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SHORT COMMUNICATIONS

Harnessing natural elicitors: enhancing post-harvest resistance in Brazilian grapes with β-D-1,6-Glucans

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ABSTRACT

This study evaluated a β-D-1,6-glucan derived from the regional oomycete *Lasiodiplodia theobromae*, comparing its effectiveness with the traditional elicitor ASM in inducing post-harvest resistance in grapes and promoting sustainable practices. Commercial grape varieties (Bordô and Niágara Rosada) were sourced locally and treated with water (Control), β-D-1,6-glucan (200 mg L^{-1}), or ASM (Bion® 500 WG, 100 mg L^{-1}). Grapes treated with β-D-1,6-glucan consistently showed a tendency for lower AUDPC (Area Under the Disease Progress Curve) values across both varieties and injury conditions. In wounded Niágara grapes, ASM initially showed a tendency to reduce AUDPC until the 8th day, but β-D-1,6-glucan had the lowest value by day 10. β-D-1,6-glucan-treated fruits also had the highest Total Phenol Content (TPC) and pulp firmness compared to those treated with ASM or water. This study underscores β-D-1,6-glucan's potential as a natural alternative to synthetic fungicides, enhancing grape resistance to *Glomerela cingulata*, extending shelf life, and reducing environmental and health impacts.

KEYWORDS: natural elicitors, β-D-1,6-glucan, oligosaccharides, plant innate immunity, fruit storage

INTRODUCTION

Brazil is an important player in global fruit production, being the third largest producer in 2023, with an annual production of 45 million tons (FAO, 2024). Grapes, a significant perennial crop, are among the top five fruits produced in the country, with the southern region being responsible for 70 % of grape production and 85 % of grapes used for processing (Macueia *et al.,* 2024). In 2022, Brazil produced 1.45 million tons of grapes, cultivated on approximately 75,000 hectares, achieving an average productivity of 19 tons per hectare (IBGE, 2024).

Agricultural pathogens and pests significantly threaten productivity and food security, with susceptible crops facing losses of over 25 % (Savary *et al.,* 2019). *Glomerella cingulata*, responsible for ripe grape rot, poses a serious challenge in southern Brazil, affecting grapes both pre- and post-harvest (Escher, 2020).

The push for sustainable practices has underscored the need for alternatives to synthetic agrochemicals, not only to improve production conditions but also to enhance post-harvest preservation of ripe fruits (Asgarian *et al.,* 2024). Natural elicitors, which mimic pathogen-associated molecular patterns (PAMPs), hold promise in enhancing plants' innate immune responses (Scariotto *et al.,* 2021; Dallacorte *et al.,* 2023). However, their commercial use is limited due to insufficient understanding of their effects on plant metabolism. Research into immune receptors and microbial effectors is crucial in bridging this gap, supporting the use of natural elicitors for crop protection (Héloir, 2019; Mendoza-Mendoza *et al.,* 2023).

β-1,3-glucans are well-known plant immune triggers, while β-D-1,6-glucans have received less attention (Robinson & Bostock, 2015; Suchoronczek *et al.,* 2018). Considering their potential as microbe-associated molecular patterns (MAMPs), it is timely to reassess the efficacy of β-glucans in enhancing pre- and post-harvest crop resistance (Fesel & Zuccaro, 2016).

Derived from oomycetes, β-D-1,6-glucans are glucose biopolymers with branched structures. Their biotechnological properties depend on chain size and branch types (Queiroz *et al.,* 2019). After extraction and purification, they induce phytoalexin biosynthesis, enhance antioxidant compounds, and modify plant cell wall composition, providing induced resistance against pathogen infections (Riseh *et al.,* 2023).

This study evaluates the efficacy of β-D-1,6-glucan from oomycete *Lasiodiplodia theobromae* compared to a traditional elicitor in inducing post-harvest resistance in two popular grape cultivars in southwest Paraná, Brazil. The goal is to enrich the literature and support the development of low-toxicity products that extend food shelf life, reduce waste, and explore new applications.

MATERIALS AND METHODS

The experiments were conducted at the Plant Biochemistry and Molecular Physiology Laboratory of UTFPR (Federal University of Technology—Paraná), Pato Branco, PR, Brazil, and the Phytopathology Laboratory of IFPR (Federal Institute of Paraná), Palmas, PR, Brazil.

1. Analysed samples and treatments

Commercial grape varieties (Bordô and Niágara Rosada) from conventional cultivation were purchased at local markets in Mariópolis, PR, Brazil. The Niágara Rosada and Bordô cultivars were selected due to their prevalence in the Pato Branco region. Uniform bunches were selected based on size (40–45 berries per bunch, with an average berry diameter of approximately 2.4 cm), firmness, colour, and absence of disease or damage, all at the same degree of ripeness. These bunches were placed in plastic trays to retain moisture and kept at an average temperature of 25 °C under a 12-hour photoperiod. The bunches were disinfected with 70 % alcohol and placed in plastic trays to create a humid chamber, where they were then sprayed with different treatments: control (Water), β-D-1,6-glucan (lasiodiplodian, 200 mg L^{-1}) (Figure 1), and Acibenzolar-S-Methyl (ASM, Bion® 500 WG, 100 mg L^{-1}) as a positive control. Treatments were applied under two conditions: with and without induced injury. Injuries were made at random, and the experiment was structured as a two-factorial design (treatment and injury) with samples arranged in a randomised block design and five replicates (one bunch per replicate). β-D-1,6-glucan (Figure 1) was produced following the protocol of Cunha *et al.* (2012), with purification conducted via dialysis using a membrane (1.3 inches, MW 11331, Sigma-Aldrich) at 4 °C for seven days.

2. *Glomerella cingulate* infection

The grape berries in the wounded treatments were punctured with an entomological pin (2 mm deep punctures on 10berries). Additionally, 12 hours after treatment application, the grapes were inoculated by spraying a suspension of *Glomerella cingulata* conidia (10^5 conidia mL^{-1}).

The fungus used in this experiment was obtained through indirect isolation from a symptomatic leaf of the Niagara Rosada grape cultivar, collected in a commercial vineyard in Mariópolis, Paraná, Brazil (26° 21′ 58″ S, 52° 33′ 32″ W) in February 2019. Following collection, a monospore isolation procedure was performed, and the isolate was maintained on potato dextrose agar (PDA) at 25 °C in darkness. It was subsequently preserved in the phytopathology laboratory of the Instituto Federal do Paraná (IFPR), Palmas, Paraná, Brazil, until the study was conducted. The number of infected berries per bunch was monitored over 10 days.

3. Content of total phenolic compounds (TPC)

The total phenolic content was determined on the 10th day after elicitation, using 3 berries per bunch, including skin and pulp, were collected, rapidly immersed in liquid nitrogen

(–180 °C), macerated into powder, and stored in Eppendorf tubes in an ultra-freezer (–80 °C, Coldlab, CL 800-86 V, Brazil). The extraction of phenolic compounds was carried out with 1 g of macerated fruit and 5 mL of extraction buffer (sodium borate buffer) with a final concentration of 0.2 mg mL^{-1}. For quantification, the Folin–Ciocalteu spectroscopic method was used at 740 nm (Shimadzu, UV-1800, Japan), and the results were expressed in mg GAE g^{-1} (GAE: gallic acid equivalent) according to the methodology proposed by Singleton *et al.* (1999).

4. Fruit firmness

Fruit firmness (pulp and fruit) was measured using 3 berries per bunch, chosen at random, on the 10th day after elicitation, using a benchtop digital texture analyzer (Stable Micro Systems, TA.XT plusC, UK) with an 8 gauge probe. mm, the results were expressed in N/m².

5. Disease severity

Disease severity was assessed daily after the first symptoms appeared by counting the number of infected berries per bunch over ten days to calculate the Area Under the Disease Progress Curve (AUDPC). The AUDPC quantifies disease intensity over time by averaging disease severity at consecutive time points and multiplying it by the time intervals between observations. This final value reflects accumulated disease throughout the study, allowing for comparison across conditions, such as locations or treatments (APS, 2007). The AUDPC was calculated using the equation from Campbell and Madden (1990):

$$AUDPC = \sum_{i=1}^{n}[(Y_{i+1}+Y_i)/2]*[(T_{i+1} - T_i)] \quad (1)$$

where:

- **n** is the number of observations,
- $\mathbf{Y_i}$ is the disease severity at the *i*-th observation,
- $\mathbf{T_i}$ is the time in days at the *i*-th observation.

6. Physical-chemical analysis

The colour of the berries was measured using a Konica Minolta Chroma Meter CR-400 (Japan), which provided the L*, a*, and b* coordinates. ºBrix measurements were corrected to 25 °C. The pH was determined using a digital pH meter.

7. Statistical data analysis

We conducted ANOVA (analysis of variance) and Tukey's mean comparison test at a significance level of 5 %. The Gene software (version 1990.2023.45) was employed for the analyses, while the OriginPro software (version 10.1.0.178) was used for graph construction.

RESULTS AND DISCUSSION

In general, the AUDPC values showed a tendency to be lower for fruits treated with β-D-1,6-Glucan, regardless of the variety (Bordô or Niagara Rosada) and whether the fruits were wounded or not (Figure 2A, 2C, and 2D). For wounded Niagara fruits, ASM showed a tendency for lower AUDPC values up to the 8th day of evaluation (Figure 2B); however, by the 10th day, β-D-1,6-Glucan exhibited the lowest AUDPC value (Figure 2B). These AUDPC values align with the visual symptoms observed in Figure 1, where both varieties treated with water show more pronounced disease symptoms compared to those treated with β-D-1,6-Glucan. American cultivars Bordô and Niágara Rosada resist *G. cingulata*, which harms fruit and vegetable quality (Bueno Júnior *et al.,* 2022; Wang *et al.,* 2015). However, extensive cultivation has increased disease incidence. Natural compounds, an alternative to synthetic fungicides, form a biofilm on fruits that stimulates defence compound production, inhibiting fungi and pathogens (Matrose *et al.,* 2021). β-D-1,6-Glucan has immunomodulatory and antioxidant properties (Kagimura *et al.,* 2015), stimulating enzymes like phenylalanine ammonia-lyase (PAL) involved in phenolic and flavonoid

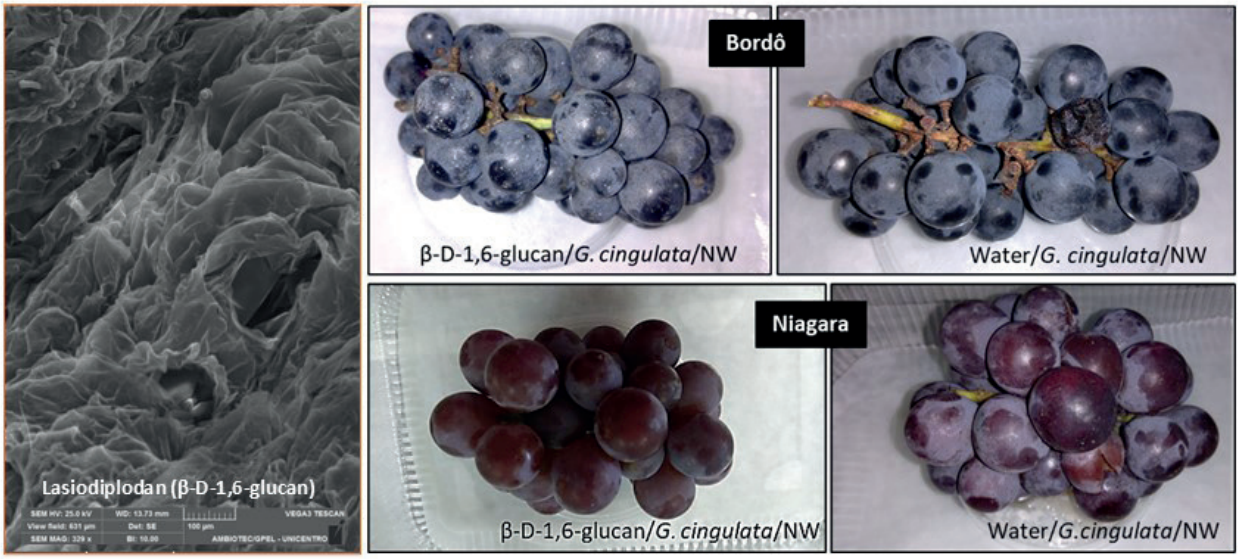


FIGURE 1. Micrographs (SEM) of native Lasiodiplodan (β-D-1,6-glucan) at 329× magnification. Grape bunches of the varieties Bordô and Niagara, treated with β-D-1,6-glucans and without (water), were challenged by *Glomerella cingulata*. NW = no injury.

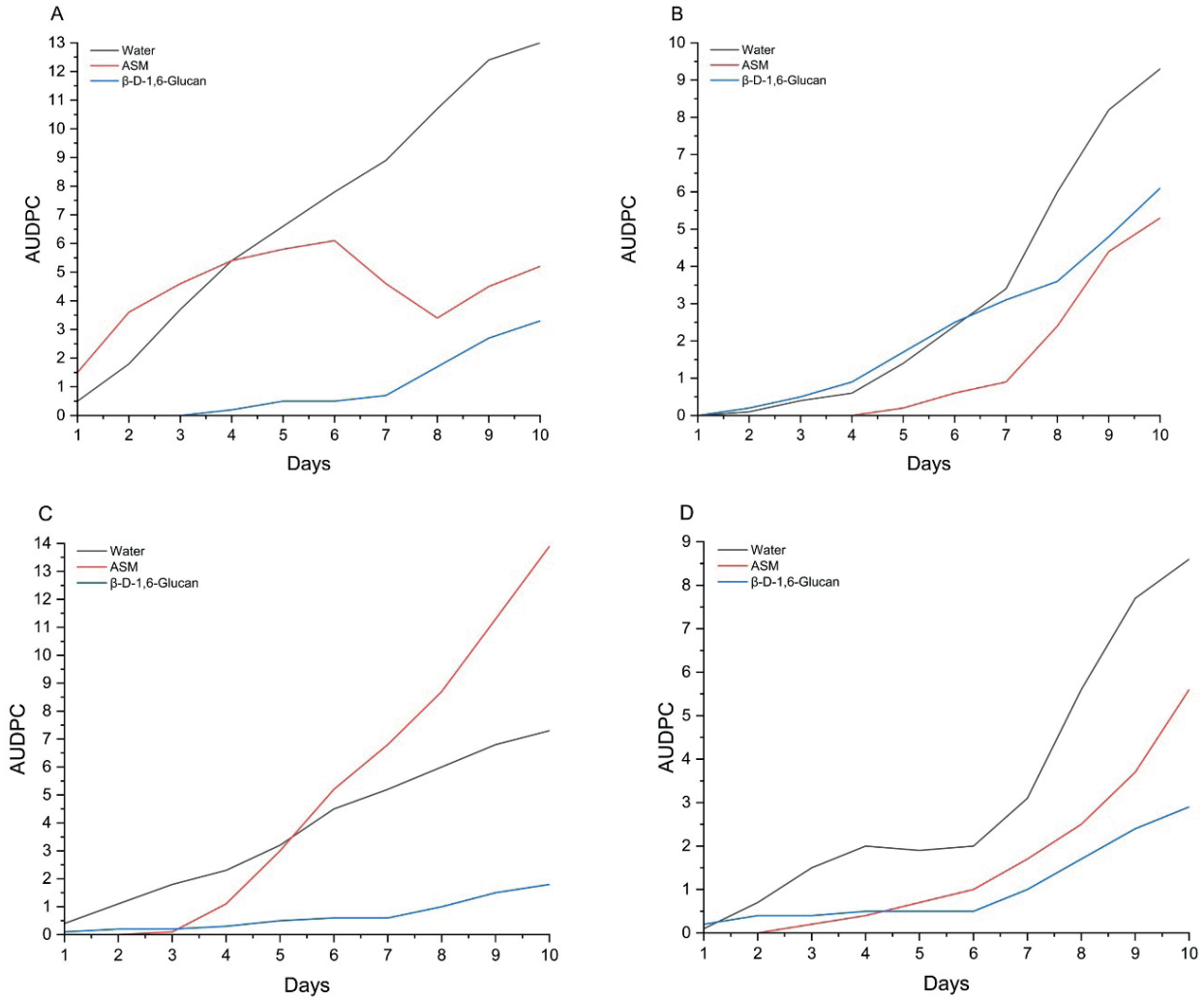


FIGURE 2. Area Under the Disease Progress Curve (AUDPC) in grape fruits treated by Acibenzolar-S-methyl (ASM), β-D-1,6-Glucan, and Water, and challenged by *Glomerella cingulata*: Niagara grapes without wounds (A) and with wounds (B); Bordô grapes without wounds (C) and with wounds (D).

biosynthesis, which protect against storage-related fungal stress (Chen *et al.,* 2006; Kong *et al.,* 2024).

As shown in Figure 3, the highest Total Phenol Content (TPC) values were also found for the elicitor β-D-1,6-Glucan. Niagara fruits treated with β-D-1,6-Glucan exhibited the greatest pulp firmness compared to those treated with ASM and water (Figure 4). Lignin deposition strengthens tissues against microbes. In studies by our group, applying elicitors (Harpin and ASM) to strawberries enhanced post-harvest defences by boosting PAL enzyme activity (Scariotto *et al.,* 2021), crucial for phenol production. This elevated PAL activity induced defence responses, leading to increased pulp firmness via lignin deposition (Danner *et al.,* 2008; Alamino *et al.,* 2013; Tomazeli *et al.,* 2016). Natural elicitors like β-D-1,6-Glucan, a pathogen-associated molecular pattern (PAMP), enhance plant immunity. Our group also found β-D-1,6-Glucan reduced disease severity, elevated antioxidant enzyme activity (e.g., superoxide dismutase), and promoted phenol accumulation in grapes and apples (Suchoronczek *et al.,* 2018). The physicochemical analysis showed no statistically significant differences among treatments. The overall averages were: Brix 12.6, pH 3.4, and colour coordinates L* 17.54, a* –0.44, and b* 3.1.

CONCLUSION

Fruits treated with β-D-1,6-Glucan consistently showed a tendency for lower AACPD values across all conditions (Bordô and Niágara Rosada, both wounded and unwounded). For wounded Niágara grapes, ASM initially showed a tendency to reduce AACPD until the 8th day, but β-D-1,6-Glucan had the lowest values by the 10th day. β-D-1,6-Glucan also produced the highest TPC values, regardless of variety or injury, leading to greater pulp firmness compared to ASM and water in Niágara grapes. This study highlights β-D-1,6-Glucan's potential as a natural alternative to synthetic fungicides for enhancing grape resistance to *G. cingulata*, extending shelf life, and minimising environmental and health impacts. Future studies should explore its role in stimulating plant immunity, as our results suggest β-D-1,6-Glucan may provide effective, low-impact fungal disease resistance.

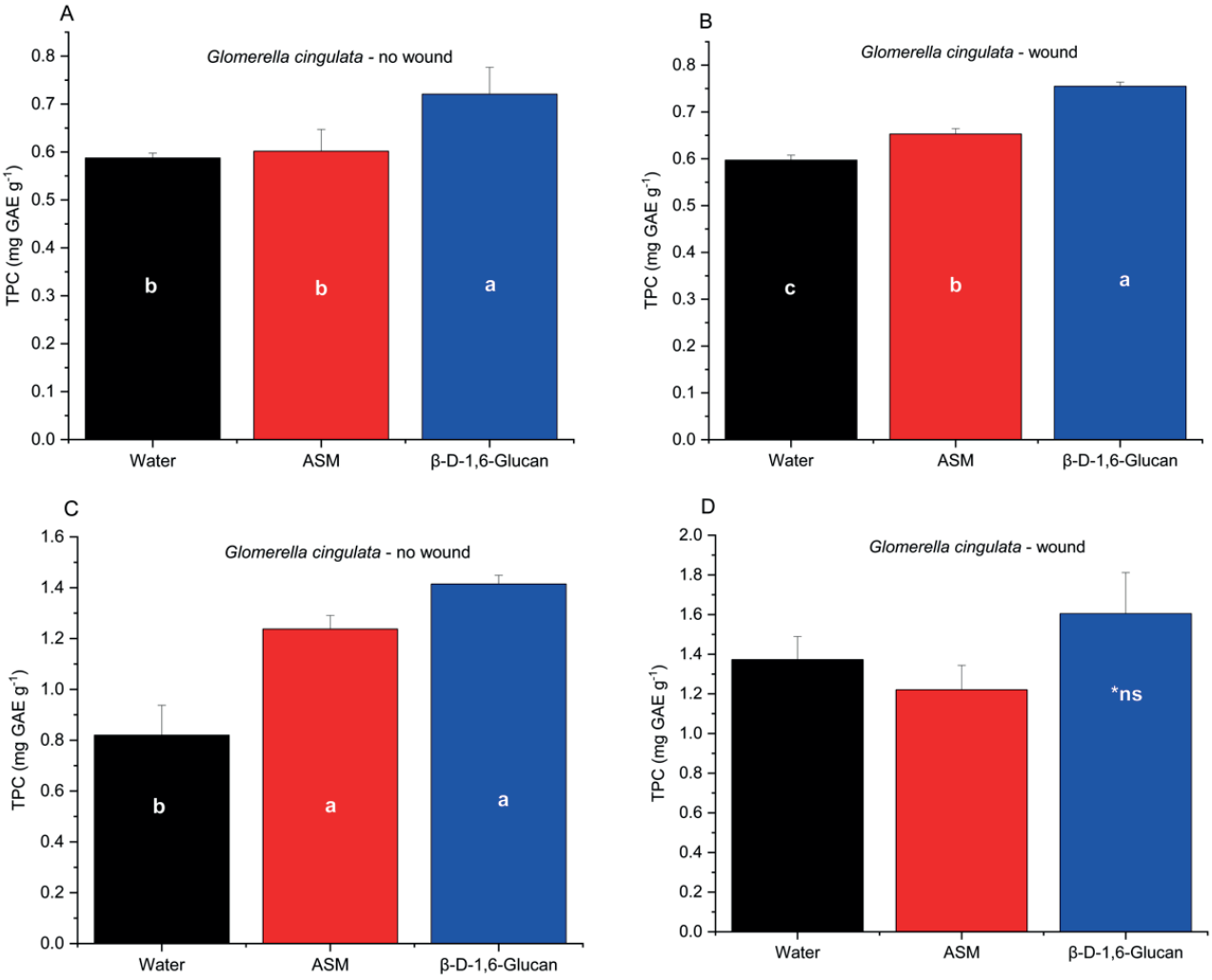


FIGURE 3. Total Phenol Content (TPC) in grape fruits treated by Acibenzolar-S-methyl (ASM), β-D-1,6-Glucan, and Water, and challenged by *Glomerella cingulata*. Histograms show Niagara grapes without wounds (A) and with wounds (B), and Bordô grapes without wounds (C) and with wounds (D). Means followed by different letters differ significantly according to Tukey's test at the 5 % significance level. *ns = not significant.

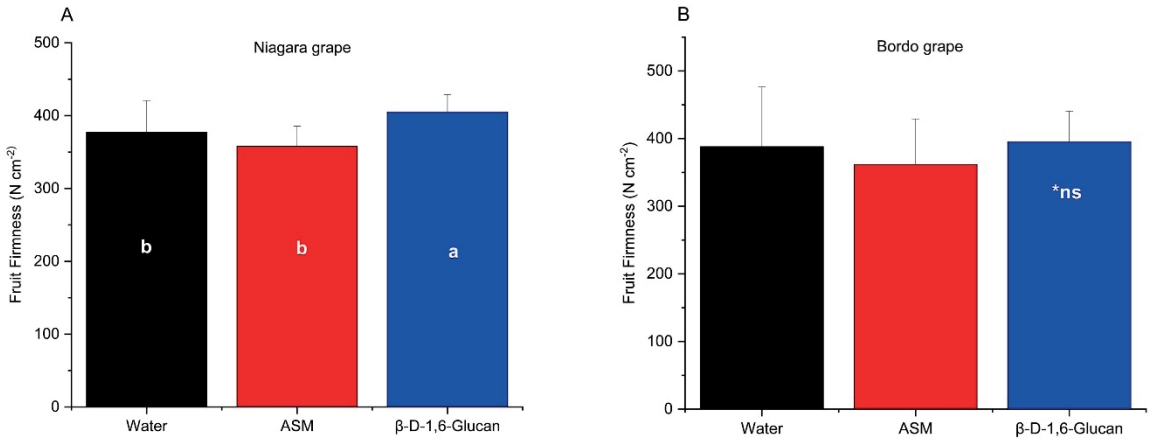


FIGURE 4. Pulp Firmness (averages of wound and not wound) in grape fruits of Bordô and Niagara varieties treated by Acibenzolar-S-methyl (ASM), β-D-1,6-Glucan and Water, and challenged by *Glomerella cingulata*. Means followed by different letters differ significantly according to Tukey's test at the 5 % significance level. *ns = not significant.

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