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Systemic Acquired Resistance: Plant Priming for Ecological Management of Mealybug-Induced Wilt in MD2 and Queen Victoria Pineapples

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Abstract: Pineapples are highly susceptible to "Wilt disease", caused by the biotrophic insect *Dysmicoccus brevipes* that also transmits several Wilt-associated viruses (PMWaVs). Conventional farms manage mealybugs and Wilt disease using chemicals. However, many of these chemicals have been banned in Europe due to safety concerns, leading to a critical need for studies on pesticide-free control methods. During their evolution, plants have developed natural defences, such as systemic acquired resistance (SAR), against pathogens and pests. In this study, salicylic acid (10^{-3} M) was applied to MD2 and Queen Victoria pineapple plants as a foliar spray or soil drench, followed by mealybug infestation. This treatment enhanced defences, assessed through mealybug multiplication rates, and biochemical and molecular responses of tissue-cultured plantlets under controlled conditions. Phenylalanine ammonia-lyase activity (PAL) was measured as a potential SAR signalling enzymatic marker. Additionally, the expression levels of four genes were analyzed, which included *AcPAL* and *AcICS2*, both linked to salicylic acid synthesis; *AcMYB*-like, a transcription factor regulating salicylic acid biosynthesis; and *AcCAT*, which is involved in H_2O_2 level control in plants. SA elicitation reduced the mealybug multiplication rate by 70% on pineapples compared to untreated plants. In this study, the biochemical marker (PAL) and three molecular markers (*AcPAL*, *AcICS2*, and *AcCAT*) showed significant differences between primed and unprimed plants, indicating SAR induction and its role in the pineapple–mealybug interaction. In MD2 and Queen Victoria, PAL increased by 2.3 and 1.5, respectively, while *AcPAL* increased by 4 and more than 10. The other molecular markers, *AcICS2*, *AcCAT*, and *AcMYB*-like (a transcription factor), increased by 3, except for the last one in Queen Victoria. The reduction in mealybug populations with SAR is less effective than with pesticides, but it provides a valuable alternative on Réunion Island, where the only remaining insecticide will soon be banned. In addition, SAR priming offers a promising, eco-friendly strategy for managing mealybug populations and reducing Wilt disease in pesticide-free pineapple cropping systems.

Keywords: pineapple wilt; *Dysmicoccus* spp.; biocontrol; SAR; integrated management of mealybugs; ecological control

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1. Introduction

Pineapples are mainly grown under intensive monoculture worldwide. Pineapple plants are susceptible to 'Wilt', a destructive disease. Wilt is triggered by a parasitic complex

that includes the mealybug *Dysmicoccus brevipes* (Cockerell), a phloem feeder, and pineapple mealybug Wilt-associated viruses, such PMWaV1, V2, and V3 [1,2], as well as potentially other virus families. *D. brevipes*, a biotrophic agent, is the most widely found mealybug on pineapples on Réunion Island, where the development of Wilt symptoms requires, according to a recent survey [3], the simultaneous presence of mealybugs and PMWaV2. Wilt is commonly controlled using conventional contact or systemic insecticides targeting mealybugs, but under pressure from consumers, the use of these chemicals is now banned in European territories, requiring the development of new management methods without using pesticides. New management methods should efficiently protect the crops from mealybugs and Wilt disease while ensuring the sustainability of pineapple production, which represents the second-largest agricultural export of La Réunion Island. Moreover, systematic insecticide applications reduce biodiversity, particularly in fragile insular ecosystems. Finally, these systematic applications in conventional production systems contaminate the environment and water resources, posing a health risk to the general public.

For pineapple, one approach involves using cover crops, such as *Crotalaria* species, to mitigate the initial global parasitic stress caused by soil-borne pathogens (e.g., nematodes and symphylans) before planting, along with the use of disease-free planting material [4]. It has been suggested that reducing environmental stresses on plants may favour the induction of efficient systemic resistances [5]. Based on this hypothesis, we developed a cropping system aimed at reducing biotic stresses and enhancing the induction of pineapples' natural defences, known as systemic resistances [6,7].

Plants detect pathogens using membrane receptors that recognize specific pathogen-associated molecular patterns (PAMPs) [8,9]. These patterns are referred to as MAMPs (microbe-associated), HAMPs (herbivore-associated), or DAMPs (damage-associated) [10–12]. The detection of these signals activates pattern-triggered immunity (PTI) as a first line of defence [13]. In addition, plants have developed systemic signalling pathways that rely on hormones such as jasmonate (JA), ethylene (ET), and salicylic acid (SAL), along with specific transcription factors and other signalling molecules [8]. The jasmonate signalling pathway, associated with induced systemic resistance (ISR), responds to necrotrophic pathogens, non-pathogenic microorganisms (e.g., bacteria or mycorrhizal fungi), and abiotic stresses. In contrast, the salicylic acid signalling pathway, associated with systemic acquired resistance (SAR), primarily targets biotrophic pathogens and pests. Following a primary infection or external elicitation, these long-distance signalling pathways activate immune responses in uninfected plant tissues, a mechanism known as "priming" [14,15], which is described as an adaptive and low-cost defensive strategy. This is because, in primed plants, defence responses are either not activated or only mildly and temporarily triggered by the priming stimulus. Under biotic or abiotic stresses, the primed plants activate their defence responses more quickly, intensely, and persistently when they detect a subsequent challenging signal. This new challenge effectively initiates their full defence mechanisms, including the activation of defence-related genes and enzymatic activities linked to oxidative burst and other defences [16–19]. Plants further enhance their defences by secreting antimicrobial pathogenesis-related (PR) proteins, such as chitinases and β-1,3-glucosidases, during pathogen interactions [14,20,21]. Other potential molecular markers of SAR include transcription factors, such as MYBs, which regulate NPR1, a key element in balancing SAR and ISR pathways. This balance reflects the antagonistic crosstalk between these two signalling pathways [22–26]. SAR has been found effective against many plant pathogens, including viruses [27], bacteria [27], fungi [28], and other biotrophic pathogens and pests such as phloem-feeding aphids [29]. The MD2 pineapple variety, like the Queen Victoria variety, has already been shown to respond to ISR and SAR stimulation for the control of the nematode *R. reniformis* under controlled conditions and in experimental field plots [5,30].

D. brevipes, a phloem-feeding mealybug, is a biotrophic insect [31] that is hypothesized to be more susceptible to SAR than to ISR. Salicylic acid (SAL) and several of its derivatives are considered SAR elicitors on different plants [32], and methyl jasmonate (MeJA) was used as an ISR elicitor in different experiments on fruit crops such as pineapple [33], vegetables, and cut flowers [34].

In this study, we tested systemic acquired resistance (SAR) and induced systemic resistance (ISR) on two pineapple varieties exported to the fresh market: MD2, which is the main variety exported in the world, and the Queen variety, which is the only variety produced on La Réunion Island. We first compared the elicitation of defences against mealybugs in MD2 and Queen Victoria pineapple plants using salicylic acid (SAL) and methyl jasmonate (MeJA). Next, we hypothesized that SAR priming against pineapple mealybugs can be induced and characterized through its biological, biochemical, and molecular effects [35]—corresponding to reduced mealybug multiplication, enhanced enzymatic defences, and the up- or downregulation of defence-related genes. The biological effect was assessed by measuring mealybug multiplication. The biochemical effect was evaluated through phenylalanine ammonia-lyase (PAL) activity, a key enzyme in SA biosynthesis and the systemic acquired resistance (SAR) signalling pathway. PAL also controls the phenolic compound biosynthesis largely involved in plant defences. Finally, the molecular effect was investigated using four potential molecular markers: *AcPAL* and *AcICS2*, *AcMYB*-like, all involved in SAL biosynthesis and its control, and *AcCAT*, which plays a dual role in plant defence. Increased catalase (CAT) activity detoxifies H_2O_2 during oxidative bursts triggered by pathogen interactions, whereas reduced CAT activity increases H_2O_2 level, creating a hostile and toxic environment for pathogens. Moreover, H_2O_2 acts as a secondary messenger in SAR, crossing membranes and activating defence-related genes throughout the plant [13]. Finally, we suggest that SAR induction could be integrated into integrated pest management (IPM) strategies to control mealybugs and mitigate the impact of Wilt disease in pineapple production.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

MD2 pineapple tissue-cultured plants were grown in 250 mL pots with a commercial potting soil mix, at 28 ± 1 °C with a 12 h photoperiod under 60 W Tarentula® diodes, simulating a natural daylight spectrum. After a 6-month acclimation period, the tissue culture plants developed an aerial mass of approximately 56.5 ± 17.5 g and 40 ± 16.5 g for MD2 and Queen Victoria, respectively. The D leaf, used as a reference for growth and mineral analysis, grew at a 45° angle to the plant's vertical axis and had just completed its length growth. The D leaves group was also identified by the more or less parallel margins at the base of the leaves, compared to older leaves (group C) with a flared base, and younger leaves (group E) with a tapered base [36].

2.2. Mealybugs

Mealybugs (*D. brevipes*) were collected from contaminated tissue-cultured pineapple plants grown in a homemade rearing system. The controlled conditions for growing pineapple that were manually contaminated with a few wild mealybugs collected from the experimental fields were the same as those previously described in Section 2.1. To eliminate gravid adult females that could release larvae immediately after inoculation (as these larvae may have developed during the rearing period), the mealybugs were kept in a sampling vial with wet filter paper for 24 h. Only 2nd- to 3rd-instar mealybugs that were actively feeding on plants were selected under binocular magnifying glass for inoculation in the

experiments of this study. Unstimulated plants without mealybug inoculation were used as external contamination controls in each experiment.

2.3. Biological Effect: Comparing ISR and SAR Efficiency and Application Methods

Five treatments were tested, each with nine replicates (nine plants per treatment), including a control (water), two elicitors—methyl jasmonate (MeJA, 0.1 mM) or salicylic acid (SAL, 1 mM)—and two application methods (15 mL applied either on the soil surface around the base of the plants or to the plant core using calibrated micropipettes). Elicitor concentrations were determined in experimentations previously published [33]. Each plant received three applications of 15 mL of the elicitor solutions or water at 4-day intervals. Three days after the final elicitor application, four mealybugs were inoculated per plant. Mealybug populations were counted 45 days post-inoculation, representing one full multiplication cycle under the experimental conditions established in a preliminary experiment. The results were expressed as the reduction in multiplication by the treatment compared to the control. The total number of dead mealybugs was counted to assess the mortality of new larvae during the 45 days of this first experiment.

The first experiment was partially repeated (biological effect only) on both varieties one year later under similar experimental conditions, except that only salicylic acid was used as the defence elicitor. The salicylic acid solution (SAL, 1 mM) was divided between the plant (5 mL) and the soil (10 mL).

2.4. Biochemical Effect: Phenylalanine Ammonia-Lyase (PAL) Activity

Two treatments—SAL (1 mM) and water (control)—were applied three times to the soil surface at a rate of 15 mL per plant at 4-day intervals, with five replicates (plants) per treatment. Three days after the final application, MD2 and Queen Victoria plants were inoculated with 20 and 40 mealybugs, respectively. To assess the effect of SAL stimulation alone, additional plants received SAL but no mealybug inoculation. Twenty days post-inoculation, phenylalanine ammonia-lyase (PAL) activity was measured on a crude extract (see Section 2.6. for sample preparation) as a biochemical marker of SAR signalling pathway and phenolic compound biosynthesis.

2.5. Molecular Effect: Candidate Genes as Molecular Markers to Characterize SAR Priming

Among genes commonly used to characterize SAR, four genes have been selected to compare their expression in primed and unprimed plants after mealybug inoculation, under our experimental conditions: *AcPAL*, *AcMYB*-like, *AcICS2*, and *AcCAT* using *AcActin* from leaves as housekeeping genes (Table 1).

Table 1. Primers and accession numbers.

Genes	Accession n°	Front (F) and Reverse (R) Primers
AcPAL	Aco010091.1 (Phytozomev3)	F-AGGTGTTTGACGCCATTTG R-CACCGTTCCAGTCCTTCAA
AcMYB-like	Aco011681.1 (Phytozomev3)	F-GTTCAAGCAAGTCAAGAACC R-GAGTCCATTGATTCGCATTG
AcICS2	XM_020232036 (NCBI)	F-AGTGAATTTGCTGTCGGTAT R-GCAATCTTGTGAACTGGGA
AcCAT	XM_020259660.1 (NCBI)	F-CAGCTATTGTGGTGCCTGGA R- CTTCCAGAGAGAACGAGGG
Housekeeping Gene *AcActin* like-fe Housekeeping gene	XM_020238587.1 (NCBI)	F-CCTACGTTGCCCTCGACTAC R-GGAAGAGCACTTCAGGACACA

2.6. Protocols for Enzymatic and Gene Expression Measurements

Sample preparation: The samples were rapidly washed under a stream of distilled water, with the roots and the upper parts of the leaves removed. The remaining portions were then rinsed quickly twice—first with 70% ethanol and then with distilled water. For the analyses, the white part of the leaves and an equivalent-sized green portion contiguous to it were frozen in dry ice, ground into powder, freeze-dried, and finally stored in a freezer at −80 °C until protein or RNA extraction.

Protein extraction: Crude extract (ce) was obtained from 50 mg of freeze-dried powder in cold 0.1 M phosphate buffer (pH 6.8), containing 40 mM PMSF as protease inhibitor (Sigma, St. Louis, MO, USA), and 62.5 mg.mL^{-1} polyvinyl-polypyrrolidone under gentle stirring on ice for one hour. The 'ce' was then filtered first on Pall A/E glass-fibre filters (1 µ), then on Whatman mini filters (0.45 µm cellulose acetate, Restek France, Lisses, France) to obtain a clear filtrate.

Enzymatic measurement (biochemical effect): The following procedures for enzymatic measurements were adapted from the protocols developed by the authors cited for micro-analysis with reaction volumes of 300 µL, each repeated twice. Absorbance was measured on 96-well quartz plates with a Powerwave HT (Biotek, Winooski, VT, USA). *PAL*: Cinnamic acid produced from L phenylalanine with 25 µL of ce was measured at 290 nm [37]. The blank was modified using D Phenylalanine (ε = 9000 M^{-1} cm^{-1}), PAL expressed in nKat. 100 µL^{-1} 'ce'.

Expression of defence genes (molecular effect): RNA was extracted on 25 mg of lyophilized material using the RNeasy Plant mini kit (Qiagen, Hilden, Germany), and the cDNA was obtained using the Reverse Transcription kit (Qiagen). RTqPCR was performed with a Fast SYBR Green Master Kit (Thermofisher, Waltham, MA, USA) on Stepone Plus (Applied Biosystem, Waltham, MA, USA) with 20 µL reaction volumes, and RTqPCR was conducted according to the recommendations included in the Kit. *Actin* genes were used as reference genes. Primers were designed for *Ananas comosus*, and differences in gene expressions between stimulated and unstimulated plants were evaluated for *AcPAL*, *AcCAT*, *AcMYB*-like, and *AcICS2*. The optimal lag time after inoculation for evaluating these differences under our conditions was determined using the *AcPAL* gene in MD2. The RNAs were extracted 5 h, 24 h, 36 h, and 48 h after inoculation with mealybugs. The results are expressed as relative quantification by normalization against negative controls (Ctrl < 0) that were neither stimulated nor inoculated, using *AcActin* leaves as the housekeeping gene.

Data analyses: The biological effect data were analyzed using Kruskal and Wallis non-parametric tests because, despite the careful selection of the fragile instars, some had their feeding stylets damaged, and some were already gravid. Dunn's tests were used to compare each treatment against the control. Data on biochemical effects and data on molecular effects were analyzed by means of separations with standard deviations. All statistical analyses were performed using XLStat software (2023.1.1 (1396)).

3. Results

3.1. Biological Effect: Comparison of Stimulation with Salicylic Acid or Methyl Jasmonate

Only SAR stimulation significantly reduced mealybug multiplication rates during the 45-day experiment, which corresponds to one mealybug development cycle under these conditions (Figure 1). SAR stimulation was effective in both pineapple varieties, MD2 and Queen Victoria. Additionally, salicylic acid (1 mM) was equally effective when applied to the plant or the soil before inoculation.

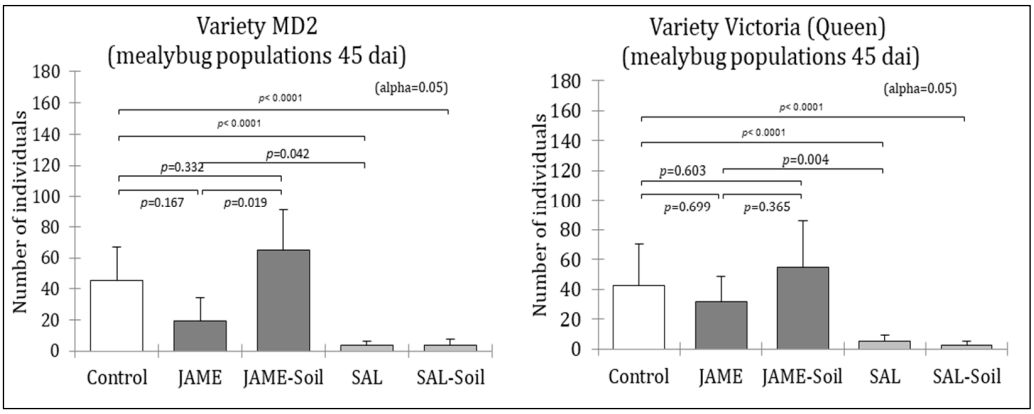

Figure 1. Comparison of the impacts of ISR and SAR on mealybug multiplication. ISR elicitor = methyl jasmonate (MeJA 0.1 mM), SAR elicitor = salicylic acid (SAL 1 mM), 15 mL of solution either on plant or on soil. Each plant was inoculated with four mealybugs. Dai = days after inoculation with the mealybugs.

In MD2, mealybug populations were significantly reduced in stimulated plants compared to positive controls, with reductions of −94% and −91% for plant and soil applications of SA, respectively. Similarly, in Queen Victoria, reductions of −88% and −93% were observed for plant and soil applications of SA, respectively. The differences between unstimulated controls and stimulated plants were highly significant ($p < 0.0001$) for both pineapple varieties, regardless of whether the stimulation was applied to the leaves or the soil.

The experiment, repeated one year later, showed a significant reduction in mealybug multiplication in both varieties (Figure 2), though the biological effect was slightly lower but still significant (*p 0.05 were 0.045 and 0.005 for MD2 and Queen Victoria, respectively*).

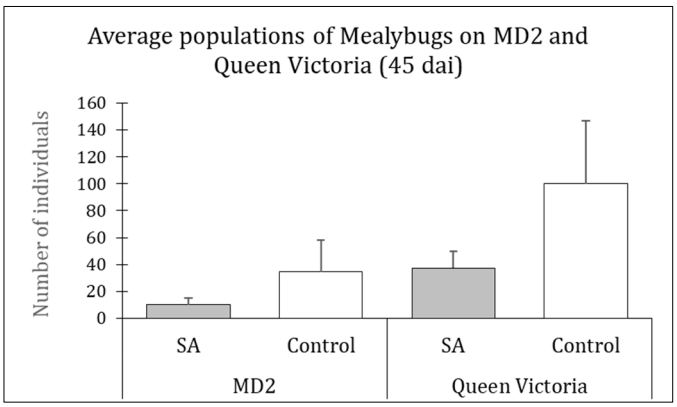

Figure 2. Impact of SAR on mealybug multiplication on MD2 and Queen Victoria varieties. SAR elicitor = salicylic acid (SAL, 1 mM). The elicitor solution was divided between the plant (5 mL) and the soil (10 mL). Four individual mealybugs were inoculated per plant; dai = days after inoculation with the mealybugs. (*p* 0.05 = 0.045 and 0.005 for MD2 and Queen Victoria, respectively).

The number of young mealybugs born during the experiment and that died during the 45-day experiment was significantly higher on stimulated Victoria (Queen) (+142.0%, *p* 0.05 = 0.004), and only tended to increase on stimulated MD2 (+48.6%, *p* 0.05 = 0.078), although not significantly.

3.2. Biochemical Effects: Enzymatic Markers of SAR Defence

As the results on biological effect showed that only salicylic acid was efficient as elicitor treatment to strongly reduce mealybug multiplication on both pineapple varieties, we evaluated PAL activity for the production of salicylic acid by the plants in the con-

text of strong stress produced by the inoculation of many mealybugs on plants (20 and 40 individual mealybugs on MD2 and Queen Victoria, respectively) (Figure 3).

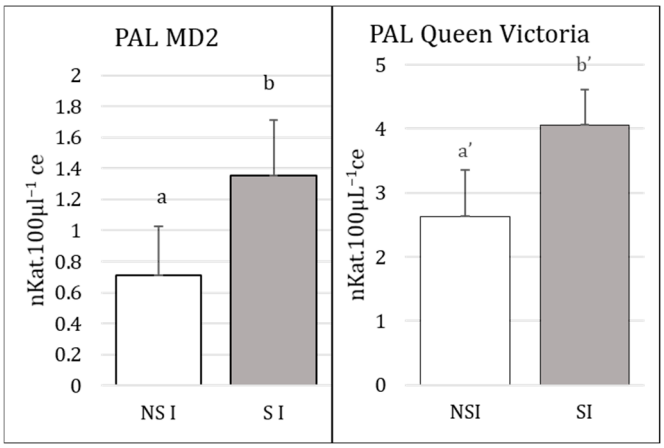

Figure 3. Quantification of PAL activity in unstimulated–inoculated (NSI = Ctrl > 0) and stimulated–inoculated (SI) plants of MD2 and Queen Victoria, inoculated with 20 and 40 mealybugs, respectively. Different letters (separate experiments were conducted for the statistical evaluation of the 2 varieties: MD2 (a, b) and Queen Victoria (a', b')) on the graphs indicate significantly different activity levels between NSI and SI. The SAR elicitor used was salicylic acid (1 mM), applied as 15 mL of solution. PAL activity was measured 20 days after inoculation (dai).

3.3. Molecular Effects

3.3.1. Timing Between Mealybug Inoculation and *AcPAL* Gene Expression in Stimulated vs. Unstimulated MD2 Plants

The optimal lag time for RNA extraction after mealybug inoculation was arbitrarily chosen to maximize *AcPAL* expression in stimulated plants while ensuring the maximum delay in response between stimulated and unstimulated plants (Ctrl > 0). To compare gene expression levels between stimulated and unstimulated plants, RNA extractions should be performed 24 h after mealybug inoculation, as this time point captures more accurately the plant responses showing maximum expression in stimulated plants as well as the delayed response in Ctrl > 0 (Figure 4).

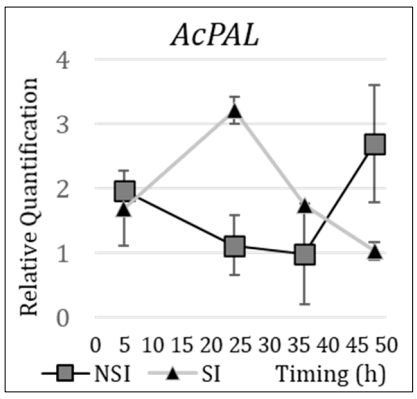

Figure 4. Relative quantification of *AcPAL* gene expression in unstimulated (NSI) and stimulated MD2 plants (SI) inoculated with 20 mealybugs. SAR elicitor = salicylic acid (1 mM), 15 mL of solution. RNA was extracted 5 h, 24 h, 36 h, and 48 h after inoculation.

3.3.2. Molecular Markers of SAR Defence in MD2 and Queen Victoria

In MD2, the expression levels of four molecular markers (*AcPAL*, *AcICS2*, *AcCAT*, and *AcMYB*-like) significantly increased after mealybug inoculation in stimulated plants

compared to unstimulated positive controls (Figure 5A—MD2). In Queen Victoria, three molecular markers (*AcPAL*, *AcICS*, and *AcCAT*) showed increased expression, while *AcMYBlike* did not, revealing significant differences in behaviour in gene expression between pineapple varieties (Figure 5B—QV). The largest increases in gene expression levels, 6-fold and 8-fold increases, were observed for PAL in MD2 and Queen Victoria, respectively. In this experiment, Queen Victoria showed lower gene expression levels than MD2, particularly for *AcICS2*, *AcCAT*, and *AcMYB*-like.

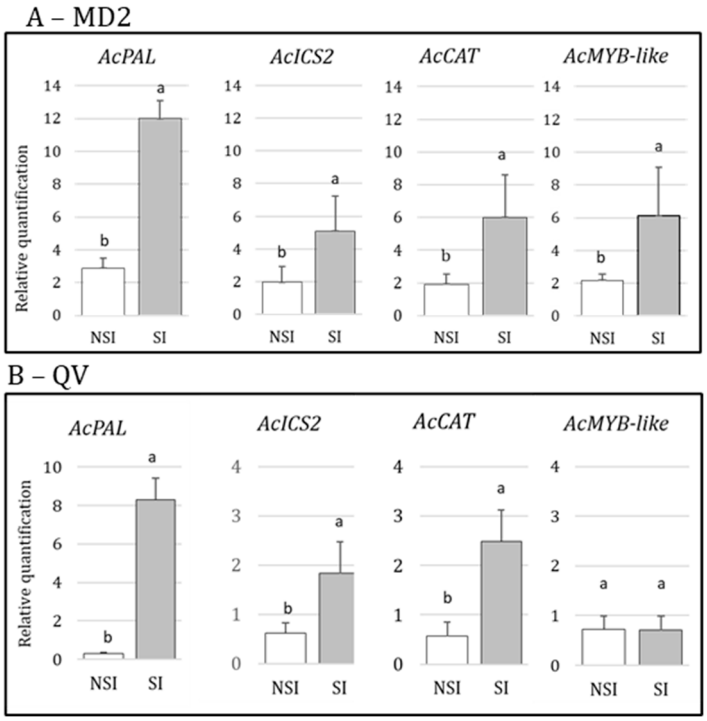

Figure 5. Relative quantification of 4 potential molecular markers of SAR, 1 h after biotic stress was applied on MD2 (**A**—MD2) and Queen Victoria (**B**—QV). Different letters (a and b) mean gene expression differed significantly between NSI and SI; the SAR elicitor was salicylic acid (SA, 1 mM), 15 mL of solution per plant. Reference gene was AcActin. Gene expressions were normalized against NS-NI (Ctrl < 0).

4. Discussion

Our findings on the biological, biochemical, and molecular effects support the hypothesis that salicylic acid (SA, 1 mM) induces systemic acquired resistance (SAR) against the mealybug associated with Wilt disease in both pineapple varieties, MD2 and Queen Victoria. However, our previous results revealed a strong differential response favouring the MD2 variety compared to Smooth Cayenne when SAR and ISR were induced with SA or MeJA, respectively, against the nematode *R. reniformis* [33]. In that publication, the authors highlighted the existence of varietal differences in the capacity to enter a primed state. Furthermore, we also established these differences in bananas by comparing their abilities to develop a primed state and systemic acquired resistance (SAR) against nematodes [38].

4.1. Biological Effect: Reducing Mealybug Multiplication

Unlike stimulation with methyl jasmonate (MeJA), treatment with salicylic acid (SA) proved more effective at inducing efficient defences against mealybug multiplication. In the first experiment, it reduced newly born larvae by −90% in both varieties. In the repeat experiment conducted a year later larval, this reduction was −70% for the MD2 variety and −65% for the Queen Victoria variety. Our results confirmed that the biotrophic insect *Dys-*

micoccus brevipes (Cockerell) is more susceptible to systemic acquired resistance (SAR) than to induced systemic resistance (ISR), similar to other biotrophic pathogens and pests [39]. SAR induced by SA elicitation in MD2 was previously found to be efficient against another pathogen, the nematode *R. reniformis* [33].

Salicylic acid (SA) applications on the soil surface to reach the roots and leaf applications were equally effective against mealybug multiplication, supporting the hypothesis that the SA-induced defences were systemic. In addition, the reduction in mealybug multiplication was likely not due to the direct toxicity of SA but instead suggests the activation of SAR defences against mealybugs. In fact, the non-toxicity of SA at low concentration (1 mM) for mealybugs was previously demonstrated through direct-contact experiments using sample tubes [6].

The number of young mealybugs that were born during the 45-day experiment and subsequently died was significantly higher on stimulated Queen Victoria plants (+142.0%, p 0.05 = 0.004) and tended to increase on stimulated MD2 plants (+48.6%, p 0.05 = 0.078), albeit not significantly and with a very low number of live mealybugs. This suggests a potential mechanism that inhibited the development of these young instars. Primed plants with SAR elicitors produced higher levels of toxic phenolic compounds and cystatin, which disrupted the digestive processes of the pathogens [40,41]. Cystatin, a natural inhibitor of the protease bromelain in pineapple [42], has been shown to alter nematode feeding and to reduce their populations by disrupting the activity of digestive enzymes in tomatoes [43]. In a former experiment, the gene *AcCystatin* showed an increased expression in MD2 pineapple fruit under abiotic stress (low temperature), as in other crops tolerant to cold or to drought stress [44]. Genetically engineered pineapples with enhanced cystatin production were studied for their potential in nematode control by introducing cystatin genes from a wild rice variety, showing promising initial results. However, at the field level, the results were insufficient to achieve the efficient control of nematodes [45]. It is hypothesized that cystatins block the digestive proteases of nematodes in tomatoes and pineapples. A similar mechanism could also affect mealybugs after SAR induction in pineapples, particularly young instars that are highly active feeders. Additionally, Queen Victoria and MD2 also naturally produce a high level of phenolic compounds such as p-coumaroyl-isocitric acid and several hydroxybenzoic acids, among other phenolic compounds that play a role in fungal disease resistance in pineapple [46].

Our results on the biological effects support the hypothesis that SAR priming is effective in reducing mealybug populations. To further validate this, enzymatic and molecular effects were assessed by measuring markers of SAR defences.

4.2. Biochemical and Molecular Effects of Salicylic Acid (SA) Treatment

The expression of the *AcPAL* gene, as well as PAL enzymatic activity, two markers of the SA signalling pathway, increased significantly in SA-treated plants compared to untreated plants in response to the stress induced by mealybug inoculation. This increase likely led to the biosynthesis of endogenous SA, initially for the SAR signalling pathway, and later for the additional synthesis of toxic compounds. The increase in PAL activity and SA biosynthesis represents one of the early biochemical events in the production of phenolic compounds, including toxic compounds required for not only defence but also structural components like lignin and callose, which reinforce cell walls as part of the SAR defence response. The *AcICS2* gene, although contributing to SA synthesis to a lesser extent, showed a significant increase in expression in both varieties, suggesting its potential as a reliable marker for SAR. The transcription factor *AcMYB*-like, involved in the regulation of SA synthesis, showed an increased expression in the MD2 variety but not in Queen Victoria, being less reliable as a general pineapple molecular marker in this case.

It is well known that ROS ignite redox signalling, but when in excess, they cause oxidative stress, leading to the damage of cellular components [47]. These authors also emphasized the redox regulation of several SAR signalling components. A burst of reactive oxygen species (ROS) at the infection site is one of the earliest cellular responses following pathogen infection. *AcCAT* expression showed an interesting increase between SA-treated plants and untreated plants in both varieties, suggesting it is also a potentially reliable marker of SAR. As mentioned earlier, CAT has a dual role in this context of defence. When CAT activity is reduced, H_2O_2 toxicity creates a hostile environment for pathogens. H_2O_2 acts also as a secondary messenger in SAR by crossing membranes and activating defence genes throughout the plant [13,47]. On the contrary, an increased CAT activity contributes to the detoxification of the plant cell environment due to the stress oxidative burst and production of ROS.

Our results on enzymatic and molecular effects confirm SAR induction by salicylic acid treatment and the establishment of SAR defences.

4.3. Integrating SAR Priming in an Ecologically Integrated Pest Management Strategy

The strategy developed to include the priming into an ecologically integrated pest management approach has been tested successfully on pineapple nematodes, *Rotylenchulus reniformis* [30], and will be applied for SAR priming against Mealybugs. The strategy involves reducing parasitism with cover crops such as Crotalaria spp. in rotation with pineapples and using disease-free planting material, particularly free of mealybugs. This approach aims to minimize the initial inoculum, before elicitation on young plants, and to rely on plant defences to maintain an acceptable level of parasitism for the sustainable production of fruits.

5. Conclusions

This study demonstrated that the treatment with 1 mM salicylic acid effectively induced systemic acquired resistance (SAR) against the mealybug *Dysmicoccus brevipes* in two pineapple varieties, MD2 and Queen Victoria, under controlled conditions. SAR induction was characterized by three key effects: (1) a biological effect significantly reducing mealybug multiplication, (2) a biochemical effect marked by increased PAL activity linked to salicylic acid biosynthesis and phenolic compound pathways, and (3) a molecular effect involving the upregulation of genes associated with SAR signalling and oxidative burst. This protocol offers a potential framework for identifying pineapple varieties with inducible natural defences. Developing new SAR- or ISR-inducing elicitors against pineapple pathogens may contribute to the development of more ecologically integrated pest management strategies for controlling Wilt disease as well as other pathogens and pests in pineapple production.

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