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Ecologically Based Management of Pineapple Mealybug Wilt: Controlling *Dysmicoccus brevipes* Mealybug Populations with Salicylic Acid Analogs and Plant Extracts

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Abstract: Mealybug wilt of pineapple (MWP) is a destructive disease worldwide caused by a parasitic complex that includes Pineapple Mealybug Wilt-associated Viruses (PMWaVs) and mealybugs (*Dysmicoccus brevipes*), which concurrently act as vectors for these viruses. Reducing the mealybug population is key to managing MWP, which is achieved in intensive production systems through the use of insecticides. SA (salicylic acid), ASM (acibenzolar-S-methyl), BABA (β-aminobutyric acid), and MeSA (methyl salicylate) are key components of systemic acquired resistance (SAR), the defense mechanism of plants against biotrophic agents such as mealybugs. In this study, these compounds were applied either as pure chemicals and/or as a major constituent of plant extracts. Both the Hawaiian hybrid MD-2 and Queen Victoria tissue culture plants, as well as suckers used for vegetative propagation, were treated with these compounds by direct application on the soil of pineapple pots. Subsequently, five mealybugs were released on each plant or each daughter plant in case of a transgenerational experiment; then, after 45 days, the number of mealybugs was counted. Exogenous SA, ASM, and MeSA reduced the population of mealybugs by a minimum of 50% and up to 80%. These SAR-inducing treatments could be an interesting alternative for controlling mealybugs and are already used in other pathosystems. The SAR mechanisms behind this effect are yet to be confirmed by molecular and enzymatic markers. ASM and MeSA are promising treatments for pineapples using tissue culture plants or traditional shoots.

Keywords: *Ananas comosus*; pineapple; wilt; mealybugs; plant defense; induced defense; SAR; transgenerational; virus disease

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1. Introduction

Mealybug wilt of pineapple (MWP) is a destructive disease worldwide affecting the pineapple industry [1–6]. *Ananas comosus* (L.) Merr is an herbaceous monocot within the bromeliad family. The spread of MWP disease is enhanced by contaminated cuttings, ants, and wind dispersion of the mealybug crawlers. Typical MWP symptoms are characterized by downward curling along the leaf margins and wilting of symptomatic leaves [7]. MWP is caused by an *Ampelovirus* complex from the *Closteroviridae* family including pineapple mealybug wilt-associated virus-1, virus-2, and virus-3 (PMWaV1, V2, and V3) [1,7]. This complex was recently reported on Queen Victoria pineapple in Réunion Island and, more widely, in the Indian Ocean [8,9]. Sether [7] highlighted the direct link between high incidence of MWP and high populations of mealybugs. To infect the plant and facilitate the spread of MWP, a group of virus-spreading organisms, including the mealybugs (vector) and ants (disseminator), is essential. In addition, the concomitant presence of mealybugs and viruses is required to express wilt symptoms in pineapple [1,10]. In

intensive pineapple production systems, the management of mealybugs is performed using insecticide applications [11,12], but more ecologically sound alternatives are needed.

Plants have developed a two-layered innate immune system to detect biotic attacks by specific receptors and to respond efficiently [13–16]. SA-mediated immunity turns this innate immune system into a whole-plant systemic immune system [13,16–19]. Salicylic acid (SA) induces systemic acquired resistance (SAR) and primes distant organs [17]. Priming enables plants to respond quicker and stronger to subsequent attacks at low energy costs. This defense can be induced by chemical compounds such as β-aminobutyric acid (BABA), SA, acibenzolar-S-methyl (ASM), or methyl salicylate (MeSA) [20–24]. SAR is a specific defense against biotrophic agents [25]. Mealybugs, *Dysmicoccus brevipes,* are biotrophic agents associated with the activation of SA-mediated immunity and SAR. The efficiency of SAR on pineapple has been demonstrated to control pathogens such as *Fusarium* sp., *Rotylenchulus reniformis,* and *Phytophthora* sp. [25–29]. In this article, we assessed the effectiveness of controlling mealybug multiplication through the application of SA or SA analogs, including ASM, BABA and MeSA, either pure chemicals or major constituents of plant extracts. These alternatives were investigated as potential substitutes for insecticides on tissue culture pineapple plants (Queen Victoria and MD2 hybrid) in controlled conditions in a culture chamber and a greenhouse.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Pineapple plants were tissue culture plantlets of MD2 and Queen Victoria (Vitropic), or plantlets of Queen Victoria produced by standard vegetative propagation (leaf budding or removal of terminal buds from stems using in-house techniques). They were grown in 0.5-L pots with a mixture of compost, peat soil, and perlite (50/40/10) in a culture chamber and a greenhouse. The temperature in the culture chamber was 28 ± 1 °C with a 12 h photoperiod under the LED light of 60 W Tarentula diodes, providing a light spectrum conducive to vegetative growth. In the greenhouse, plants were grown under average conditions of 20 °C night and 29 °C during the day and with a 12.25 h photoperiod (records from local weather station). The plantlets were acclimated for 1 month in both the culture chamber and greenhouse before the onset of the experimental treatments. They were grown with daily automatic irrigation, except during treatment periods. To assess plant vigor, chlorophyll content was measured with a SPAD-502 chlorophyll meter (MINOLTA), and the length of the longest leaf of the plant recorded on each plant.

2.2. Mealybugs

Virus-free pineapple mealybugs (*Dysmicoccus brevipes*) were reared according to the method described by Pandey and Johnson [30]. Butternut squashes were rinsed with antifungal (nipagin and benzoate: 2 g/L each) and placed on vermiculite in insect boxes with insect vent grids. Mealybugs were released inside this rearing system and grown at 25 ± 3 °C in the dark. The absence of PMWaV1, V2, and V3 was controlled on subsamples of mealybug populations by multiplex real-time PCR by ANSES (Agence Nationale de Sécurité Sanitaire) [8]. The presence of both viruses and mealybugs on the plants is necessary to observe the symptoms of PMW; however, viruses may interfere with the control of mealybugs using plant defenses. Instars exhibiting wax synthesis, specifically stage 2 and stage 3, but not yet at the adult stage were used for all the experiments [31]. The instars were carefully picked up with soft brushes to preserve the integrity of their three stylets, and five individuals were placed on each experimental plant in each experiment. These mealybugs exhibit a life cycle lasting approximately 45 to 60 days under our experimental conditions, as confirmed by a preliminary experiment. The applied mealybugs developed into adults and reproduced, but after 45 days, no adults apart from those originating from the initial five mealybugs that were released could be detected. This observation suggests that only one generation of new mealybugs was recorded during each experimental period. All stages of mealybug present on the plants were counted.

2.3. Treatments

Solutions for treatments were prepared as follows: each chemical was dissolved in ethanol (2% of the final volume), then diluted with water (98% of the final volume). The resulting solutions applied on plants had final concentrations of 1 mM. Control solution contained ethanol (2%) and water (98%).

Treatments with plant extracts (herbal maceration and essential oil) and chemicals used for biological assay are presented in Table 1. Herbal maceration was adapted from phenolic compound extraction methods [32]. Bion, 50 WG (Syngenta Crop Protection), is composed of 50% ASM. Essential oil of *Gaultheria fragrantissima* (fragrant wintergreen) sourced from Pure Essential Bio is mainly composed of methyl salicylate (>98% of MeSA) [33]. In the article, we coded the treatments by their active ingredients, except herbal macerations, which are not known (Table 1). Both herbal macerations contained SA, which was quantified by HPLC-MS at 100 mg/kg of dry matter for *Hypericum lanceolatum* and at 10 mg/kg of dry matter for *Flacourtia indica*. The concentrations were determined considering these quantities and the dry matter saturation of preparations (Table 1).

Table 1. List of all treatments for biological assays in controlled and greenhouse conditions.

Treatment	Concentration	Source
SA (salicylic acid)	1 mM	Sigma-Aldrich (St. Louis, MO, USA)
ASM (acibenzolar-S-methyl)	1 mM	Bion 50WG Syngenta (Basel, Switzerland)
BABA (β-aminobutyric acid)	1 mM	Sigma-Aldrich (St. Louis, MO, USA)
MeSA (essential oil of *Gaultheria fragrantissima*)	1 mM	Pure Essential Bio (Washington, DC, USA)
Hypericum lanceolatum (herbal maceration)	20 g/L	Tisane Bourbon (Le Tampon, France)
Flacourtia indica (herbal maceration)	150 g/L	Collected in Réunion

2.3.1. In Vitro Toxicity of Compounds against Mealybugs

In a preliminary experiment, all treatments were subjected to a toxicity test on mealybugs. Ten mealybugs (instar stage 3) were introduced in closed 15-mL sample jars, with filter paper soaked in each treatment, then the same mealybugs were also sprayed by the solutions applied to plants. The experiment had three replicates, and the numbers of mealybugs alive were counted at day 1 and day 8 after application.

2.3.2. Biological Assay in Culture Chambers

Biological assays were conducted over two years (2019 and 2022) using tissue culture plants of the MD2 hybrid and Queen Victoria variety. These assays took place in culture chambers, where treatments consisted of applying either SA (salicylic acid) or water only. In 2019, there were 7 (Queen Victoria) and 9 (MD2) replicate plantlets per treatment. In 2022, experimental units of five plantlets per treatment were replicated four times (a total of 20 plantlets per treatment). Treatments, including the control, were directly applied on the soil at a rate of 15 mL for each 0.5-L pot. All treatment applications were done on the soil and were repeated three times at 3-day intervals.

Three days after treatments, five mealybug instars were released on the leaves of each plant. Populations of mealybugs were counted 45 days after release (dar). Plants were stripped of leaves to count each mealybug, including crawlers. Control plants without mealybugs were used to assess if mealybug contaminations occurred inside culture chambers. Mechanical barriers against aerial insects using plastic sheeting and against ants using glue were added to reduce the risk of contamination.

2.3.3. Biological Assay in the Greenhouse

The second assay was conducted with suckers of Queen Victoria in the greenhouse (2022). Plantlets were distributed in sets of five plants in three replicates for each treatment in the greenhouse (a total of 15 plants per treatment). The treatments (Table 1) were prepared and applied as described before. Then, five mealybug instars were released and the resulting populations were evaluated using the same methodology as before. Contamination protections were the same as in the previous assays.

2.3.4. Biological Assays on Daughter Plants from Treated Parental Plants in the Greenhouse

In the third biological assay, which was conducted in the greenhouse, parental plants of Queen Victoria were grown in 6-L pots for 8 months. Then, 30 mL of each treatment solution (Table 1) or water for the control were applied to the soil of each pot containing the pineapple plants. There were three replicates per treatment, except for the SA reference treatment and the water control, each of which had six replicates. After 3 days, the apical buds of the parental plants were removed to eliminate apical dominance, allowing the parental plants to produce daughter plants (usually known as suckers). Subsequently, each parental plant produced five suckers, resulting in a total of 15 suckers per treatment for the treated group and 30 suckers for the reference SA and control groups. After 8 weeks, the suckers (~30 g) were harvested and transferred in 0.5-L pots for a 10-week acclimation period, allowing for root growth. Then, five mealybug instars were released on the aerial part of the suckers, and the resulting populations were counted 45 days later. Populations were evaluated as described before. Contamination protections were the same as the previous assays.

2.4. Statistics

Calculations were made in R with RStudio interface. Mealybug population data in the three experiments were analyzed using negative binomial models due to overdispersion. Tests on mealybug populations were performed using log-transformed data, and each model was validated by plotting residuals.

In the first experiment on the toxicity of compounds, no statistical analysis was necessary (Section 2.3.1).

In the second experiment (Section 2.3.2), conducted in culture chambers during 2019 and 2022, we investigated the factors influencing mealybug populations. These factors included treatment, variety, and year, along with their interactions; this was analyzed using post hoc mean comparison tests, specifically Tukey's test. In addition, in the conditions of this assay, the risk for a plant of harboring more than 25 mealybugs was calculated (based on the best treatment results), and was analyzed by a binomial model.

In the third experiment (Section 2.3.3), the biological assay on pineapple plants in the greenhouse, we evaluated the effects of treatment, set of five replicate plants, and their interaction. Post hoc mean comparisons were conducted using Dunnett's test, comparing each treatment against the control.

In the fourth experiment, (Section 2.3.4), the biological assay on pineapple daughter plants of treated parental plants in the greenhouse, we considered the treatment as a fixed effect and the parental plant as a random effect. Post hoc mean comparisons were again performed using Dunnett's test to compare each treatment against the control.

In Sections 2.3.3 and 2.3.4, we assessed the treatment effect on plant size (height) using classical ANOVA. Post-hoc mean comparisons were conducted using Tukey's test where necessary. For the evaluation of treatment effects on chlorophyll level (SPAD), we employed the Kruskal–Wallis non-parametric test. In cases where significant differences were detected, we performed pairwise comparisons using the Wilcoxon Signed-Rank Test. Each model was validated by plotting residuals.

3. Results

3.1. In Vitro Toxicity of Compounds against Mealybugs

All treatments, SA, natural extract, and chemicals were subjected to an in vitro toxicity test on mealybugs. The results showed no mealybug mortality under these conditions on day 1 and day 8 (Table 2).

Table 2. Direct toxicity of treatments on mealybugs in vitro, based on three replicates with ten mealybugs each.

Treatments	% Mortality of Mealybugs	
	1 Day	8 Days
SA (salicylic acid)	0	0
ASM (acibenzolar-S-methyl)	0	0
BABA (β-aminobutyric acid)	0	0
MeSA (essential oil of *Gaultheria fragrantissima*)	0	0
Hypericum lanceolatum (herbal maceration)	0	0
Flacourtia indica (herbal maceration)	0	0
Control (water)	0	0

3.2. Biological Efficacy of Soil Application of SA against Mealybug Populations in Controlled Conditions

In the culture chamber, SA (1 mM) significantly reduced (by 51.1%) the number of mealybugs at 45 dar compared with the control ($p < 0.001$) (Table 3). The mean of mealybug populations counted in pineapples treated with SA was 18.9, and that of the control was 40.9. These results were averaged for the two varieties and the two experiments (Figure 1). Indeed, data analysis revealed a significant interaction between variety and year for Queen Victoria ($p < 0.001$), but no interaction on treatments with either variety or year. Regarding SA, 32 data points (red dots in Figure 1) were obtained in the 2019 trial, while 80 data points (blue dots) were recorded in the 2022 trial. SA treatment reduced the occurrence of extreme values and lowered the risk of plants harboring more than 25 mealybugs. There was a 22% decrease in risk for MD2 and a 17% decrease for Queen Victoria. The treatments ASM and MeSA, which were highly effective in controlling mealybug multiplication, resulted in most of the plants harboring 25 mealybugs or fewer (Figure 2). This explains the rationale for choosing 25 mealybugs as a limit for measuring the risk of further infestation.

Table 3. Biological efficacy of soil-applied salicylic acid (SA) for reducing mealybug numbers per pineapple plant, averaged for MD2 hybrid and Queen Victoria.

Treatment	Mean Mealybugs/Plant	Percentage of Reduction Based on Control (%)	Risk of Plants Harboring More than 25 Mealybugs (%)
SA	18.9	51.1% ($p < 0.001$)	26.7% (* $p = 0.001$)
Control	40.9	-	53.6% (* $p = 0.002$)

* Analysis: ANOVA.

3.3. Biological Efficacy of Soil Application of SA, Natural Extracts, and Chemicals on Queen Victoria against Mealybug Populations in the Greenhouse

In the greenhouse, the mean of mealybugs counted on pineapple treated with soil-applied SA was 18.1 per plant, and that of the control was 35.9 per plant (Table 4, Figure 2). These results are the averages of three replicates. SA application on soil tended to reduce the number of mealybugs at 45 dar by 51.1%. These results confirmed the previous findings obtained in the experiment in the culture chamber, although they were not statistically significant. Two extreme values corresponding to numbers >350 mealybugs were removed (one for BABA and one for SA). The reason behind this was that among 105 records of

mealybug multiplication, no other value exceeded 350 mealybugs, including those of the controls. In addition, an entire contaminated replication of BABA was removed.

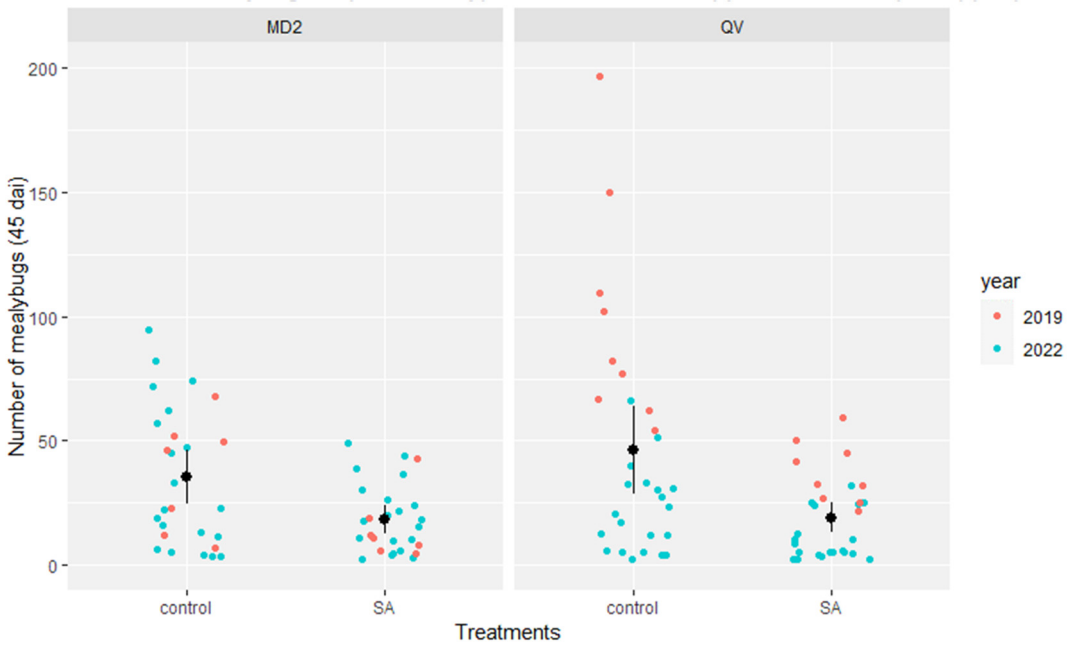

Figure 1. Number of mealybugs counted 45 days after release of five mealybugs on pineapple treated by soil application of exogenous salicylic acid (SA) for the two pineapple varieties MD2 hybrid (MD2) and Queen Victoria (QV). Black dots correspond to the means and standard deviations.

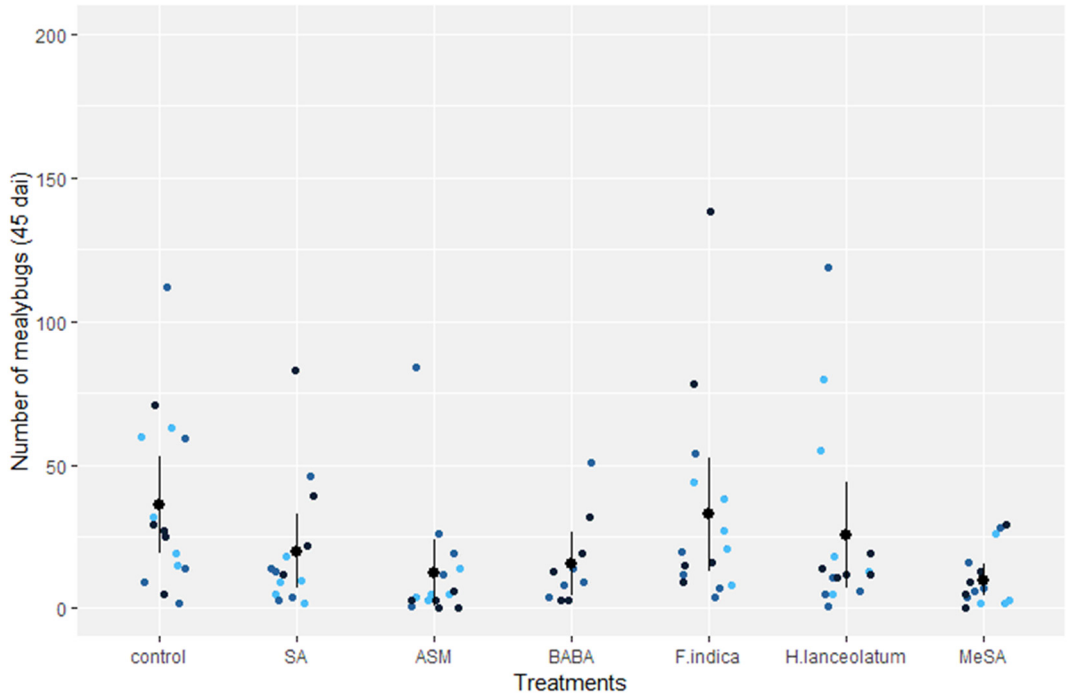

Figure 2. Number of mealybugs per plant counted 45 days after releasing five mealybugs on pineapple treated by soil application of exogenous salicylic acid (SA), acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA), *Flacourtia indica* (plant extract), *Hypericum lanceolatum* (plant extract), essential oil of *Gaultheria fragrantissima* rich in methyl salicylate (MeSA), or the control on soil of pineapple (Queen Victoria). Blue dots correspond to one replicate of five plants. Black dots correspond to the means and standard deviations.

Table 4. Biological efficacy of soil-applied treatments with salicylic acid analogs and plant extracts on Queen Victoria pineapple plants for mealybug population reduction.

Treatment	Mean Mealybugs/Plant	Percentage of Reduction Based on Control (%)	Risk of Plants Harboring More than 25 Mealybugs (%)
SA (salicylic acid)	18.1	51%	27%
ASM (acibenzolar-S-methyl)	11.9	67% (* $p < 0.01$)	13%
BABA (β-aminobutyric acid)	17.1	52%	20%
Flacourtia indica (plant extract)	31.9	11%	40%
Hypericum lanceolatum (plant extract)	23.4	35%	20%
MeSA (essential oil of *Gaultheria fragrantissima*)	9.9	72% (* $p < 0.002$)	20%
Control	35.9	0%	53%

* Analysis: ANOVA ($p < 0.001$); Dunnett's test.

The two leading treatments, ASM and MeSA, significantly reduced the mealybug population by 67% ($p < 0.01$) and 72% ($p < 0.002$), respectively (Table 4). These treatments proved more effective than SA, resulting in an additional reduction in the mealybug populations by 16 to 21%. In this type of experiment, the mealybug population values generally exhibit a broad range and a heterogeneous distribution within each group of plants (Figure 2). Nevertheless, the ASM and MeSA treatments effectively minimized extreme values and mitigated the risk of plants harboring more than 25 mealybugs by 13% and 20%, respectively, compared to 53% for the control.

The other treatments showed limited efficacy in reducing the number of mealybugs (Figure 2). BABA did not significantly reduce populations compared with the control. Regarding the two plant extracts, *F. indica* and *H. lanceolatum*, an exotic and an endemic plant, respectively, the mealybug count decreased slightly, by 11% (31.9 mealybugs per plant) and 35% (23.4 mealybugs per plant), respectively, compared with 35.9 in the control (Table 4). The endemic plant treatment exhibited reduced extreme values and fewer instances of plants with more than 25 mealybugs, a trend closely resembling that of ASM and MeSA treatments (Figure 2).

3.4. Biological Efficacy of Parental Application of SA, Natural Extracts, and Chemicals against Mealybug Populations on Daughter Plants in the Greenhouse

Applying ASM or MeSA to parental plants (transgenerational induction treatment) significantly reduced (by 76% and 80%, respectively) ($p < 0.001$) the number of mealybugs on daughter plants at 45 dar (Table 5). These transgenerational treatments reduced the mealybug population as effectively as direct application. The populations of mealybugs on treated plants were compared with the control for each treatment (Figure 3, Table 4). In contrast, the transgenerational induction treatment by SA was inefficient and did not reduce the population. Concerning extreme values, the risk of plants harboring more than 25 mealybugs reached 32% for SA treatment and 46% for the control group, whereas only 7% of plants treated with ASM or MeSA exhibited more than 25 mealybugs per plant.

BABA treatment showed the lower significant difference according to the model. Nevertheless, BABA minimized extreme values and mitigated the risk of plants harboring more than 25 mealybugs to 7%, similar to ASM and MeSA (Table 5). Finally, plant extracts were not effective, with higher mealybug population averages than the control. The *H. lanceolatum* and *F. indica* extracts displayed an extremely high average of 49.3 and 81.6 mealybugs per plant, respectively. In addition, *H. lanceolatum* had only two replicates, with variable results (Figure 3).

Table 5. Biological efficacy of transgenerational application of natural and chemical treatments on Queen Victoria pineapple plants to reduce mealybug populations.

Treatment	Mean Mealybugs/Plant	Percentage of Reduction Based on Control (%)	Risk of Plants Harboring More than 25 Mealybugs (%)
SA (salicylic acid)	36.4	0%	32%
ASM (acibenzolar-S-methyl)	8.5	76% (* $p < 0.001$)	7%
BABA (β-aminobutyric acid)	16.0	54% (* $p = 0.01$)	7%
Flacourtia indica (plant extract)	75.0	-	100%
Hypericum lanceolatum (plant extract)	49.3	-	60%
MeSA (essential oil of *Gaultheria fragrantissima*)	6.9	80% (* $p < 0.001$)	7%
Control	35.1	0%	46%

Analysis by ANOVA ($p < 0.001$); Dunnett's test *.

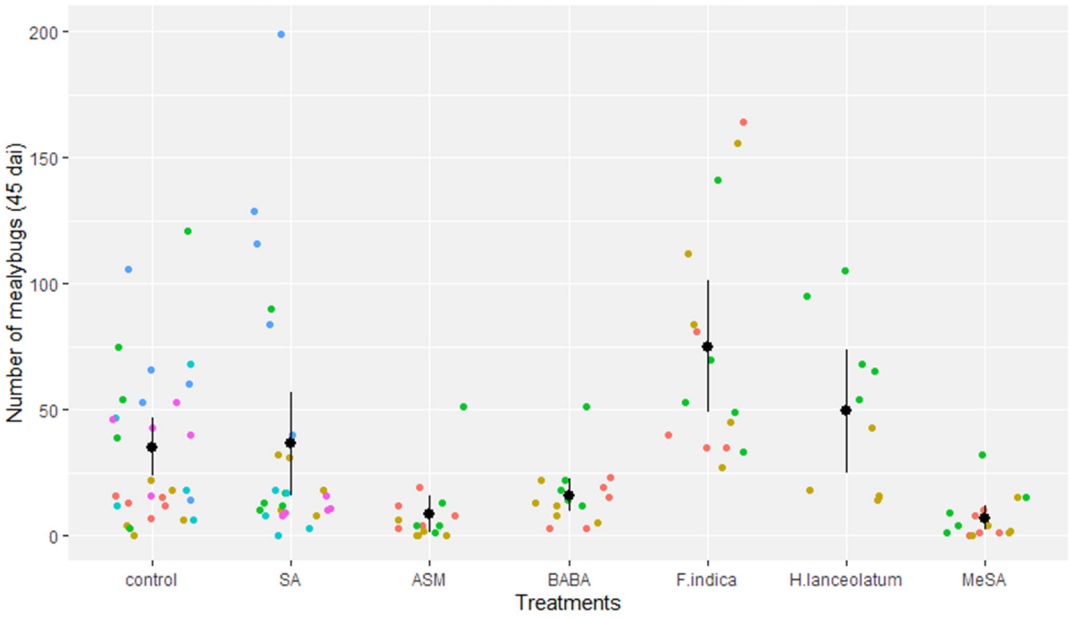

Figure 3. Number of mealybugs per Queen Victoria pineapple plant counted 45 days after releasing five mealybugs on pineapple treated by transgenerational application with exogenous salicylic acid (SA), acibenzolar-S-methyl (ASM), β-aminobutyric acid (BABA), *Flacourtia indica* (plant extract), *Hypericum lanceolatum* (plant extract), essential oil of *Gaultheria fragrantissima* rich in methyl salicylate (MeSA), or control. Transgenerational application refers to soil application of the treatment to parent plants, from which suckers (daughter plants) were taken for experimentation with mealybugs. Colored dots correspond to one replica of five plants of each parental plant. Black dots correspond to the means and standard deviations.

3.5. Effects of Treatments on Plant Physiology in the Greenhouse

Forty-five days after the release of mealybugs, plant chlorophyll levels were measured using the SPAD-502 chlorophyll meter, and plant size (height) was assessed (Table 6). With plant extracts, minor differences were observed for the chlorophyll level and height of plants treated by direct soil application (Section 2.3.3), although *F. indica* induced a significantly larger size ($p = 0.002$) than the control. Concerning plants treated by transgenerational application (Section 2.3.4), the *H. lanceolatum* plant extract significantly affected the chlorophyll level in leaves ($p = 0.007$). Based on these observations, natural treatments, essential oils, and plant extracts had a greater impact on plant physiology in our study than chemical treatments.

Table 6. Plant height and chlorophyll levels of Queen Victoria pineapple plants 45 days after the release of five mealybugs in greenhouse in experiments with direct soil application or transgenerational application of natural or chemical treatments.

Treatment	n (Plants)	Direct Soil Application		Transgenerational Application	
		Plant Height (cm)	Chlorophyll Level (SPAD)	Plant Height (cm)	Chlorophyll Level (SPAD)
		$p < 0.001$ **	$p = 0.28$	$p = 0.09$	$p < 0.001$ **
SA (salicylic acid)	30	42 ± 2.2	72	24 ± 2.2	68
ASM (Acibenzolar-S-Methyl)	15	36 ± 2.2	71	20 ± 2.3	61
BABA (β-Aminobutyric acid)	15	42 ± 2.5	73	26 ± 3.2	68
Flacourtia indica (plant extract)	15	45 ± 2.2 * $p = 0.002$	68	22 ± 2.6	64
Hypericum lanceolatum (plant extract)	15	37 ± 2.2	69	29 ± 4.2 *	78 * $p = 0.007$
MeSA (essential oil of *Gaultheria fragrantissima*)	15	39 ± 2.2	73	17 ± 2.0 *	57
Control	30	36 ± 2.2	73	23 ±1.9	64

Analysis: * ANOVA and Dunnett's test; ** Kruskal–Wallis and Wilcoxon Signed-Rank test.

4. Discussion

Our results demonstrated that soil application of exogenous SA or some of its analogs, ASM or MeSA, significantly reduced the population of mealybugs at 45 dar by 51%, 66.5%, and 72.8%, respectively. In addition, these treatments minimized extreme values and mitigated the risk of plants harboring more than 25 mealybugs. These promising results show the biological efficacy of these treatments on pineapple tissue culture plants or traditional suckers against an aerial biotrophic insect such as mealybugs in both a culture chamber and greenhouse. The present study did not analyze the induction of systemic acquired resistance (SAR) using molecular or enzymatic markers, but SA, MeSA, and ASM are assumed to prime the plants for SAR after the subsequent release of mealybugs. Although monocots have received less research attention, the mechanisms underlying SAR induction appear to be similar between monocots and dicots [34]. According to Holeski's definition [35], the priming defense is a state in which plants that have been previously attacked respond faster and stronger to a subsequent attack with low-cost energy. Other publications confirmed that this type of defense is induced by BABA, SA, ASM, and MeSA [20,21,23,24,36]. In our study, it would be necessary to optimize the concentrations of some of the treatments in response to which plants appear to have been affected in terms of size and chlorophyll levels and could, consequently, have been energetically affected (Table 6). Our data were consistent with a previous study that showed that the direct application of exogenous SA on the MD2 hybrid, whether applied to soil or leaves, significantly reduced mealybug multiplication through SAR induction as characterized by using molecular and enzymatic markers [37]. No variety difference was observed in our study in terms of mealybug multiplication.

Low doses of SA are known to prime the tissue for enhanced defense gene expression during subsequent pathogen attacks [38]. Furthermore, ASM is a well-known SAR inducer of numerous plants and a commercially available priming agent [39]. Parkinson [40] demonstrated that ASM efficiently improved resistance against a virus of passion fruit. Chinnasri [26] confirmed the efficiency of ASM in pineapple defense against nematodes. They also suggested the potential of SAR as part of an integrated management program to control Fusariosis and other pineapple diseases. Both studies validated SAR induction by analyzing molecular markers for PR protein [27,40]. MeSA was also described as a mobile signal required for the signal perception of SAR in distal tissue [20]. Jeon [41] showed that SAR effectively managed pine wilt disease caused by pinewood nematodes through foliar application of MeSA without direct nematicidal activity. Several studies using molecular markers confirmed that ASM may induce SAR on pineapple and other plants, and our

results demonstrate that SA, ASM, and MeSA successfully decreased mealybug populations on pineapple without any toxic effect. In our experiments, MeSA derived from the essential oil of *Gaultheria fragrantissima* proved to be as effective as the well-known SAR inducer ASM. Our results demonstrated variations in biological efficiency against mealybugs based on the treatments used.

Our study demonstrates that an application to the soil impacts the mealybug population localized in aerial tissues. Other studies on pineapple utilizing foliar spray have shown efficiency on root tissues. These findings contribute to the hypothesis of the SA signaling pathway's involvement in the defense mechanism. These previous studies have shown that exogenous SA could reduce the symptoms caused by pineapple root pests such as nematodes or oomycetes [25–29]. Soler et al. [28] showed that SA at 1 mM reduced the population of the nematode *Rotylenchulus reniformis* on MD2 pineapple at 45 days by 58.8%, while SA provided only a 14.3% reduction for Smooth Cayenne pineapple. Another study showed that with different concentrations (0.5 to 5 mM), SA reduced symptoms caused by *Phytophthora cinnanomi* at 6 dar [29]. It would be interesting to evaluate other concentrations to control mealybugs in order to determine the optimal dose and enhance the treatment efficiency. Finally, the next step would be to move from the greenhouse to the field. Treatments inducing SAR are under extensive investigation in greenhouses and fields with various crops, showing promising potential for pineapple production [38,39,42,43].

Our study demonstrated that ASM and MeSA continued to be effective on offspring shoots when the treatments were applied 18 weeks prior on the parental plant, significantly reducing the mealybug populations by 84.3% and 78.3%, respectively, at 45 dar. Transgenerational applications of these treatments were as effective as direct soil applications. Priming could be the mechanism behind the mealybug control induced by ASM and MeSA, either in the direct application or in the transgenerational application. ASM and MeSA applications primed distal tissue, inducing SAR in the entire plant. Priming can be passed down in the offspring generation, indicating an epigenetic component of transgenerational priming [23,35,44]. This phenomenon, called "next generation SAR", could act as a plant memory of disease stress encountered in the parental generation [44]. The response to biotic stress can depend on the mode of application of the treatments [45]. Seed priming and foliar application are the modes of application that are the most common in terms of transgenerational priming [46,47]. It appeared that parental plants could transmit the priming to the next generation of buds formed after the removal of the heart of the plant. However, buds could have already formed before the removal and would have been directly primed at the same time as parental plants. In both cases, the treatments maintained a primed state in the next generation of pineapple for 18 weeks until the mealybugs were released. In our study, we observed that the duration of the primed state on pineapple (a monocot) treated with ASM appeared to surpass what was observed in certain dicots [40]. In 2006 [25], Matos demonstrated that ASM controlled *Fusarium subglutinans* in pineapple, resulting in a reduction in associated symptoms on fruit and slips. Disease development in pineapple slips increased when inoculation was performed 6 or 8 weeks after ASM treatment. The results suggest that acquired resistance is short-lived. In contrast, our results showed that ASM and MeSA were still effective 18 weeks after treatment. In our conditions, BABA did not appear to exhibit significant efficiency through direct soil application, but when applied transgenerationally, it resulted in a remarkable 51.2% reduction in the number of mealybugs. Another study showed the effect of BABA on the pea aphid, where BABA induced PR protein and enables long-term defense [48].

Another noteworthy aspect is the ability of the Queen Victoria pineapple to experience vegetative propagation and develop many axillary buds in traditional shoots, unlike other cultivars [49]. In addition, although it is customary to use pineapple shoots as planting material, tissue culture plants and healthy shoots from nurseries could also contribute to mealybug control. The latter two are known to be free of viruses and mealybugs. This is why inducing SAR in pineapple shoots or tissue culture plants could be a good approach to reducing the population of mealybugs.

Lastly, it could be interesting to study other exotic and endemic plants rich in SA or its derivates from Réunion Island, analyzing and determining their content. Herbal macerations and essential oils require only simple preparations and are easy to use. As most phytosanitary products come from the mainland, herbal macerations or essential oils could be locally developed alternatives for producers. In 2022 [50], Avila reviewed many articles on insecticidal activity against mealybugs, including some essential oils. In our study, the application of the essential oil of *Gaultheria fragrantissima*, rich in MeSA, strongly reduced mealybug populations on pineapple while not showing a directly toxic effect on mealybugs. This type of treatment may offer an opportunity for pineapple producers on Réunion Island.

5. Conclusions

In conclusion, treatments consisting of compounds that induce systemic acquired resistance (SAR) are of interest for pineapple production. Our study showed that two treatments, ASM and MeSA, significantly reduced the population of mealybugs through direct and transgenerational applications. It would be interesting to plant pre-treated pineapples in the field to improve the plant's defense response against mealybugs. Pre-treated pineapples or primed pineapples may be promising alternatives to reduce mealybug populations and the incidence of pineapple mealybugs wilt. The results obtained in the greenhouse need to be optimized and confirmed in the field in ecologically-based friendly pineapple cropping systems.

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