effect of the biocontrol product

Although FV's protective effect was confirmed, its underlying mechanisms required further investigation. Based on previous studies reporting the elicitor activity of plant extracts on plant defense gene expression, it was hypothesized that FV might also act as an elicitor (Medeiros et al., 2009; Goupil et al., 2012; Moushib et al., 2013; Malo et al., 2017). Using the qRT-PCR-based biomolecular tool developed by Brisset and Dugé de Bernonville (2011), expression of 28 tomato defense-related genes was analyzed.

As reported in several studies, defense gene induction by biocontrol products can vary over time (Bodin et al., 2020; Burra et al., 2014; Gaucher et al., 2022; Malo et al., 2017). In potato, Burra et al. (2014) showed that phosphite application triggered early transcriptomic responses from 3 hpt, peaking at 6 hpt. Accordingly, gene expression was assessed at 6, 24, and 48 hpt. FV acted as an elicitor at all time points, with the strongest response at 6 hpt. Among the up-regulated genes, *PR2*, *PR5*, and *POX* were the only ones significantly induced across all time points. *PR2* and *PR5* are markers of systemic acquired resistance (SAR) and are involved in the salicylic acid (SA) signaling pathway (van Loon and van Strien, 1999). *PR2* encodes a β-1,3-glucanase that hydrolyses β-1,3-glycosidic bonds in β-1,3-glucans, major constituents of *P. infestans* cell walls, releasing fragments that act as elicitors to trigger defense responses (Bartnicki-Garcia, 1968; Balasubramanian et al.,

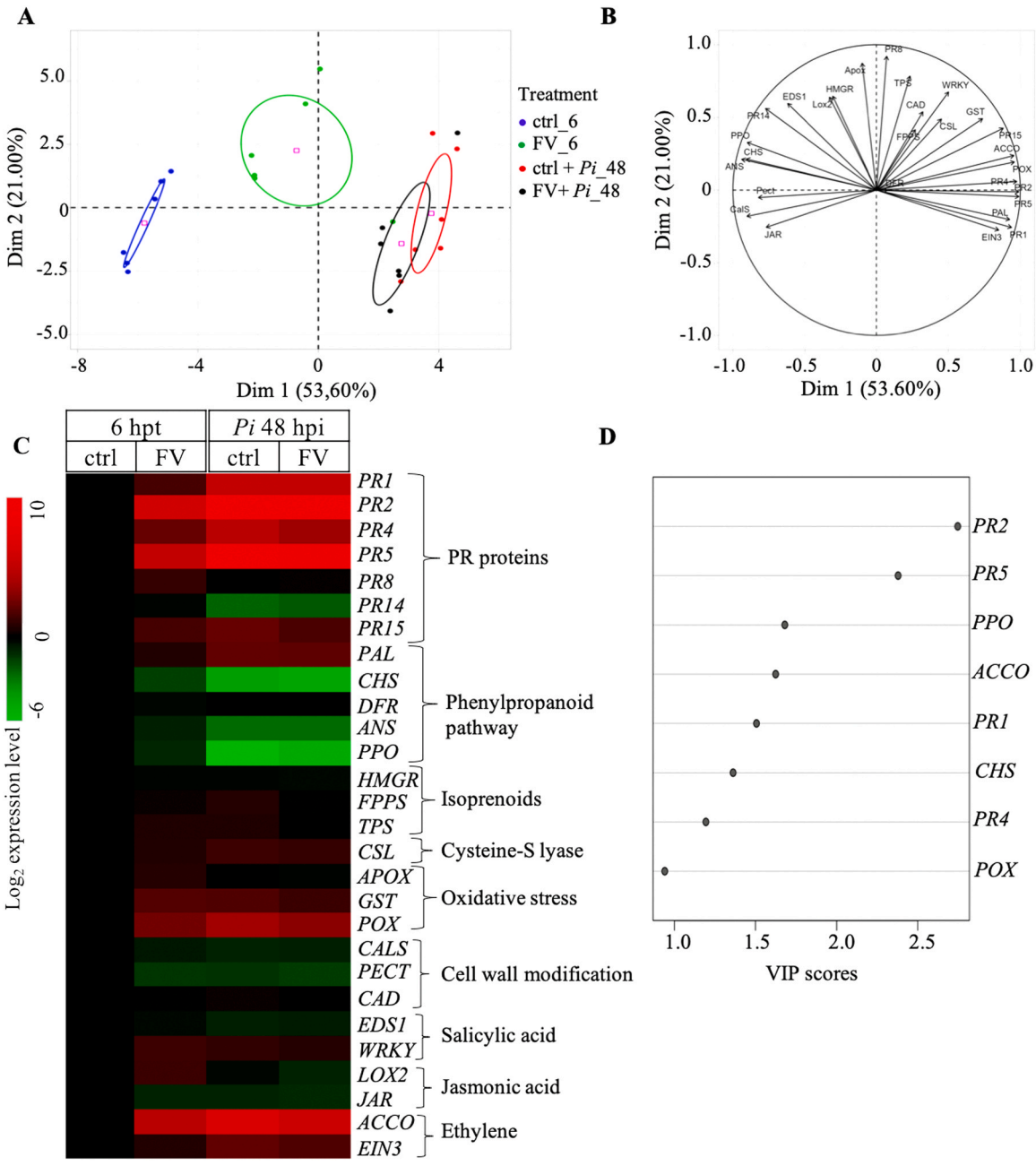

Fig. 5. Evaluation of the priming by formulated biocontrol product on 28 tomato defense gene expression. Defense gene expression profiles in treated and infected leaves (leaf ranks 3, 4, and 5) following application of the formulated version of the biocontrol product (FV) or water (ctrl). Treated leaves were collected 6 h post-treatment (ctrl/FV_6), while infected leaves, inoculated to *Phytophthora infestans* at this time point, were collected 48 h post-inoculation (*Pi*_48). Data were obtained from two independent experiments, each including three biological replicates per treatment per time point ($n = 3$ per experiment), where each biological replicate consisted of a composite sample from three plants. Since data from both experiments were pooled, the final sample size was $n = 6$ per treatment per time point. The mean value of water-treated leaves (ctrl) at 6 hpt was used for the calculation of the log2 ratio of each defense gene. Principal Component Analysis (PCA) was performed, with score plot **(A)** and biplot **(B)** representing all treatments at the two time points. Each treatment is depicted in a specific color in the score plot. The percentage of variance explained by each principal component is indicated on the respective axes (PC1 and PC2). Arrows in the biplots indicate the effect and direction of each factor's contribution to treatment dispersion. **(C)** Heatmap profiling of the average expression levels of 28 defense-related genes in tomato across all experimental conditions; treatment (FV or ctrl) and time points (6, and *Pi*_48). The $\log_2$ expression levels above or below 0 represent an induction (in red) or a repression (in green), respectively. **(D)** Analysis of discriminant genes across treatment and two time points using the Variable Importance in Projection (VIP) score plot, derived from Partial Least Squares-Discriminant Analysis (PLS-DA). Data represents genes with VIP score ≥ 1. *ACCO* = 1-aminocyclopropane-1-carboxylic acid oxidase; *ANS* = anthocyanidin synthase; *APOX* = ascorbate peroxidase; *CALS* = callose synthase; *CAD* = cinnamyl alcohol dehydrogenase; *CHS* = chalcone synthase; *CSL* = cysteine sulfoxide lyase; *DFR* = dihydroflavonol reductase; *EDS1* = enhanced disease susceptibility 1; *EIN3* = ethylene-insensitive 3; *FPPS* = Farnesyl pyrophosphate; *GST* = glutathione-*S*-transferase; *HMGR* = hydroxymethyl glutarate-CoA reductase; *JAR* = jasmonate resistant 1; *LOX* = 13-lipoxygenase 2; *PAL* = phenylalanine ammonia-lyase; ***PECT*** = pectin methyl esterase; *POX* = peroxidase; *PPO* = polyphenol oxidase; *PR1*, *PR2*, *PR4*, *PR5*, *PR8*, *PR10*, *PR14*, and *PR15* = pathogenesis-related protein; *TPS* = terpene synthase; and *WRKY* = WRKY DNA-binding protein 41.

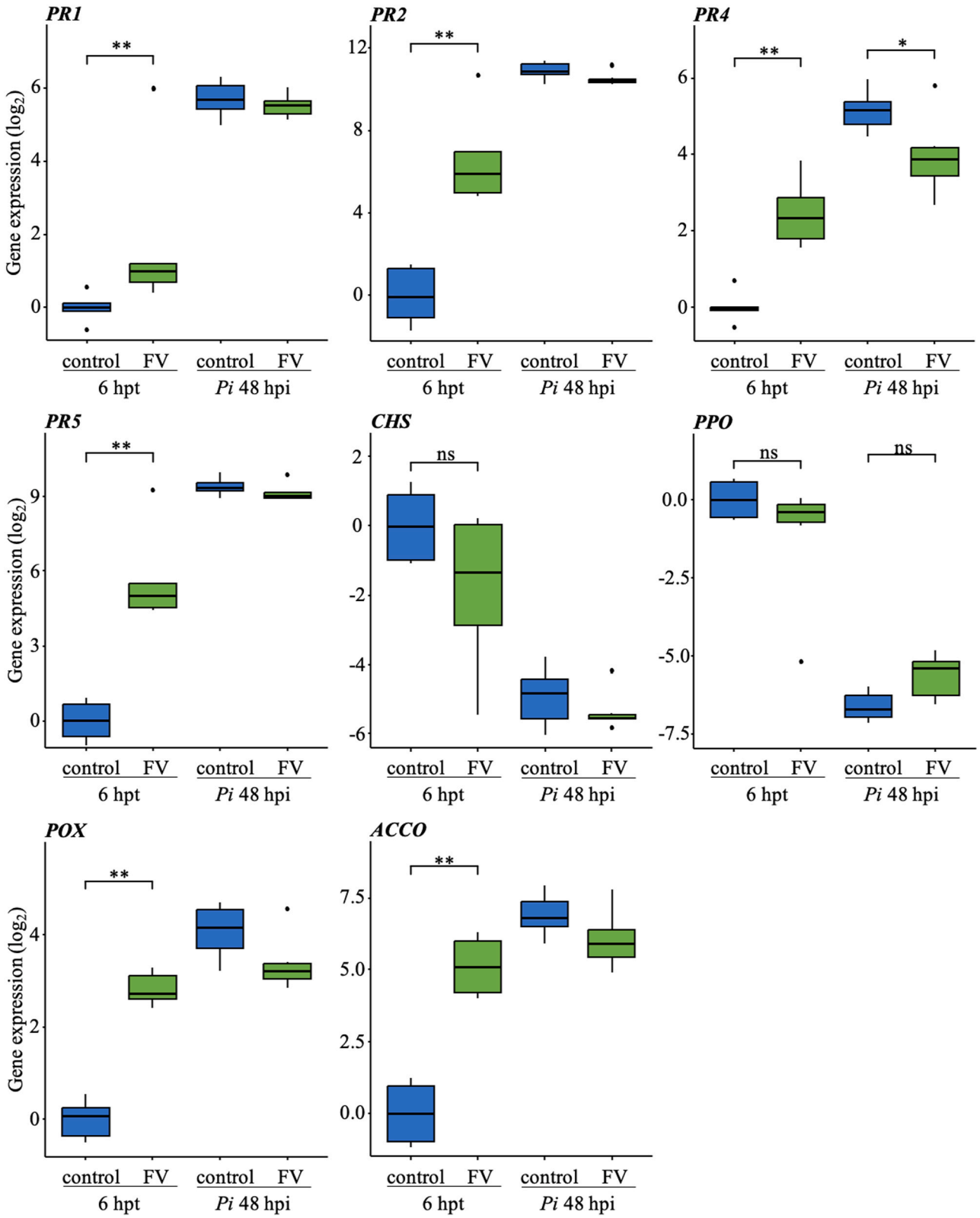

Fig. 6. Expression levels of the most discriminant genes (*PR1*, *PR2, PR4, PR5, CHS, PPO,* and *ACCO*), selected based on the VIP score across treatments (FV and control) and time points (6 hpt, and *Pi* 48 hpi). Gene *POX* was manually added based on its VIP score and expression profile. Gene expression levels are represented as $\log_2$ ratios, calculated using the mean expression value of water-treated leaves at 6 hpt as the calibrator. Gene expression data were obtained from leaves of ranks 3, 4 and 5 across 2 independent experiments, $n = 6$. To compare the expression levels of gene between FV and water treatment at each time point, statistical tests were conducted when gene expression levels showed an induction (> 1 $\log_2$) or a repression (< -1 $\log_2$) compared to the average control value at each time point. Stars indicate significant differences between FV and water treatments (Wilcoxon-Mann-Whitney test: * $p < 0.05$; ** $p < 0.01$).

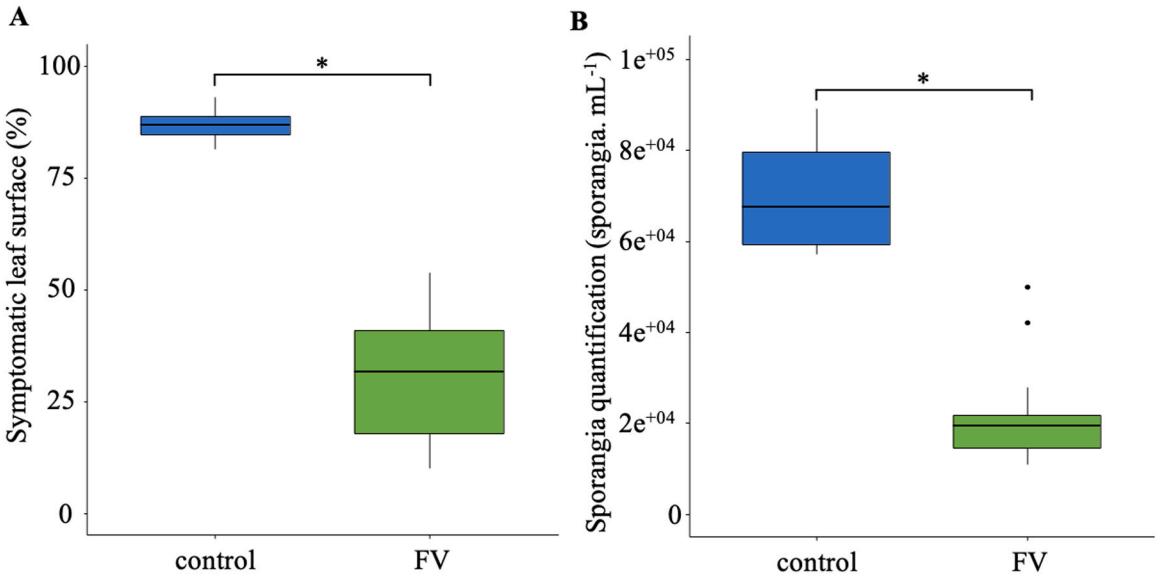

Fig. 7. Evaluation of the formulated version (FV) protection via the percentage of infected leaf area per plant (A) and the number of sporangia per milliliter of leaf extract per plant (B) following *P. infestans* inoculation. Plants were treated with FV or water (control) 6 h before detached leaflet inoculation. **(A)** The percentage of infected area was calculated by relating the infected surface to the total leaflet area 7 days post-inoculation (dpi). Data were collected from 14 plants (combined from two independent experiments, each with 7 plants, $n = 14$). For each plant, the value represents the average infected area across 12 inoculated leaflets, with 4 leaflets per rank (leaf ranks 3, 4, and 5). **(B)** Sporangia quantification was performed on the same leaflets used for symptoms analysis but assessed 3 days later (10 dpi). The number of sporangia per plant was determined from the average count of 12 inoculated leaflets, divided into two batches per plant. Asterisks indicate significant differences in infected foliar surface between FV and water treatment (*$p < 0.0001$ for both comparisons). Statistical analyses were performed using Student's *t*-test with Welch's correction for (A) and the Wilcoxon test for (B).

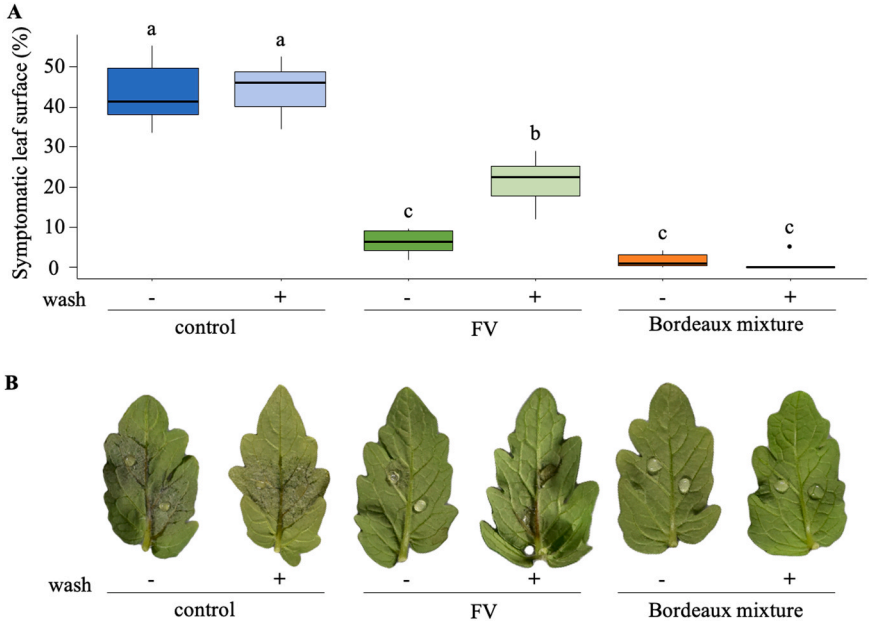

Fig. 8. Evaluation of the wash-off sensitivity of formulated (FV) version and the contact effect against *Phytophthora infestans*, inoculated on detached leaflets 1-day post-wash plants. **(A)** The boxplots show the percentage of infected leaflet area per plant, including both sporulation and lesion zones. This percentage is obtained by relating the infected area to the total surface area of each leaflet. For each plant, the value corresponds to the average infected area measured on all leaflets inoculated. Five leaflets per rank (rank 4 and 5), totaling 10 leaflets per plant, were analyzed with 7 plants per treatment ($n = 7$). This experiment was repeated twice. Letters indicate significant differences between treatments (Kruskal-Wallis test, and Wilcoxon rank-sum test for multiple comparisons, $p \leq 0.05$). - = no washed; + = washed. **(B)** Representative symptoms of *P. infestans* observed on washed and unwashed leaflets and treated with water (control), the biocontrol (FV at 0.1%) and Bordeaux mixture (500 $^{mg.L^{-1}}$) after 7 days post-inoculation. Bordeaux mixture was used as positive control.

2012; Gauthier et al., 2014; Leubner-Metzger and Meins, 1999). *PR5* encodes a thaumatin-like protein capable of disrupting pathogen membranes, while *POX* encodes a peroxidase associated with oxidative stress responses (Hiraga et al., 2001; van Loon et al., 2006).

Another gene of interest, *TPS*, involved in terpene biosynthesis, was significantly induced at 24 hpt, suggesting its contribution to defense metabolite production (Falara et al., 2011). *ACCO*, encoding a key enzyme in the final step of ethylene biosynthesis, was strongly induced

exclusively at 6 hpt, indicating a possible early role for ethylene in FV-induced defense (Houben and Van de Poel, 2019). Other genes, such as *PR4*, *PR15*, *GST*, *WRKY41*, and *LOX2*, although less induced by FV than the other genes, were significantly up-regulated only at 6 hpt. These genes encode proteins involved in several processes, including the chitin-binding domain (*PR4*), the oxidative burst (*PR15*), cellular detoxification (*GST*), JA biosynthesis (*LOX2*) and SA/JA signaling crosstalk (*WRKY41*) (Gullner et al., 2018; Higashi et al., 2008; van Loon et al., 2006; Montesinos et al., 2021; Turner et al., 2002).

The gradual increase in gene expression in control plants suggests an enhancement of basal defenses, possibly linked to plant age. The transient induction of *TPS* by FV and its later increase in control plants suggests that terpene biosynthesis is dynamically regulated. Certain terpenes act as volatile organic compounds (VOCs) which are known to act as signalling molecules between distant plants (Heil and Ton, 2008; Rosenkranz et al., 2021). Notably, β-caryophyllene, a sesquiterpene emitted by tomato leaves, can enhance salicylic acid levels in the root exudates of neighboring plants (Kong et al., 2021).

Based on these results, the elicitation signature of FV is a rapid activation of plant defenses as early as 6 hpt, with some transient responses. This early elicitation leads to a prolonged gene expression induction of SA-dependent defenses (*PR2*, *PR5*), as well as the induction of genes related to the production of reactive oxygen species (*PR15*, *POX*). More transiently, FV also appears to induce the JA-dependent defense pathway (*LOX2*) and the ethylene pathway (*ACCO*), with the latter remaining active for a longer duration. The elicitation signature observed likely depends on the product composition and the host genotype. Dugé de Bernonville et al. (2014) showed that expression patterns of defense genes in apple varied among 11 tested plant resistance inducers (PRI). For example, the elicitor effects of Fosetyl-aluminum (Aliette® Flash) and 1-naphthaleneacetamide (Amdthin® W) were weaker than those of acibenzolar-*S*-methyl (Bion® 50 WG) and laminarin (Iodus® 2 CS). None of the tested PRIs strongly induced *LOX* gene expression, suggesting a limited activation of the JA pathway. Conversely, surfactin triggered SA- and JA-related defense responses in wheat by increasing the expression of *PR5* and *LOX2* (Le Mire et al., 2018). Similarly to FV, λ-carrageenan induced early and transient *LOX2* activation and sustained *PR5* expression in wheat, reflecting an early JA- and prolonged salicylic SA-response (Le Mire et al., 2019). To further characterize FV, its elicitor activity should be evaluated across different tomato genotypes and plant species to determine whether its defense-inducing effects are species- or genotype-specific. Such investigation is crucial, as elicitor responses often vary with the intrinsic resistance level of the host (Saubeau et al., 2016). Comparative studies with other plant inducers (PRIs) provide a useful framework for interpretation. For instance, acibenzolar-*S*-methyl (ASM) has been shown to activate both SA- and JA-dependent pathways in wheat, with JA signaling predominating (Le Mire et al., 2019, 2018). In contrast, in apple and grapevine, ASM-induced defense was mainly associated with SA-related genes, notably *PR2* and *PR5* (Bodin et al., 2020; Dugé de Bernonville et al., 2014). More broadly, ASM is well recognized for its ability to activate SA-responsive genes, particularly the SAR markers such as *PR1*, *PR2*, and *PR5* (Brisset et al., 2000; van Loon and van Strien, 1999).

4.3. No evidence of a priming effect

To evaluate the potential priming effect of FV, plants were inoculated with *P. infestans* at 6 hpt, corresponding to the peak of elicitation, and gene expression was analyzed at 6 hpt and 2 dpi. At 6 hpt, FV induced a gene expression profile as observed in the elicitation experiment, though with generally higher expression levels. Some differences were noted: *PR8* (chitinase) was induced by FV, whereas *TPS* was not. After inoculation (48 hpi), however, no genes were more strongly expressed in FV-treated plants than in controls, suggesting that FV did not prime tomato defense gene expression at this stage, except for the down-regulation of *PR4*. Nevertheless, FV-treated plants still exhibited fewer symptoms and lower sporangial production, indicating protection.

Earlier sampling combined with microscopic observations could clarify whether priming occurs at an early infection stage. Indeed, several studies have shown that defense activation kinetics vary depending on the target gene and the inducing compound. For example, *PR3* induction by COS-OGA and *PR1* induction by phosphites peaked at 24 and 48 hpi, respectively, before declining to levels similar to those of the controls at 72 hpi (Machinandiarena et al., 2012; van Aubel et al., 2018). Although no priming was detected at 48 hpi in this study, several defense-related genes (*PR1*, *PR2*, *PR5*, *PR4*, *POX*, and *ACCO*) were up-regulated following inoculation (ctrl and FV at 48 hpi). However, these genes had already been activated by FV at 6 hpt, possibly contributing to reduced disease symptoms. However, it remains unclear whether these genes play a direct role in protecting FV-treated leaves against *P. infestans*.

It is well established that SA-related genes such as *PR1*, *PR2*, and *PR5* are pathogen-responsive and have sometimes been associated with resistance to *P. infestans* in tomato and potato (Enkerli et al., 1993; Faino et al., 2010; Halim et al., 2007). The expression levels of these genes have been linked to resistance, with stronger induction of *PR1* and *PR5* observed in resistant compared to susceptible potato cultivars (Vleeshouwers et al., 2000; Wang et al., 2008). However, this correlation is not universal. Studies using concentrated culture filtrates (CCF) of *P. infestans* have shown that SA pathway does not always correspond to resistance levels and may vary depending on the pathogen strain or plant genotype (Saubeau et al., 2016; Thomas et al., 2019). In tomato, *PR1* induction occurred at similar times in both compatible and incompatible interactions, suggesting that faster activation of this gene is not necessarily linked to resistance (Smart et al., 2003). These findings underscore the complexity of the SA signaling pathway in *P. infestans* resistance and highlight the need for functional studies using mutant lines to elucidate the specific role of SA-related genes in this interaction. Interestingly, *CHS* and *PPO* were repressed after inoculation, consistent with earlier findings showing *CHS* down-regulation during the biotrophic phase of infection (Zuluaga et al., 2016a). Regarding *PPO*, its silencing in potato lines has been associated with reduced growth of *P. infestans* in tubers (Llorente et al., 2014).

It should be noted that the absence of an observable priming effect may be related to experimental design. Plants were inoculated at 6 hpt, corresponding to the peak of FV-induced defense responses. Given the strong direct antimicrobial activity of FV observed in the wash-off experiment and in the previous study, pathogen survival and colonization may have been substantially reduced (Penaud et al., 2025). As a result, pathogen pressure may have been insufficient to trigger a measurable priming response at 48 hpi. In this context, a potential priming effect could have been masked by the predominant direct action of the product. In addition, the absence of mock-inoculated controls at 48 hpi represents a limitation of this study. Without FV + mock plants at this time point, it is not possible to fully discriminate between gene expression changes driven solely by infection and those potentially influenced by FV treatment. Consequently, although no enhanced response was observed in FV-treated plants after infection compared with infected controls, the possibility that infection-induced responses masked treatment-related differences cannot be formally excluded. This limitation will be addressed in future experiments aimed at better assessing priming effects, including the analysis of plants treated with the plant extract alone.

4.4. Part of the protection involves a direct effect of FV

The efficacy of biocontrol products is often inconsistent under field conditions due to environmental factors such as temperature, UV radiation, humidity, and rainfall (Benner, 1993; Dorn et al., 2007; Pressecq et al., 2025). Understanding both their direct and indirect mechanisms and the conditions that affect them is therefore essential. In the wash-off

experiment, Bordeaux mixture maintained its efficacy, confirming its persistence on the leaf surface and contact-based activity (Gessler et al., 2011). In contrast, FV efficacy decreased after washing, indicating susceptibility to leaching and suggesting that part of its protective effect depends on direct contact with *P. infestans*. This observation is consistent with a predominantly direct mode of action for FV, in agreement with previous results showing that the formulated product impacts infection structures and mycelial development of *P. infestans* (Penaud et al., 2025). The remaining protection observed may result from either defense induction or the presence of residual product on the leaf. Previous studies have shown that phosphites and COS-OGA maintain their protective effect after washing, suggesting that a direct surface contact with *P. infestans* is not required, which is consistent with the fact that phosphites are systemic and move internally in the plant (Borza et al., 2014; Burra et al., 2014; van Aubel et al., 2018). In our study, the contribution of defense induction to the remaining efficacy after washing should be interpreted cautiously, as defense gene activation may reflect a response to the plant extract treatment rather than a direct contribution to disease control. To confirm the indirect implication of elicitor-based action of FV, further studies using silenced plant lines for key defense genes would be informative. Additionally, improving FV's formulation, including adhesive agents, could improve rainfastness and ensure more consistent field performance.

5. Conclusion

This study demonstrated the *in planta* efficacy of a plant extract-based biocontrol product (FV) against tomato late blight, providing both rapid and sustained preventive protection following treatment. FV conferred significant protection as early as 2 h post-treatment (hpt) and maintained efficacy up to 8 days post-treatment (dpt). However, no protection was observed on newly growing leaves at 8 dpt, suggesting that protection was primarily due to the direct effect of FV, with enhanced defense gene expression prior to inoculation, rather than to a significant systemic activity. Indeed, the elicitor potential of FV was confirmed through the up-regulation of several defense-related gene expressions, particularly at 6 hpt, indicating activation of early plant defense responses. In contrast, no priming effect was detected at 2 days post-inoculation, implying that FV primarily acts as an elicitor rather than as a priming agent. Additionally, FV's efficacy decreased after washing leaf surfaces, supporting the hypothesis that a direct contact effect contributes significantly to its mode of action. Together, these results provide an improved understanding of the conditions and mechanisms underlying the efficacy of this biocontrol product. Future research should aim to further distinguish between the direct and indirect effects of FV using microscopic and cytochemical approaches, such as callose deposition and reactive oxygen species (ROS) detection at different infection stages. Additionally, evaluating FV on diverse tomato genotypes and potato plants will help identify potential host-specific responses. Finally, investigating possible systemic and curative effects will contribute to optimizing FV's integration into sustainable and environmentally friendly management strategies against *Phytophthora infestans*.

Research statement

This research was conducted as part of the BIOMES Chair (Biomechanisms for Soil Life and Plant Nutriprotection), a long-term collaboration between UniLaSalle Polytechnic Institute and Gaïago SAS. The objective of the "Biocontrol and Plant Protection" work package is to elucidate the mode of action of a plant extract-based biocontrol product against *Phytophthora infestans* on tomato. The project combines both applied and fundamental approaches, focusing on the conditions required for the product's efficacy and its role in activating plant defense responses. Doctoral students from the University of Rouen also contribute to this collaboration research initiative.

CRediT authorship contribution statement

Aude Bernardon-Mery: Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Abdelrahman Alahmad:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Data curation, Conceptualization. **Adrien Gauthier:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Karine Laval:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Valentin Penaud:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The author, Aude Bernardon-Mery is the CEO of the company Biom InnoV. The only financial interest to note is that the product studied is currently under research and development process by the company funding the project. In addition to financial support, the funder assisted in designing the study and provided technical advice regarding the product. They also contributed to revising the manuscript and to the decision to submit the results for publication. Apart from these specific roles, the funder had no additional involvement in the research process. The UniLaSalle research unit AGHYLE retains the right to terminate the collaboration contract immediately if the company's expectations and or demands conflict with ethical standards, research integrity, or scientific principles. Beyond these disclosures, the authors declare no other relevant financial or non-financial interests

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2026.106355.

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