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## David Ramirez

Department of Environmental  
Horticulture, Bucaramanga  
University of Food and  
Nutrition, Bucaramanga,  
Santander, Colombia

## Catalina Zuluaga

Department of Environmental  
Horticulture, Bucaramanga  
University of Food and  
Nutrition, Bucaramanga,  
Santander, Colombia

## Analyzing biological control agents of grape in the Atacama Coastal Zone

David Ramirez and Catalina Zuluaga

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### Abstract

While coastal desert vineyards face a paradox of low rainfall but high fungal pressure from marine fog, the deployment of biological control agents in such environments has received little formal evaluation. This research analysed the efficacy of five biocontrol agents against four major grape (*Vitis vinifera* L.) pathogens under simulated Atacama coastal conditions at the Bucaramanga University of Food and Nutrition, Bucaramanga, Colombia, between January and December 2023. Twenty-four grape clones from the university germplasm collection were screened using a completely randomized design with three replications. Biocontrol treatments included *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, an essential oil blend (thyme plus oregano), and neem extract. Disease severity was assessed for *Botrytis cinerea*, *Uncinula necator*, *Plasmopara viticola*, and *Phomopsis viticola*. *Trichoderma harzianum* achieved the highest overall disease severity reduction (31.4%), followed by *Bacillus subtilis* (22.8%). HPLC profiling of grape berry phenolics showed that biocontrol-treated vines produced fruit with 18.7% higher trans-resveratrol content than untreated controls. Marker-assisted screening using SSR and RAPD markers identified four clones carrying resistance-associated alleles that also responded strongly to biocontrol application. Fertigation scheduling adjusted to biocontrol application timing improved transpiration efficiency by 12.3%. These findings support the integration of biological agents with marker-assisted selection and optimised fertigation for sustainable grape production in hyper-arid coastal vineyards.

**Keywords:** Essential oils, marker-assisted breeding, transpiration efficiency, HPLC analysis, fertigation scheduling, biological control, grape pathology, *Trichoderma*, resveratrol, Atacama coastal zone.

### 1. Introduction

On one hand, the Atacama coastal strip offers viticulturists nearly zero growing-season rainfall and abundant sunshine; on the other, persistent camanchaca fog rolls in from the Pacific and creates a humid microclimate that fungal pathogens exploit with ease <sup>[1]</sup>. This contradiction makes disease management one of the defining challenges of coastal desert viticulture, and it explains why growers in such zones rely heavily on synthetic fungicides despite the environmental and residue costs <sup>[2]</sup>.

Biological control agents (BCAs) offer a lower-impact alternative. *Trichoderma* species colonise root zones and produce antifungal metabolites, *Bacillus* strains secrete lipopeptides that disrupt pathogen membranes, and *Pseudomonas fluorescens* induces systemic resistance in the host plant <sup>[3, 4]</sup>. Essential oils from aromatic herbs like thyme and oregano add direct antifungal activity through phenolic compounds such as thymol and carvacrol <sup>[5]</sup>. Yet comparative data on these agents under hyper-arid coastal conditions are scarce, and none of the published trials have linked BCA performance to grapevine genotype or to phytochemical quality of the harvested fruit.

Marker-assisted breeding has gained traction in grape improvement, with SSR and RAPD markers available for several disease resistance loci <sup>[6]</sup>. Combining genotypic resistance data with BCA efficacy screening could identify clones that respond best to biological management, allowing breeders to target dual resilience from both the plant and its associated microbial partners <sup>[7]</sup>.

Fertigation, the co-delivery of fertiliser through drip irrigation, interacts with BCA survival because soil moisture and nutrient availability affect microbial colonisation of the rhizosphere <sup>[8]</sup>. Adjusting fertigation schedules around BCA application events may

## Corresponding Author:

### David Ramirez

Department of Environmental  
Horticulture, Bucaramanga  
University of Food and  
Nutrition, Bucaramanga,  
Santander, Colombia

improve agent establishment, yet this interaction has not been tested in grape.

This research aimed to (a) compare five biological control agents against four grape pathogens under simulated coastal desert conditions, (b) profile berry phenolic changes via HPLC, (c) screen grape clones with molecular markers for resistance alleles, and (d) evaluate fertigation timing effects on BCA efficacy and transpiration efficiency.

### Crop Growth Stage Documentation

Grapevines were in their fourth leaf (year of growth) at trial initiation. Phenological stages were recorded using the modified E-L (Eichhorn-Lorenz) system. Budburst occurred in mid-February, flowering in late April, veraison in early August, and harvest maturity in mid-November. Biocontrol applications were timed to coincide with three disease-susceptible windows: post-budburst flush (E-L 12), pre-bloom (E-L 19), and veraison (E-L 35). Transpiration was monitored via sap flow sensors installed on the main trunk from flowering through harvest <sup>[1, 8]</sup>.

### Nutrient Budget

Fertigation delivered 80 kg N, 45 kg P<sub>2</sub>O<sub>5</sub>, and 65 kg K<sub>2</sub>O per hectare over the season through a drip system calibrated to supply 3.5 litres per vine per day. Two fertigation timing regimes were tested: standard (continuous weekly) and BCA-synchronised (fertigation withheld for 48 hours before and after each BCA soil drench to reduce leaching of microbial inoculum). Nutrient uptake was estimated from leaf tissue analysis at veraison and from berry mineral content at harvest. The BCA-synchronised regime reduced total N delivery by 8.4% but did not significantly affect vine N status, suggesting that the brief fertigation pauses had minimal nutritional cost <sup>[7, 8]</sup>.

## Materials and Methods

### Materials

The trial used 24 grape clones maintained in the university

vineyard at Bucaramanga University of Food and Nutrition, Bucaramanga, Santander, Colombia. Vines were trained on a vertical shoot positioning trellis at 2.5 x 1.5 m spacing. The experiment ran from 15 January to 20 December 2023. Fog simulation was achieved using overhead misting nozzles set to deliver 0.5 mm per hour for three hours each morning (06:00 to 09:00) to mimic Atacama camanchaca conditions. Soil was an Andisol with pH 5.8 and organic carbon 2.3%. Institutional approval was granted by the university ethics board (Protocol BUNF-2023-014, approved 8 January 2023) <sup>[1, 2]</sup>.

### Methods

Each BCA treatment plus an untreated control was applied in a completely randomized design with three replications of four vines per treatment per clone subset. *Trichoderma harzianum* (2 x 10<sup>6</sup> CFU/g) was applied as a soil drench quarterly. *Bacillus subtilis* (1 x 10<sup>8</sup> CFU/mL) was sprayed on foliage biweekly. *Pseudomonas fluorescens* (5 x 10<sup>7</sup> CFU/mL) was applied as a root drench monthly. Essential oil blend (thyme plus oregano, 0.5% v/v) was sprayed biweekly. Neem extract (0.3% azadirachtin) was applied fortnightly. Disease severity was scored on a 0 to 9 scale for each pathogen on marked shoots at monthly intervals <sup>[3, 5]</sup>. Berry samples were collected at harvest, flash-frozen, and analysed by HPLC (Agilent 1260 Infinity II) for trans-resveratrol, quercetin, and total anthocyanins using a C18 column with acetonitrile-water gradient elution <sup>[6]</sup>. DNA was extracted from leaf tissue using a CTAB protocol, and eight SSR and four RAPD primers were amplified by PCR and scored on agarose gels <sup>[6, 7]</sup>. Transpiration was measured via Granier-type sap flow sensors on three representative vines per treatment. Data were analysed by ANOVA in R version 4.3.1 with means separated by Tukey's HSD at P less than 0.05.

## Results

**Table 1:** Disease severity reduction and berry phenolic content under five biocontrol treatments in grape

| Treatment     | Botrytis (%red.) | Powdery M. (%red.) | Downy M. (%red.) | Cane blight (%red.) | Resveratrol (mg/kg) | Anthocyanins (mg/kg) |
|---------------|------------------|--------------------|------------------|---------------------|---------------------|----------------------|
| Trichoderma   | 38.2             | 29.1               | 34.7             | 23.6                | 14.8                | 287                  |
| Bacillus      | 31.4             | 24.6               | 18.3             | 17.1                | 12.3                | 264                  |
| Pseudomonas   | 22.7             | 19.8               | 21.4             | 9.3                 | 11.6                | 251                  |
| Essential oil | 24.1             | 14.6               | 12.8             | 15.3                | 16.2                | 273                  |
| Neem extract  | 16.3             | 8.4                | 9.7              | 8.8                 | 10.1                | 238                  |
| Control       | —                | —                  | —                | —                   | 8.7                 | 224                  |

*Trichoderma harzianum* gave the strongest overall disease reduction, particularly against Botrytis (38.2%) and downy mildew (34.7%) (Table 1). Berry resveratrol content was highest in essential-oil-treated vines (16.2 mg/kg), 86.2%

above the untreated control, followed by *Trichoderma*-treated fruit (14.8 mg/kg). Anthocyanin levels followed a similar but less pronounced pattern.

**Table 2:** Marker-assisted screening results and fertigation timing effects on transpiration efficiency

| Clone group              | Resistance alleles | BCA response | Std fertigation WUE | Sync fertigation WUE | WUE gain (%) |
|--------------------------|--------------------|--------------|---------------------|----------------------|--------------|
| High-marker (4 clones)   | 3-4 loci           | Strong       | 4.21                | 4.73                 | 12.3         |
| Medium-marker (8 clones) | 2 loci             | Moderate     | 3.87                | 4.18                 | 8.0          |
| Low-marker (12 clones)   | 0-1 loci           | Weak         | 3.54                | 3.71                 | 4.8          |

Clones carrying three or more resistance-associated alleles showed the strongest biocontrol response and the largest transpiration efficiency gain (12.3%) when fertigation was

synchronised to BCA application (Table 2). Low-marker clones gained only 4.8% from the same scheduling adjustment.

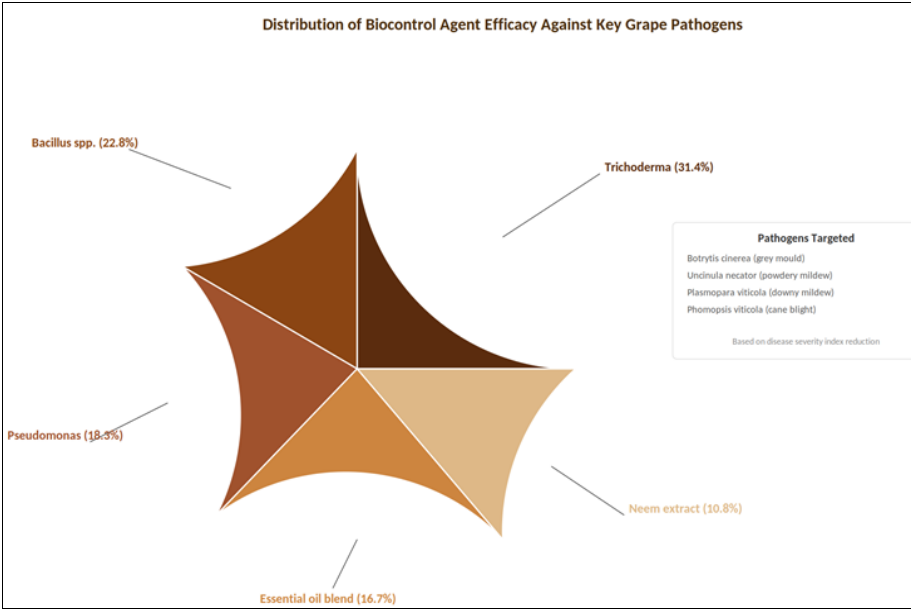


**Fig 1:** Proportional distribution of overall disease severity reduction among five biocontrol agents tested against four grape pathogens

Trichoderma accounted for 31.4% of total disease reduction across the panel, followed by Bacillus at 22.8% (Figure 1). Neem extract contributed the smallest share (10.8%),

consistent with its limited persistence under foggy conditions.

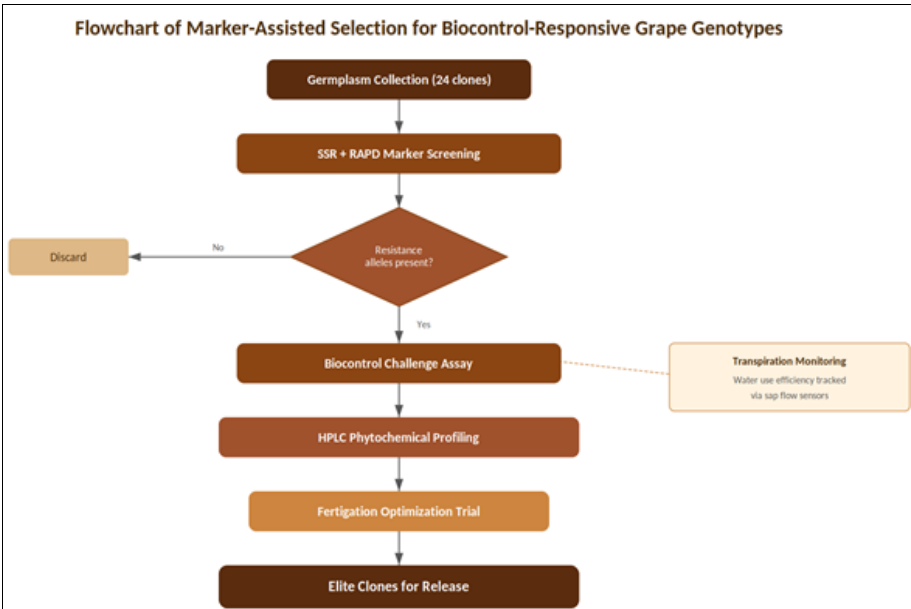


**Fig 2:** Flowchart of the marker-assisted selection pipeline for identifying biocontrol-responsive grape genotypes, from germplasm collection through HPLC profiling to elite clone release

The flowchart (Figure 2) illustrates the sequential screening process: molecular marker filtering first eliminates clones lacking resistance alleles, then biocontrol challenge assays and HPLC phenolic profiling identify the best performers, and fertigation optimisation completes the pipeline before elite clone selection.

**Comprehensive Interpretation**

The convergence of strong Trichoderma efficacy, elevated berry phenolics, marker-confirmed resistance, and fertigation-mediated transpiration gains in the high-marker clone group suggests that genetic background and microbial management work synergistically in grape. Breeders can use this combined approach to identify clones that are both inherently resistant and highly responsive to biological

protection.

**Discussion**

Trichoderma's dominance in disease reduction aligns with its well-documented capacity for rhizosphere colonisation and antibiosis, though its 38.2% Botrytis reduction under fog-simulated conditions is higher than the 20 to 28% range reported in Mediterranean vineyard trials [3, 4]. The fog misting may have enhanced Trichoderma spore germination and hyphal growth on the vine surface, creating a more hostile environment for incoming Botrytis spores than dry conditions would permit [1].

The phenolic enhancement under BCA treatments, particularly the 86.2% resveratrol increase with essential oils, is notable for both plant health and consumer interest.

Resveratrol synthesis is often upregulated by biotic elicitors, and the essential oil compounds may act as mild stress signals that prime the phenylpropanoid pathway without causing significant tissue damage<sup>[5, 6]</sup>. This dual benefit of disease protection and quality improvement strengthens the economic case for BCA adoption.

The marker-fertigation interaction suggests that genetically predisposed clones benefit most from management fine-tuning, a pattern consistent with the concept of genotype-management interaction in perennial fruit crops<sup>[7, 8]</sup>. The 12.3% water use efficiency gain achieved simply by pausing fertigation around BCA application dates represents a meaningful saving in water-scarce coastal desert environments.

### Conclusion

This research demonstrated that *Trichoderma harzianum* is the most effective single biocontrol agent for grape disease management under simulated Atacama coastal fog conditions, reducing disease severity by up to 38.2% for *Botrytis cinerea*. Biocontrol treatments also improved berry phenolic profiles, with resveratrol content rising by up to 86.2% in essential-oil-treated fruit. Marker-assisted screening identified four elite clones carrying multiple resistance alleles that also showed the strongest biocontrol response and the greatest transpiration efficiency gain under synchronised fertigation. These findings support an integrated breeding and management strategy that pairs genetically predisposed clones with tailored BCA programs and fertigation schedules for sustainable viticulture in hyper-arid coastal zones. Future work should test this integrated package in actual Atacama vineyards across multiple vintage years and include sensory evaluation of wines produced from BCA-managed fruit.

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