

## Article

# Interactive Effects of Rootstock and Training System on Photosynthesis, Biochemical Responses, and Yield in *Vitis labrusca* Under Subtropical Climate Conditions

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**Abstract:** Climate change imposes significant challenges on viticulture, especially in subtropical regions, where thermal and water stresses impact vine physiology and yield. This study evaluated the effects of two rootstocks ('IAC 766 Campinas' and '106-8 Mgt') and two training systems (low and high trellis) on the photosynthesis, biochemical parameters, and productivity of *Vitis labrusca* ('Bordô' and 'Isabel'). The interaction between rootstock and training system significantly influenced gas exchange, chlorophyll fluorescence, antioxidant enzyme activity, and yield components. In 'Bordô', grapevines trained on high trellis and grafted onto 'IAC 766 Campinas' showed a 45.1% higher electron transport rate and 39.8% greater total chlorophyll content at flowering compared to the low trellis and '106-8 Mgt' combination. Productivity increased by 49% under this combination. In 'Isabel', low trellis combined with 'IAC 766 Campinas' enhanced water use efficiency by 50% and SPAD index by 11%. These results highlight that selecting suitable rootstock and training system combinations can optimize physiological efficiency and yield, representing an effective adaptation strategy for viticulture under subtropical conditions.

**Keywords:** *Vitis* spp.; gas exchange; water use efficiency; rubisco enzyme; antioxidant enzymes



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## 1. Introduction

The grapevine (*Vitis* spp.) is one of the most economically and socially important fruit crops, widely cultivated for the production of table grapes, juices, and wines. Among the cultivated species, *Vitis labrusca* stands out in tropical and subtropical regions due to its greater pathogen resistance and better adaptation to a wide range of edaphoclimatic conditions compared to *Vitis vinifera* cultivars [1]. However, the productivity and fruit quality of *V. labrusca* grapevines can be significantly influenced by agronomic factors, such as rootstock selection and training system, which directly affect photosynthetic efficiency, secondary metabolite biosynthesis, and overall plant performance [2].

The rootstock plays a central role in regulating scion growth, enhancing water uptake, and nutrient absorption, while also modulating plant tolerance to biotic and abiotic stresses. Studies have shown that different scion–rootstock combinations can influence xylem hydraulic conductivity and water use efficiency, thereby impacting carbon assimilation rates and key physiological parameters associated with the grapevine’s energy metabolism [3–6]. Furthermore, rootstocks may alter the biochemical profile of plant tissues by modulating the synthesis of antioxidant compounds such as polyphenols, flavonoids, and anthocyanins, which are essential for fruit quality and the stability of grape-derived products [2].

Simultaneously, the training system shapes canopy architecture, light interception, and leaf biomass distribution, thereby influencing photosynthetic activity and berry biochemical composition. For instance, high trellis systems tend to increase canopy exposure to solar radiation, favoring higher photosynthetic rates, improved water use efficiency, and greater accumulation of phenolic compounds and sugars in the berries [2,3,7]. In contrast, low trellis systems may alter the canopy microclimate, affecting vegetative growth dynamics and the translocation of photoassimilates [7,8].

The intensification of climate change imposes significant physiological challenges on viticulture, particularly in tropical and subtropical regions, where rising average temperatures, irregular rainfall patterns, and increased solar radiation can compromise key physiological processes in grapevines. These stressors negatively affect photosynthetic metabolism, water use efficiency, and antioxidant regulation, directly influencing productivity and fruit quality [7,8]. Thus, selecting appropriate rootstocks and training systems is crucial for mitigating these impacts and ensuring sustainable production.

Given the physiological and agronomic relevance of these factors, this study aimed to investigate the interactive effects of rootstocks and training systems on photosynthesis, biochemical responses, and yield in *Vitis labrusca* grapevines cultivated under subtropical climate conditions. The assessment of these interactions provides valuable insights for optimizing vineyard management practices aimed at enhancing physiological efficiency and productive performance in tropical and subtropical environments.

## 2. Materials and Methods

### 2.1. Location and Climatic Conditions of the Experimental Area

The experiment was conducted at the Fruit Center of the Agronomic Institute of Campinas (IAC), located in Jundiaí, São Paulo State, Brazil (23°06′ S, 46°55′ W, 745 m altitude). According to the Köppen climate classification, the region is classified as Cfb, characterized by an average annual precipitation of 1400 mm, a mean annual temperature of 19.5 °C, and relative humidity of approximately 70.6%. The soil of the experimental area was classified as dystrophic Haplic Cambisol.

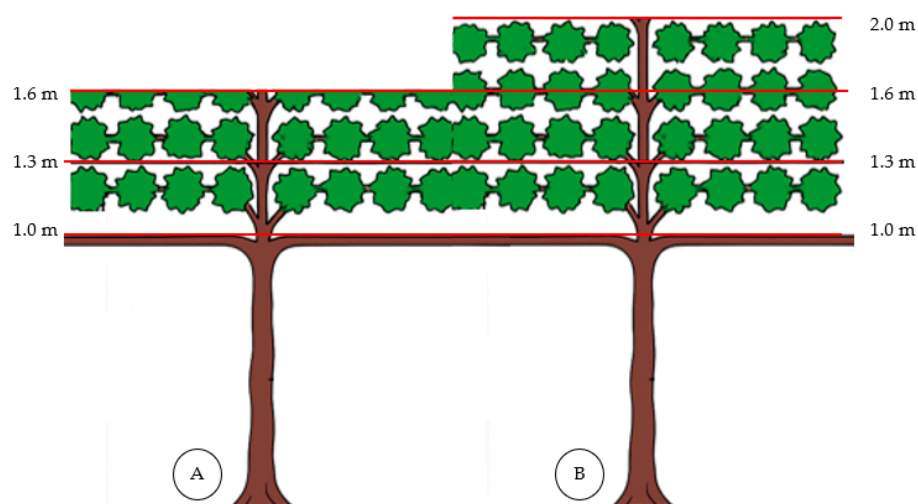
Rootstock cuttings were planted in September 2009 at a spacing of 2.5 m between rows and 1.0 m between plants. Scion grafting was carried out in July 2010 using the full cleft grafting technique. Pruning was performed annually in July, maintaining a single bud per cane, followed by the application of 5% hydrogen cyanamide (Dormex®, BASF, Ludwigshafen am Rhein, Germany) to promote uniform budbreak. Harvesting took place in December, according to the physiological maturation stage of the berries.

### 2.2. Experimental Design and Treatments

The experiment was conducted in a randomized block design with a 2 × 2 factorial arrangement, consisting of two rootstocks—‘IAC 766 Campinas’ (*Riparia do Traviú* × *Vitis caribaea*) and ‘106-8 Mgt’ [*Riparia* × (*Cordifolia* × *Rupestris*)]—and two training systems representing different trellis heights: 1.6 m and 2.0 m.

The ‘IAC 766 Campinas’ rootstock is characterized by moderate to high vigor, good drought tolerance, and adaptability to a wide range of soil types, including those with low fertility and high acidity. It also provides resistance to certain soil-borne pathogens, making it a versatile choice for tropical and subtropical climates. In contrast, the ‘106-8 Mgt’ rootstock exhibits moderate vigor, excellent rooting capacity, and high resistance to phylloxera (*Daktulosphaira vitifoliae*), with moderate tolerance to drought. This rootstock is often selected for its compatibility with high-quality wine grape cultivars and its adaptability to well-drained, nutrient-rich soils.

The two training systems used were adaptations of the vertical shoot positioning (VSP) system, differing in canopy height and structural configuration. The Low Trellis system consisted of three support wires positioned at 1.0, 1.3, and 1.6 m above ground. In contrast, the High Trellis system included four support wires positioned at 1.0, 1.3, 1.6, and 2.0 m, distributed vertically to allow greater canopy volume (Figure 1). This multi-cordon approach was designed to optimize light interception and canopy distribution, as previously described in studies by [2,6]. Both systems utilized a double cordon, spur-pruned structure, with shoots distributed vertically along the trellis wires, promoting uniform canopy exposure. This design minimizes shading and allows precise control over the vegetative and reproductive growth of the vines, which can significantly influence fruit quality.



**Figure 1.** Schematic representation of two vertical shoot positioning (VSP) training systems with different heights: (A) Low trellis system (1.6 m) with three support wires positioned at 1.0 m, 1.3 m, and 1.6 m; (B) High trellis system (2.0 m) with four support wires positioned at 1.0 m, 1.3 m, 1.6 m, and 2.0 m.

The *Vitis labrusca* cultivars evaluated were ‘Bordô’ and ‘Isabel’. The experimental area was arranged in five blocks, each comprising three plants per plot. Canopy height was maintained throughout the vegetative cycle through shoot thinning, apical trimming, and shoot tying.

### 2.3. Sample Collection

Physiological and biochemical assessments were performed during full flowering. Four fully expanded leaves (lamina and petiole) were collected from each plant, positioned opposite to the cluster, totaling 60 leaves per treatment. Additionally, SPAD index measurements and biochemical analyses were repeated at the onset of berry ripening. Photosynthetic measurements were performed in situ on intact leaves. For biochemical

analyses, leaves were collected, wrapped in aluminum foil, immediately frozen in liquid nitrogen, and stored in an ultra-freezer ( $-82^{\circ}\text{C}$ ) until analysis.

### 2.3.1. Photosynthetic Evaluation

Photosynthetic activity was measured using an open gas exchange system (Infra Red Gas Analyzer—IRGA, model LI-6400, LI-COR Biosciences, Lincoln, NE, USA). The following physiological variables were assessed: net  $\text{CO}_2$  assimilation rate ( $A$ ), transpiration rate ( $E$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), and stomatal conductance ( $g_s$ ), according to the method proposed by Von Caemmerer; Farquhar (1981) [9]. Water use efficiency (WUE) was determined as the ratio of  $\text{CO}_2$  assimilation to transpiration. Rubisco carboxylation efficiency ( $A/C_i$ ) was calculated based on the ratio between  $\text{CO}_2$  assimilation rate and internal  $\text{CO}_2$  concentration in the leaf.

For chlorophyll *a* fluorescence, leaves were dark-adapted for 30 min and then exposed to a saturation pulse of  $10,000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$  of photosynthetically active photon flux density (PPFD) to obtain the following fluorescence parameters: maximum fluorescence in the dark ( $F_m$ ), maximum fluorescence in light ( $F_m'$ ), minimum fluorescence in the dark ( $F_o$ ), and minimum fluorescence in light ( $F_o'$ ). These measurements were conducted in the morning, between 8:00 and 11:00 a.m., to ensure more stable environmental conditions. The total measurement time for all treatments was approximately 2 h, with each leaf being evaluated within this timeframe to reduce the influence of diurnal variation.

These measurements were used to calculate the following:

- Maximum quantum efficiency of photosystem II ( $F_v/F_m$ ) [10];
- Effective quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) [11];
- Photochemical quenching ( $q_P$ ) [12];
- Non-photochemical quenching (NPQ) [13];
- Electron transport rate (ETR) [14];
- Non-regulated energy dissipation in PSII ( $\Phi_{\text{NO}}$ ) [14];
- Regulated non-photochemical energy dissipation in PSII ( $\Phi_{\text{NPQ}}$ ) [14].

The SPAD index was determined using a portable chlorophyll meter (Model 502, Konica Minolta, Tokyo, Japan), with three readings per leaf (left, center, and right), totaling 180 measurements.

### 2.3.2. Biochemical Analyses

Biochemical analyses of leaves were performed via spectrophotometry (BEL Photonics, SP UV/VIS, Brazil), with all measurements carried out in triplicate. Chlorophyll concentrations (*a*, *b*, and total) were quantified from 100 g of fresh tissue according to Sims and Gamon (2002) [15]. For protein and enzymatic activity analyses, 100 mg of fresh leaves were homogenized with 2 mL of  $0.1\ \text{mol L}^{-1}$  potassium phosphate buffer (pH 6.8) and 100 mg of polyvinylpolypyrrolidone (PVPP). Total soluble proteins were determined according to Bradford (1976) [16]. Superoxide dismutase (SOD) activity was measured following Giannopolitis and Ries (1977) [17]. Peroxidase (POD) activity was determined according to Teisseire and Guy (2000) [18], and catalase (CAT) activity was assessed using the methodology described by Peixoto et al. (1999) [19]. Lipid peroxidation was estimated using the TBARs method, as described by Rama Devi and Prasad (1986) [20].

### 2.3.3. Harvest, Yield, and Must Quality

Harvesting was performed when clusters reached technological maturity. At harvest, all clusters were counted and weighed to determine the total number of clusters per plant and to estimate yield ( $\text{t ha}^{-1}$ ).

For physicochemical analyses of the must, 250 berries per experimental plot ( $n = 250$ ) were used. Must was obtained by berry pressing, and soluble solids content (SS) was determined via direct refractometry using a digital refractometer (Atago<sup>®</sup>, Schmidt Haensch, Berlin, Germany) with automatic temperature compensation, with results expressed in °Brix. Must pH was measured directly using a pH meter (Micronal B-274, Mettler Toledo, Barueri, Brazil). Titratable acidity (TA) was determined by titrating 5 g of must diluted in 100 mL of distilled water with standardized 0.1 N sodium hydroxide solution, using phenolphthalein as an indicator, and expressed as g/L of tartaric acid.

Reducing sugars were quantified by the Somogyi–Nelson colorimetric method based on a glucose standard curve, with absorbance readings at 510 nm in a BEL Photonics<sup>®</sup> SP 2000 UV/Vis spectrophotometer. Results were expressed as percentage of reducing sugars per mL of must [21].

#### 2.4. Statistical Analyses

Data were subjected to analysis of variance (ANOVA), normality testing, and mean comparison using Tukey's test ( $p < 0.05$ ) in Sisvar software version 6.0 [22]. In addition, principal component analysis (PCA) was performed to characterize the interaction between grapevine cultivars, rootstocks, and training systems using Statistical Analysis Software version 4.0 (SAS).

### 3. Results and Discussion

#### 3.1. Physiological and Biochemical Performance of 'Bordô' Grapevines

A significant interaction was observed between rootstocks and training systems for physiological and biochemical parameters in 'Bordô' grapevines. Notable differences were found in the quantum efficiency of photosystem II (Fv/Fm), photochemical energy dissipation (qP), non-photochemical dissipation (NPQ), electron transport rate (ETR), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE), Rubisco carboxylation efficiency (A/Ci), chlorophyll content (Chl a, b, and total), and superoxide dismutase (SOD) activity during flowering (Table 1). Moreover, at the onset of berry ripening, significant interactions were detected for Chl a and total Chl contents, as well as for the activity of the antioxidant enzymes peroxidase (POD), SOD, and catalase (CAT) (Table 1).

The rootstocks significantly influenced the quantum efficiency, with higher values observed in grapevines grafted onto '106-8 Mgt'. Regarding the training system, the low trellis resulted in higher values for this variable. Despite these differences, all combinations exhibited values within the optimal range for photosystem II (PSII) functionality, indicating no damage to the photosynthetic apparatus of 'Bordô' grapevines (Table 1). This suggests that both rootstock/training system combinations can be recommended for the cultivation of 'Bordô' without compromising photochemical efficiency.

In addition, the high ETR values observed in grapevines trained on a high trellis and grafted onto 'IAC 766 Campinas' may reflect a more efficient electron transport capacity, possibly associated with greater light interception. However, this did not necessarily translate into higher net CO<sub>2</sub> assimilation (A), indicating that other physiological or biochemical factors may be limiting the overall photosynthetic capacity under these conditions. This indicates that this combination may be beneficial for maximizing the efficiency of light energy conversion, especially in regions with high solar radiation.



**Table 1.** Quantum efficiency of photosystem II (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), stomatal conductance (gs), transpiration rate (E), water use efficiency (WUE), Rubisco carboxylation efficiency (A/Ci), chlorophyll (Chl) a, b, and total, and superoxide dismutase (SOD) activity at flowering, and chlorophyll (Chl) a and total, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) activity at the berry ripening in ‘Bordô’ grapevines under different rootstocks and training systems.

| Flowering                                                            |                |                    |                    |        |
|----------------------------------------------------------------------|----------------|--------------------|--------------------|--------|
| Variables                                                            | Trellis System | Rootstocks         |                    | CV (%) |
|                                                                      |                | ‘IAC 766 Campinas’ | ‘106-8 Mgt’        |        |
| F <sub>v</sub> /F <sub>m</sub>                                       | Low            | 0.87 ± 0.002 aB    | 0.91 ± 0.005 aA    | 0.38   |
|                                                                      | High           | 0.82 ± 0.003 bB    | 0.91 ± 0.004 aA    |        |
| qP                                                                   | Low            | 0.43 ± 0.01 bB     | 0.50 ± 0.004 aA    | 3.19   |
|                                                                      | High           | 0.62 ± 0.02 aA     | 0.52 ± 0.016 aB    |        |
| NPQ                                                                  | Low            | 3.10 ± 0.13 aB     | 3.24 ± 0.19 aA     | 5.68   |
|                                                                      | High           | 2.31 ± 0.13 bB     | 2.94 ± 0.09 bA     |        |
| ETR<br>(μmol m <sup>-2</sup> s <sup>-1</sup> electrons)              | Low            | 101.70 ± 3.86 bA   | 106.72 ± 5.46 bA   | 4.66   |
|                                                                      | High           | 147.54 ± 9.37 aA   | 117.25 ± 4.06 aB   |        |
| g <sub>s</sub><br>(mol m <sup>-2</sup> s <sup>-1</sup> )             | Low            | 0.11 ± 0.01 aB     | 0.13 ± 0.01 aA     | 4.81   |
|                                                                      | High           | 0.10 ± 0.001 bB    | 0.13 ± 0.001 aA    |        |
| E<br>(mmol m <sup>-2</sup> s <sup>-1</sup> water vapor)              | Low            | 4.15 ± 0.19 aB     | 5.93 ± 0.24 aA     | 3.22   |
|                                                                      | High           | 3.42 ± 0.10 bB     | 5.79 ± 0.05 aA     |        |
| WUE<br>(μmol CO <sub>2</sub> (mmol H <sub>2</sub> O) <sup>-1</sup> ) | Low            | 7.40 ± 0.44 bA     | 5.94 ± 0.28 aB     | 2.03   |
|                                                                      | High           | 7.83 ± 0.29 aA     | 6.03 ± 0.28 aB     |        |
| A/Ci                                                                 | Low            | 0.21 ± 0.01 aB     | 0.29 ± 0.01 bA     | 3.65   |
|                                                                      | High           | 0.18 ± 0.01 bB     | 0.32 ± 0.02 aA     |        |
| Chl a<br>(mg 100 g <sup>-1</sup> leaves)                             | Low            | 29.75 ± 0.79 aA    | 25.68 ± 2.68 bB    | 5.99   |
|                                                                      | High           | 28.91 ± 1.74 aB    | 38.83 ± 1.97 aA    |        |
| Chl b<br>(mg 100 g <sup>-1</sup> leaves)                             | Low            | 13.07 ± 0.21 aA    | 11.43 ± 0.92 bB    | 5.18   |
|                                                                      | High           | 12.97 ± 1.29 aB    | 26.55 ± 0.50 aA    |        |
| Chl total<br>(mg 100 g <sup>-1</sup> leaves)                         | Low            | 42.82 ± 0.81 aA    | 37.12 ± 3.59 bB    | 5.39   |
|                                                                      | High           | 41.88 ± 2.78 aB    | 65.39 ± 2.44 aA    |        |
| SOD<br>(mg U <sup>-1</sup> protein)                                  | Low            | 2793.95 ± 55.74 bA | 2858.31 ± 48.46 aA | 10.30  |
|                                                                      | High           | 3526.43 ± 52.66 aA | 2577.19 ± 50.38 aB |        |
| Berry Ripening                                                       |                |                    |                    |        |
| Variables                                                            | Trellis System | Rootstocks         |                    | CV (%) |
|                                                                      |                | ‘IAC 766 Campinas’ | ‘106-8 Mgt’        |        |
| Chl a<br>(mg 100 g <sup>-1</sup> leaves)                             | Low            | 29.15 ± 3.91 bB    | 42.47 ± 0.85 bA    | 4.74   |
|                                                                      | High           | 48.44 ± 0.67 aA    | 50.54 ± 0.30 aA    |        |
| Chl total<br>(mg 100 g <sup>-1</sup> leaves)                         | Low            | 43.50 ± 5.32 bB    | 61.32 ± 1.25 bA    | 6.04   |
|                                                                      | High           | 69.03 ± 1.24 aA    | 74.94 ± 3.75 aA    |        |
| POD<br>(μmol mg <sup>-1</sup> min <sup>-1</sup> protein)             | Low            | 19.46 ± 3.03 bB    | 23.91 ± 1.02 aA    | 7.68   |
|                                                                      | High           | 24.68 ± 1.56 aA    | 18.74 ± 0.71 bB    |        |
| SOD<br>(mg U <sup>-1</sup> protein)                                  | Low            | 4036.05 ± 54.17 aA | 3824.72 ± 56.26 aA | 3.74   |
|                                                                      | High           | 4183.20 ± 50.14 aA | 3499.31 ± 55.22 bB |        |
| CAT<br>(μg mKat <sup>-1</sup> protein)                               | Low            | 18.78 ± 1.54 aB    | 30.01 ± 3.37 aA    | 10.64  |
|                                                                      | High           | 21.68 ± 1.36 aA    | 11.01 ± 0.71 bB    |        |

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

Fv/Fm is a widely used parameter to evaluate PSII efficiency and detect potential disturbances caused by environmental stress [23]. A decrease in this parameter is associated

with reduced PSII capacity to reduce the primary electron acceptor QA (quinone A), which may impair photosynthetic efficiency [24,25]. However, the results indicate that 'Bordô' grapevines did not experience significant photoinhibitory stress regardless of the rootstock and training system combination. This stability in photochemical efficiency suggests that both combinations can be used under different management conditions without negatively affecting plant physiology.

Photochemical energy dissipation (qP) was significantly influenced by the interaction between rootstock and training system. The combination of 'IAC 766 Campinas' rootstock with the low trellis presented the lowest qP values, suggesting greater energy dissipation and, therefore, more effective protection of the photosynthetic apparatus (Table 1). Although reduced qP values were observed in the combination of 'IAC 766 Campinas' rootstock and low trellis, these lower values did not indicate severe physiological stress, as the overall photochemical performance remained within optimal ranges. This suggests that the observed differences are more likely related to the specific energy dissipation strategies influenced by the training system, rather than indicating significant photodamage or functional impairment.

Non-photochemical quenching (NPQ) was higher in grapevines grafted onto '106-8 Mgt' and trained on a low trellis (Table 1). NPQ is a key photoprotective mechanism that dissipates excess absorbed light energy as heat, preventing damage to PSII [26,27]. This mechanism is related to the activation of the PsbS protein, which facilitates the conversion of chlorophyll to zeaxanthin, thereby promoting efficient dissipation of excess energy.

The electron transport rate (ETR) was higher in grapevines grafted onto 'IAC 766 Campinas' and trained on a high trellis (Table 1). High ETR values indicate greater production of ATP and NADPH, which are essential for the Calvin cycle and carbon assimilation. Therefore, the combination of 'IAC 766 Campinas' and high trellis may be associated with greater photosynthetic efficiency and, consequently, better productive performance of the vine [6].

Stomatal conductance (gs) was significantly higher in grapevines grafted onto '106-8 Mgt', reflecting greater stomatal opening and, consequently, increased CO<sub>2</sub> uptake for photosynthesis (Table 1). This increase in gs also resulted in a higher transpiration rate (E), demonstrating the close relationship between these processes. Since stomatal opening regulates CO<sub>2</sub> influx and water vapor efflux, this factor should be considered in irrigation management, especially in regions with limited water availability [28].

Water use efficiency (WUE) was higher in grapevines grafted onto 'IAC 766 Campinas', regardless of the training system (Table 1). This result suggests that this combination may be more suitable for conditions of low water availability, as it enables more efficient use of plant water resources. Furthermore, the lower transpiration rate observed in these grapevines indicates an efficient water regulation mechanism, which may contribute to reduced water consumption without compromising CO<sub>2</sub> assimilation. This characteristic may be crucial for vine adaptation in environments subject to moderate drought.

Rubisco carboxylation efficiency (A/Ci) was higher in grapevines trained on a high trellis and grafted onto '106-8 Mgt', while under the low trellis, the best results were observed with 'IAC 766 Campinas' (Table 1). As Rubisco is the key enzyme for carbon fixation in the Calvin cycle, these results indicate that the choice of rootstock and training system can directly impact the photosynthetic efficiency of the vine.

At the beginning of berry ripening, the SPAD index was significantly influenced by the rootstock, with higher values recorded in grapevines grafted onto 'IAC 766 Campinas' (Table 2). This index is directly related to chlorophyll content and serves as an indirect indicator of the plant's photosynthetic capacity. Moreover, Chl b content was also higher in this combination, suggesting better maintenance of photosynthetic activity during

this developmental phase (Table 2). Sustained high chlorophyll levels throughout the reproductive cycle may be associated with delayed leaf senescence, allowing for continued carbon assimilation until the end of fruit ripening. This trait may be advantageous in grape production aimed at achieving higher sugar accumulation, which is particularly important for ‘Bordô’, a *Vitis labrusca* cultivar used in juice and wine production.

**Table 2.** CO<sub>2</sub> assimilation rate (A), intercellular CO<sub>2</sub> concentration (Ci), SPAD index, peroxidase (POD), and catalase (CAT) activity at flowering, and SPAD index and chlorophyll (Chl) b content at the berry ripening in ‘Bordô’ grapevines under different rootstocks and training systems.

| Flowering                                                      |                      |                      |                     |                     |        |
|----------------------------------------------------------------|----------------------|----------------------|---------------------|---------------------|--------|
| Variables                                                      | Trellis System       |                      | Rootstocks          |                     | CV (%) |
|                                                                | Low                  | High                 | ‘IAC 766 Campinas’  | ‘106-8 Mgt’         |        |
| A ( $\mu\text{mol m}^{-2}\text{s}^{-1}\text{ CO}_2$ )          | 29.59 $\pm$ 4.04 a   | 27.47 $\pm$ 3.71 b   | 25.12 $\pm$ 1.60 b  | 31.94 $\pm$ 1.86 a  | 5.26   |
| Ci ( $\mu\text{mol mol}^{-1}\text{ CO}_2$ )                    | 117.06 $\pm$ 18.09 a | 117.15 $\pm$ 18.69 a | 133.87 $\pm$ 1.67 a | 100.35 $\pm$ 5.61 b | 2.76   |
| SPAD                                                           | 39.06 $\pm$ 2.29 b   | 40.34 $\pm$ 3.32 a   | 42.05 $\pm$ 1.39 a  | 37.36 $\pm$ 1.66 b  | 2.27   |
| POD ( $\mu\text{mol mg}^{-1}\text{ min}^{-1}\text{ protein}$ ) | 27.96 $\pm$ 2.47 a   | 27.19 $\pm$ 3.97 a   | 29.34 $\pm$ 3.27 a  | 25.82 $\pm$ 2.10 b  | 10.69  |
| CAT ( $\mu\text{g mKat}^{-1}\text{ protein}$ )                 | 4.74 $\pm$ 0.62 a    | 3.98 $\pm$ 0.49 b    | 4.43 $\pm$ 0.72 a   | 4.29 $\pm$ 0.66 a   | 11.49  |
| Berry Ripening                                                 |                      |                      |                     |                     |        |
| Variables                                                      | Trellis System       |                      | Rootstocks          |                     | CV (%) |
|                                                                | Low                  | High                 | ‘IAC 766 Campinas’  | ‘106-8 Mgt’         |        |
| SPAD                                                           | 40.92 $\pm$ 1.65 a   | 41.98 $\pm$ 2.02 a   | 42.56 $\pm$ 0.93 a  | 40.35 $\pm$ 1.94 b  | 3.86   |
| Chl b ( $\text{mg } 100\text{ g}^{-1}\text{ leaves}$ )         | 16.59 $\pm$ 2.87 b   | 22.50 $\pm$ 3.12 a   | 21.62 $\pm$ 3.90 a  | 17.47 $\pm$ 3.58 b  | 12.71  |

Means followed by the same lowercase letter in the row, within the same factor, do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

The activities of the antioxidant enzymes POD and CAT were significantly influenced by both the rootstock and training system (Table 2). CAT activity was higher in grapevines trained on a low trellis, whereas POD activity was greater in vines grafted onto ‘IAC 766 Campinas’, demonstrating that different management combinations can modulate the plant’s antioxidant defense mechanisms.

These results demonstrate that both rootstocks and training systems significantly affect the physiology of ‘Bordô’ grapevines. The choice of the most suitable combination should consider not only yield performance but also physiological efficiency and adaptability to the specific environmental conditions of the cultivation region.

### 3.2. Yield and Quality of ‘Bordô’ Grapevines

There was no significant interaction between rootstocks and training systems for the number of clusters per plant, yield per plant, or overall productivity of ‘Bordô’ grapevines. Therefore, the effects of each factor were analyzed independently. The highest number of clusters per plant (20.07) was observed in grapevines grafted onto the ‘IAC 766 Campinas’ rootstock, which also resulted in greater yield (2.62 kg plant<sup>−1</sup>) and productivity (10.49 t ha<sup>−1</sup>) (Table 3). These results indicate a stronger affinity between this rootstock and the scion cultivar, possibly due to its greater vigor compared to ‘106-8 Mgt’ [2]. This effect may be associated with the enhanced ability of ‘IAC 766 Campinas’ to promote a hormonal balance favorable to reproductive development, increasing the number of viable fruiting buds and raising the productive potential of the vine.



**Table 3.** Number of clusters per plant, yield, productivity, soluble solids, pH, titratable acidity, and reducing sugars of ‘Bordô’ grapes under different training systems and rootstocks.

| Variables                              | Trellis System |                | Rootstocks         |                | CV (%) |
|----------------------------------------|----------------|----------------|--------------------|----------------|--------|
|                                        | Low            | High           | ‘IAC 766 Campinas’ | ‘106-8 Mgt’    |        |
| Number of clusters per plant           | 18.25 ± 3.78 a | 19.61 ± 3.01 a | 20.07 ± 3.67 a     | 17.79 ± 2.85 b | 17.66  |
| Yield (kg per plant)                   | 2.10 ± 0.82 a  | 2.27 ± 0.71 a  | 2.62 ± 0.74 a      | 1.76 ± 0.50 b  | 24.13  |
| Productivity (t ha <sup>−1</sup> )     | 8.42 ± 3.26 a  | 9.11 ± 2.86 a  | 10.49 ± 2.98 a     | 7.04 ± 1.98 b  | 24.14  |
| Soluble solids (°Brix)                 | 15.94 ± 0.65 b | 16.88 ± 0.71 a | 16.28 ± 0.94 a     | 16.54 ± 0.69 a | 4.09   |
| pH                                     | 3.56 ± 0.10 a  | 3.59 ± 0.05 a  | 3.57 ± 0.08 a      | 3.58 ± 0.08 a  | 2.15   |
| Titratable acidity (g/L tartaric acid) | 6.1 ± 0.11 a   | 4.2 ± 0.11 b   | 5.8 ± 0.21 a       | 5.7 ± 0.14 a   | 2.98   |
| Reducing sugars (%)                    | 12.86 ± 1.70 b | 14.60 ± 1.16 a | 11.41 ± 1.06 a     | 11.19 ± 0.76 a | 3.04   |

Means followed by the same lowercase letter in the row, within the same factor, do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

The higher productivity associated with the ‘IAC 766 Campinas’ rootstock may be related to its positive effect on water and nutrient absorption and translocation, promoting greater availability of essential metabolites for plant growth. The high vigor conferred by this rootstock reduces the need for training systems that promote a larger leaf area, such as high trellises, which are primarily intended to increase the plant’s photosynthetic capacity. This is because more vigorous rootstocks typically have a greater capacity to produce growth-regulating substances, which favor both yield and grapevine quality [2,6].

Training systems did not significantly influence the number of clusters per plant, yield, or productivity of ‘Bordô’ grapevines. Therefore, the choice of training system may be based on economic and management considerations, since low trellises can reduce vineyard establishment and maintenance costs. However, high trellises resulted in higher soluble solids content in the must (Table 3), suggesting that this system may be more suitable for grape production aimed at winemaking or high-quality juice production.

Regardless of the training system or rootstock used, ‘Bordô’ grapevines exhibited high productivity. The average productivity observed in this study ranged from 7.04 to 10.49 t ha<sup>−1</sup> (Table 3), which falls within the typical range for grape cultivars used in processing, varying between 5 and 15 t ha<sup>−1</sup>, depending on vineyard density and cultural practices [29].

Soluble solids content (°Brix) and must pH showed no significant interaction between the studied factors. However, soluble solids were higher in the must of grapevines trained on high trellises (Table 3). This effect may be associated with the influence of the training system on photosynthetic rate, resulting in greater carbohydrate accumulation, which is subsequently converted into sugars. This increase in sugar content is essential for ‘Bordô’ grapes, as this variety is widely used for juice and wine production, and its final quality is directly related to the accumulation of primary and secondary metabolites [2,30].

The soluble solids values observed under all experimental conditions met the standards established by Brazilian regulations for grapes intended for processing, which require a minimum of 14 °Brix [31,32]. Moreover, the balance between titratable acidity and sugar content in the must is a key determinant of the sensory and technological quality of grape-derived products, playing a fundamental role in defining the organoleptic characteristics of juices and wines. The must pH ranged from 3.56 to 3.59, regardless of the training system or rootstock used. These values are slightly higher than the ideal range for grapes intended for processing, which is between 3.2 and 3.4. pH is an important variable in determining the quality of juices and wines, as it directly influences anthocyanin stability and color intensity in beverages [33].

Higher pH values may be associated with increased potassium accumulation in the berries, which can affect the color stability of juices and wines and influence consumer

perception of taste. This relationship should be considered when selecting appropriate vineyard management practices for specific end products.

Thus, the results demonstrate that the ‘IAC 766 Campinas’ rootstock promotes higher productivity, while the high trellis system enhances sugar accumulation—both essential traits for the quality of ‘Bordô’ grapes. Therefore, the appropriate selection of rootstocks and training systems can serve as a strategic tool for optimizing the production of grapes with desirable characteristics for different market segments.

### 3.3. Physiological and Biochemical Performance of ‘Isabel’ Grapevines

There was no significant interaction between training systems and rootstocks for the quantum efficiency of photosystem II (Fv/Fm), photochemical quenching (qP), or water use efficiency (WUE) in ‘Isabel’ grapevines. Therefore, the effects of these factors were analyzed independently (Table 4). Although Fv/Fm and qP showed statistical differences among treatments, the values obtained indicate that no damage occurred to the photochemical apparatus of the plants. This demonstrates that, regardless of the training system or rootstock used, ‘Isabel’ grapevines did not experience significant photoinhibitory stress.

**Table 4.** Quantum efficiency of PSII (Fv/Fm), photochemical quenching (qP), and water use efficiency (WUE) at flowering, and SPAD index, chlorophyll (Chl) a, b, and total content, and peroxidase (POD) activity at the berry ripening in ‘Isabel’ grapevines under different rootstocks and training systems.

| Flowering                                                         |                |                |                    |                |        |
|-------------------------------------------------------------------|----------------|----------------|--------------------|----------------|--------|
| Variables                                                         | Trellis System |                | Rootstocks         |                | CV (%) |
|                                                                   | Low            | High           | ‘IAC 766 Campinas’ | ‘106-8 Mgt’    |        |
| Fv/Fm                                                             | 0.91 ± 0.01 b  | 0.93 ± 0.01 a  | 0.93 ± 0.01 a      | 0.91 ± 0.01 b  | 0.43   |
| qP                                                                | 0.55 ± 0.07 a  | 0.46 ± 0.07 b  | 0.57 ± 0.06 a      | 0.44 ± 0.05 b  | 3.79   |
| WUE (μmol CO <sub>2</sub> (mmol H <sub>2</sub> O) <sup>−1</sup> ) | 12.31 ± 1.80 a | 4.93 ± 1.98 b  | 10.35 ± 3.86 a     | 6.89 ± 4.07 b  | 5.78   |
| Berry Ripening                                                    |                |                |                    |                |        |
| Variables                                                         | Trellis System |                | Rootstocks         |                | CV (%) |
|                                                                   | Low            | High           | ‘IAC 766 Campinas’ | ‘106-8 Mgt’    |        |
| SPAD                                                              | 45.30 ± 2.74 a | 45.93 ± 2.27 a | 47.30 ± 1.45 a     | 43.93 ± 2.09 b | 2.13   |
| Chl a (mg 100 g <sup>−1</sup> leaves)                             | 40.07 ± 3.29 a | 37.02 ± 2.13 b | 39.78 ± 3.15 a     | 37.32 ± 2.71 a | 6.17   |
| Chl b (mg 100 g <sup>−1</sup> leaves)                             | 17.47 ± 2.77 a | 17.49 ± 0.83 a | 17.26 ± 1.55 a     | 17.70 ± 2.42 a | 10.74  |
| Chl total (mg 100 g <sup>−1</sup> leaves)                         | 57.54 ± 4.46 a | 54.52 ± 2.45 a | 57.04 ± 2.29 a     | 55.02 ± 4.85 a | 6.51   |
| POD (μmol mg <sup>−1</sup> min <sup>−1</sup> protein)             | 17.12 ± 1.98 a | 15.38 ± 1.34 b | 17.42 ± 1.70 a     | 15.08 ± 1.20 b | 6.56   |

Means followed by the same lowercase letter in the row, within the same factor, do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

Water use efficiency was higher in grapevines trained on a low trellis and grafted onto the ‘IAC 766 Campinas’ rootstock (Table 4). This parameter is fundamental for the adaptation of grapevines to diverse edaphoclimatic conditions and is one of the main determinants of long-term sustainable production. In perennial crops, the selection of training systems and rootstocks that promote higher water use efficiency can be decisive for mitigating the impacts of water stress. The superior performance of the low trellis system in this regard may be associated with reduced canopy exposure to strong winds and excessive radiation, decreasing transpiration rates and contributing to a more efficient water balance.

Grapevines are frequently exposed to various adverse environmental factors, with water deficit being one of the main causes of reduced fruit yield and quality. Therefore, plants with higher water use efficiency tend to exhibit greater resilience during periods of limited water availability and climatic variability, such as fluctuations in the distribution

and intensity of precipitation. In this context, the 'IAC 766 Campinas' rootstock, when combined with the low trellis system, proved to be more effective in water conservation, making it a viable alternative for regions where water availability is a limiting factor for grapevine cultivation. Additionally, selecting rootstocks adapted to water-restricted conditions may reduce the need for supplemental irrigation, lowering production costs and contributing to a more sustainable viticulture.

There was a significant interaction between training systems and rootstocks for non-photochemical quenching (NPQ) and electron transport rate (ETR) (Table 5). The lowest NPQ value was observed in the combination of high trellis and the '106-8 Mgt' rootstock, indicating lower non-photochemical dissipation of light energy and, consequently, reduced activation of photoprotective mechanisms. However, the isolated analysis of NPQ is not sufficient to determine the occurrence of stress in the photosystems, and the evaluation of other parameters associated with photochemical efficiency is necessary. The lower non-photochemical dissipation observed may be associated with greater efficiency in light energy conversion, favoring carbon assimilation and grapevine productivity [34].

The electron transport rate (ETR) was higher in the combination of high trellis and the '106-8 Mgt' rootstock, suggesting greater efficiency in the conversion of light energy into chemical energy and in electron transport through the Calvin cycle (Table 5). Additionally, this same combination resulted in a high CO<sub>2</sub> assimilation rate, reinforcing the positive relationship between photochemical efficiency and the plant's photosynthetic capacity. This indicates that this specific interaction maximizes the efficiency of the photosynthetic apparatus, promoting better utilization of available light energy and enhancing the production of photoassimilates.

The combined assessment of gas exchange and chlorophyll a fluorescence has been widely used to differentiate stomatal and non-stomatal limitations to photosynthesis under stress conditions [35]. The interaction between high irradiance and an inadequate training system can compromise the photosynthetic apparatus's ability to efficiently utilize incident radiation, resulting in oxidative damage and degradation of photosynthetic pigments [36]. Under adverse environmental conditions, when photon absorption exceeds CO<sub>2</sub> assimilation capacity, photoinhibition of photosystem II (PSII) may occur, significantly reducing photosynthetic efficiency [37]. These findings highlight the importance of proper canopy management to optimize photosynthetic efficiency and minimize potential oxidative damage in grapevines.

A significant interaction was also observed between training systems and rootstocks for stomatal conductance (gs), transpiration rate (E), Rubisco carboxylation efficiency (A/Ci), net assimilation rate (A), and intercellular CO<sub>2</sub> concentration (Ci) (Table 5). The high trellis system promoted greater stomatal conductance when combined with the '106-8 Mgt' rootstock, indicating greater stomatal opening and, consequently, greater CO<sub>2</sub> diffusion into the leaf. On the other hand, grapevines trained on a low trellis exhibited lower gs values, suggesting more restricted stomatal aperture.

Stomatal closure, particularly under adverse environmental conditions, can reduce internal CO<sub>2</sub> concentration, limiting photosynthetic activity due to decreased substrate availability for the Calvin cycle. This limitation reduces NADPH and ATP consumption, increases NADP<sup>+</sup> concentration, and decreases the availability of electron acceptors in the transport chain, which may trigger oxidative stress.

The '106-8 Mgt' rootstock also conferred greater Rubisco efficiency (A/Ci) to the scion cultivar, although it was associated with a higher transpiration rate, resulting in lower water use efficiency (Tables 4 and 5). This outcome highlights the need to select rootstocks and training systems based on management conditions, such as irrigation availability and the climatic characteristics of the cultivation region.

**Table 5.** Non-photochemical quenching (NPQ), electron transport rate (ETR), stomatal conductance (gs), transpiration rate (E), Rubisco carboxylation efficiency (A/Ci), net assimilation rate (A), intercellular CO<sub>2</sub> concentration (Ci), SPAD index, chlorophylls a, b, and total, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) at flowering, and superoxide dismutase (SOD) and catalase (CAT) at the berry ripening in ‘Isabel’ grapevines under different rootstocks and training systems.

| Flowering                                                 |                |                    |                    |        |
|-----------------------------------------------------------|----------------|--------------------|--------------------|--------|
| Variables                                                 | Trellis System | Rootstocks         |                    | CV (%) |
|                                                           |                | ‘IAC 766 Campinas’ | ‘106-8 Mgt’        |        |
| NPQ                                                       | Low            | 3.42 ± 0.03 aB     | 4.24 ± 0.12 aA     | 3.15   |
|                                                           | High           | 3.43 ± 0.15 aA     | 2.39 ± 0.09 bB     |        |
| ETR<br>(μmol m <sup>-2</sup> s <sup>-1</sup> electrons)   | Low            | 100.63 ± 3.54 aA   | 65.03 ± 1.87 bB    | 5.01   |
|                                                           | High           | 87.12 ± 5.44 bB    | 103.57 ± 4.58 aA   |        |
| g <sup>s</sup><br>(mol m <sup>-2</sup> s <sup>-1</sup> )  | Low            | 0.03 ± 0.001 bA    | 0.04 ± 0.001 bA    | 5.34   |
|                                                           | High           | 0.10 ± 0.01 aB     | 0.31 ± 0.01 aA     |        |
| E<br>(mmol m <sup>-2</sup> s <sup>-1</sup> water vapor)   | Low            | 1.67 ± 0.02 bA     | 1.93 ± 0.13 bA     | 3.49   |
|                                                           | High           | 4.25 ± 0.13 aB     | 10.90 ± 0.25 aA    |        |
| A/Ci                                                      | Low            | 0.18 ± 0.01 aA     | 0.10 ± 0.001 bB    | 3.17   |
|                                                           | High           | 0.18 ± 0.001 aA    | 0.17 ± 0.001 aA    |        |
| A (μmol m <sup>-2</sup> s <sup>-1</sup> CO <sub>2</sub> ) | Low            | 24.69 ± 1.07 bA    | 22.44 ± 0.57 bB    | 4.68   |
|                                                           | High           | 26.83 ± 1.05 aB    | 35.13 ± 1.70 aA    |        |
| Ci (μmol mol <sup>-1</sup> CO <sub>2</sub> )              | Low            | 139.33 ± 2.96 aB   | 208.17 ± 11.49 aA  | 4.41   |
|                                                           | High           | 150.74 ± 4.32 aB   | 177.86 ± 4.54 bA   |        |
| SPAD                                                      | Low            | 36.67 ± 1.89 bA    | 35.95 ± 1.03 aA    | 3.20   |
|                                                           | High           | 42.34 ± 0.57 aA    | 34.89 ± 0.71 aB    |        |
| Chl a<br>(mg 100 g <sup>-1</sup> leaves)                  | Low            | 46.22 ± 3.92 aA    | 33.55 ± 1.07 bB    | 5.23   |
|                                                           | High           | 41.16 ± 1.96 bB    | 48.10 ± 1.68 aA    |        |
| Chl b<br>(mg 100 g <sup>-1</sup> leaves)                  | Low            | 21.94 ± 1.38 aA    | 16.62 ± 1.02 bB    | 6.80   |
|                                                           | High           | 17.37 ± 1.50 bB    | 21.92 ± 1.58 aA    |        |
| Chl total<br>(mg 100 g <sup>-1</sup> leaves)              | Low            | 68.16 ± 4.96 aA    | 50.18 ± 1.83 bB    | 4.63   |
|                                                           | High           | 58.54 ± 3.40 bB    | 70.02 ± 2.05 aA    |        |
| POD<br>(μmol mg <sup>-1</sup> min <sup>-1</sup> protein)  | Low            | 33.38 ± 4.78 aA    | 16.44 ± 1.19 bB    | 8.23   |
|                                                           | High           | 27.69 ± 1.48 bA    | 24.33 ± 0.75 aB    |        |
| SOD<br>(mg U <sup>-1</sup> protein)                       | Low            | 5040.23 ± 57.56 bA | 4824.10 ± 57.27 aA | 11.62  |
|                                                           | High           | 7193.32 ± 38.84 aA | 4769.08 ± 49.44 aB |        |
| CAT<br>(μg mKat <sup>-1</sup> protein)                    | Low            | 129.68 ± 1.93 aA   | 47.04 ± 3.33 aB    | 5.21   |
|                                                           | High           | 52.01 ± 5.11 bA    | 46.06 ± 1.92 aB    |        |
| Berry Ripening                                            |                |                    |                    |        |
| Variables                                                 | Trellis System | Rootstocks         |                    | CV (%) |
|                                                           |                | ‘IAC 766 Campinas’ | ‘106-8 Mgt’        |        |
| SOD<br>(mg U <sup>-1</sup> protein)                       | Low            | 3533.86 ± 55.18 aA | 2900.90 ± 60.04 aB | 4.69   |
|                                                           | High           | 2741.31 ± 57.92 bA | 2698.74 ± 60.09 aA |        |
| CAT<br>(μg mKat <sup>-1</sup> protein)                    | Low            | 36.80 ± 2.91 aA    | 12.95 ± 0.63 bB    | 4.75   |
|                                                           | High           | 10.98 ± 1.15 bB    | 43.29 ± 1.50 aA    |        |

Means followed by the same lowercase letter in the column and uppercase letter in the row do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

Enzymatic activity at flowering also showed an interaction between factors, with lower values observed in the combination of high trellis and ‘106-8 Mgt’ rootstock (Table 5). The lower antioxidant enzyme activity suggests reduced oxidative stress under these conditions, which is essential for achieving high yield potential, as flowering is a key phenological stage in determining the crop’s productive capacity.

SPAD index and chlorophyll contents at the beginning of berry ripening were not significantly affected by treatments (Table 4), indicating that, unlike what was observed in ‘Bordô’, leaf senescence in ‘Isabel’ was not delayed by the rootstocks evaluated.

POD activity was lower at the beginning of berry ripening in grapevines trained on high trellis and grafted onto the ‘106-8 Mgt’ rootstock (Table 4), suggesting less stressful conditions for the scion under these conditions. SOD and CAT activity also showed interactions between factors. The lowest SOD activity was observed in the combination of high trellis and ‘106-8 Mgt’, while CAT activity was lowest in the high trellis combined with the ‘IAC 766 Campinas’ rootstock (Table 4), reinforcing the influence of management factors on oxidative stress modulation in ‘Isabel’ grapevines.

### 3.4. Yield and Quality of ‘Isabel’ Grapevines

There was no significant interaction between rootstocks and training systems for the number of clusters per plant, yield, productivity, soluble solids, titratable acidity (TA), or reducing sugars (RS) in ‘Isabel’ grapevines. Therefore, the factors were analyzed independently (Table 6). The absence of interaction between factors indicates that the productive responses of this cultivar are more influenced by the individual effect of each management component, without a strong dependence between rootstock and training system.

**Table 6.** Number of clusters per plant, yield, productivity, soluble solids, pH, titratable acidity, and reducing sugars of ‘Isabel’ grapes under different training systems and rootstocks.

| Variables                              | Trellis System |                | Rootstocks         |                | CV (%) |
|----------------------------------------|----------------|----------------|--------------------|----------------|--------|
|                                        | Low            | High           | ‘IAC 766 Campinas’ | ‘106-8 Mgt’    |        |
| Number of clusters per plant           | 15.15 ± 4.88 b | 21.26 ± 5.20 a | 15.05 ± 5.05 b     | 21.37 ± 4.89 a | 19.14  |
| Yield (kg per plant)                   | 2.21 ± 0.97 b  | 2.80 ± 1.00 a  | 2.00 ± 0.99 b      | 3.01 ± 0.78 a  | 24.57  |
| Productivity (t ha <sup>−1</sup> )     | 8.86 ± 3.90 b  | 11.21 ± 3.99 a | 8.01 ± 3.94 b      | 12.06 ± 3.13 a | 24.57  |
| Soluble solids (°Brix)                 | 16.83 ± 1.18 b | 17.34 ± 1.14 a | 17.04 ± 1.01 a     | 16.93 ± 1.24 a | 3.94   |
| pH                                     | 3.52 ± 0.08 a  | 3.65 ± 0.14 a  | 3.55 ± 0.06 a      | 3.53 ± 0.09 a  | 2.01   |
| Titratable acidity (g/L tartaric acid) | 8.6 ± 0.15 a   | 8.7 ± 0.11 a   | 8.9 ± 0.15 a       | 8.4 ± 0.10 a   | 4.89   |
| Reducing sugars (%)                    | 10.40 ± 1.88 b | 13.17 ± 2.84 a | 12.44 ± 3.07 a     | 11.93 ± 2.23 a | 3.61   |

Means followed by the same lowercase letter in the row, within the same factor, do not differ significantly according to Tukey’s test at 5% probability. Note: CV (%): coefficient of variation.

Grapevines trained on a high trellis exhibited a higher number of clusters per plant, resulting in increases of 21.07% in yield and 20.96% in productivity compared to the low trellis system. These results suggest that this training system provides greater compatibility with the ‘Isabel’ cultivar, promoting greater shoot growth and, consequently, a higher number of fruitful and fertile buds. The literature indicates that bud fertility is a key factor for grapevine fruiting [38,39]. This may be related to greater light incidence on the shoots during the bud differentiation period, promoting a physiological stimulus for floral induction and enhancing the reproductive potential of the plant.

A significant effect of rootstocks was observed on the number of clusters per plant, yield, and productivity, with the best results obtained in grapevines grafted onto the ‘106-8 Mgt’ rootstock (Table 6). This rootstock led to a 4.05 t ha<sup>−1</sup> increase in productivity in ‘Isabel’ grapevines, suggesting better compatibility with this cultivar. Although ‘IAC 766 Campinas’ is recognized for its high vigor [2], the best results were observed with ‘106-8 Mgt’, indicating that the genetic interaction between scion and rootstock may be more relevant than the isolated vigor of the rootstock. Furthermore, the grapevine’s response to grafting is highly influenced by soil fertility levels, making it challenging to extrapolate these results to other environmental conditions [40].



Regardless of the training system or rootstock used, 'Isabel' grapevines exhibited high productivity, ranging from 8.01 to 12.06 t ha<sup>-1</sup>, values consistent with the average productivity of grape cultivars intended for processing, which typically ranges from 5 to 15 t ha<sup>-1</sup> [29].

Soluble solids content (SS) was significantly higher in grapes from vines trained on high trellises (17.34 °Brix), which may be associated with enhanced photosynthetic rate due to the greater leaf area promoted by this training system. Higher photosynthetic efficiency leads to greater carbohydrate accumulation in the berries, resulting in sweeter grapes [2], a key trait for 'Isabel' grapes, widely used in the production of Brazilian juices and wines [33,41]. The impact of the training system on the chemical composition of the berries may have direct implications for must quality and, consequently, for consumer acceptance of the final product.

Regardless of the training system or rootstock used, soluble solids content exceeded the minimum required by Brazilian legislation for grapes intended for processing, which establishes a minimum of 14 °Brix [31,32]. The same was observed for titratable acidity, with values ranging from 8.4 to 8.9 g/L tartaric acid, within the legal limit of 9.0 g/L [31,32].

These results indicate that both the high trellis system and the '106-8 Mgt' rootstock provide advantages for 'Isabel' grapevines, promoting higher productivity and improved fruit quality. The selection of the most suitable management system should consider both commercial production goals and local environmental conditions, ensuring a balance between yield and fruit quality.

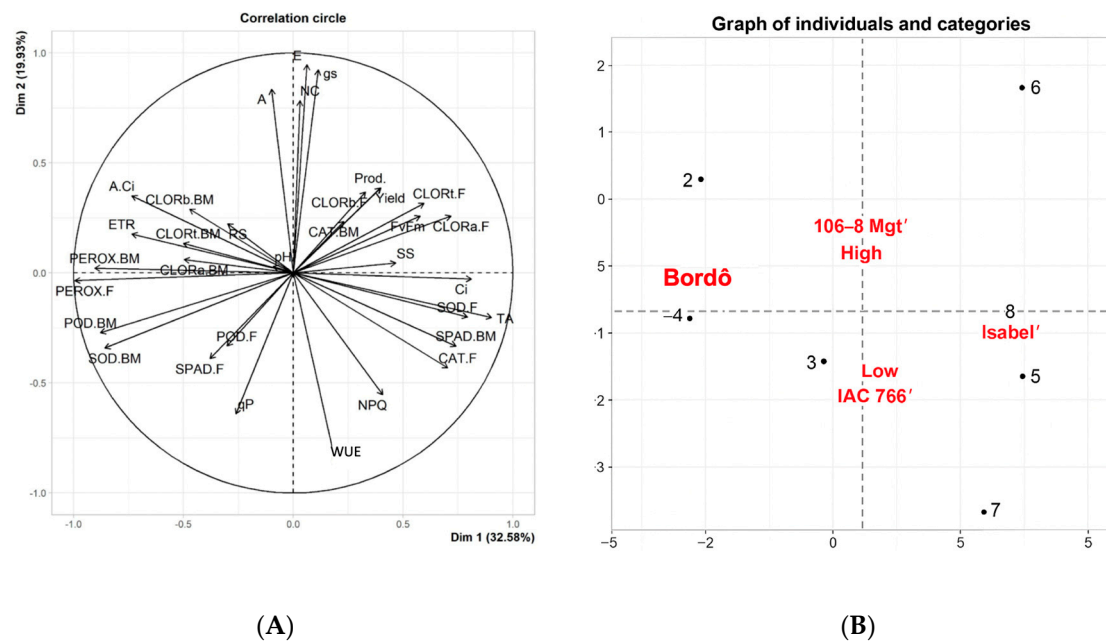
### 3.5. Principal Component Analysis of *Vitis labrusca* Grapevines

Principal component analysis (PCA) explained 52.51% of the total variance among treatments, with Dim 1 accounting for 32.58% and Dim 2 for 19.93% (Figure 2A,B). Dim 1 was mainly associated with higher photosynthetic efficiency and productive variables, such as Fv/Fm, soluble solids (SS), yield, productivity, total chlorophyll at flowering (ChlT.F), and Rubisco carboxylation efficiency (A/Ci). Dim 2 was positively correlated with gas exchange traits, including transpiration rate (E), stomatal conductance (gs), and net CO<sub>2</sub> assimilation (A), reflecting stomatal activity under varying water demand.

The distribution of treatments (Figure 2B) showed that 'Isabel' grafted onto 'IAC 766 Campinas' under a low trellis was positioned in the positive quadrant of Dim 1, associated with higher WUE, SPAD index, and reduced E, indicating better water use and pigment stability. In contrast, 'Bordô' grafted onto '106-8 Mgt' and trained on high trellis clustered with higher A, A/Ci, ETR, and chlorophyll content, reflecting increased photosynthetic capacity and productivity.

Antioxidant enzyme activities (POD, SOD, and CAT) were projected opposite to yield components, indicating that higher oxidative stress responses were linked to lower productivity. This pattern supports a trade-off between energy allocation to defense mechanisms and yield formation.

These findings confirm the influence of rootstock and training system combinations on physiological and productive responses in *Vitis labrusca*, highlighting the importance of targeted selection based on cultivar behavior and environmental conditions.



**Figure 2.** Principal components of photochemical, biochemical, and productive variables for the interaction between two *Vitis labrusca* grapevines ('Bordô' (B) and 'Isabel' (I)) combined with two rootstocks ('IAC 766 Campinas' (766) and 106-8 'Mgt' (106)) under two training systems (low trellis (b) and high trellis (a)). (A) Correlation circle of physiological, biochemical, and productive variables. (B) Distribution of the interaction between cultivar, rootstock, and training system along the principal components. Note: Quantum efficiency of PSII (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), transpiration rate (E), water use efficiency (WUE), Rubisco carboxylation efficiency (A/Ci), stomatal conductance (gs), net CO<sub>2</sub> assimilation rate (A), and intercellular CO<sub>2</sub> concentration (Ci); SPAD index at flowering (SPAD.F) and at the beginning of berry ripening (SPAD.BM); chlorophyll a (CLORa.F; CLORa.BM), chlorophyll b (CLORb.F; CLORb.BM), and total chlorophyll (CLORT.F; CLORT.BM); peroxidase (POD.F; POD.BM), superoxide dismutase (SOD.F; SOD.BM), catalase (CAT.F; CAT.BM), and lipid peroxidation (PEROX.F; PEROX.BM) at flowering (F) and at the berry maturation (BM); soluble solids (SS), titratable acidity (TA), and yield components: production (Prod.) and yield (Yield).

#### 4. Conclusions

The interaction between rootstocks and training systems influences photosynthetic efficiency, water use, and productivity in *Vitis labrusca* grapevines. In 'Bordô', grapevines grafted onto '106-8 Mgt' and trained on a high trellis showed a 27.1% higher net CO<sub>2</sub> assimilation rate (A) and a 77.8% greater Rubisco carboxylation efficiency (A/Ci) compared to the least efficient combination. In contrast, 'Isabel' grapevines grafted onto 'IAC 766 Campinas' and trained on a low trellis exhibited a 77.8% increase in water use efficiency (WUE), making it more suitable for regions with limited water availability.

Regarding yield performance, 'IAC 766 Campinas' promoted a 48.9% increase in productivity in 'Bordô' compared to '106-8 Mgt', while in 'Isabel', the use of '106-8 Mgt' resulted in a 50.6% higher productivity compared to 'IAC 766 Campinas'. Additionally, training 'Isabel' grapevines on a high trellis increased yield by 26.5% and the number of clusters per plant by 41.3% relative to the low trellis system.

These results reinforce that the strategic selection of rootstock and training system combinations can substantially enhance grapevine physiological efficiency and yield, contributing to the sustainability and productivity of viticulture under subtropical climate conditions.

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