EXTENDED ABSTRACT

Effect of cytokinin and auxin application on double cropping performance in *Vitis vinifera* L.: preliminary findings

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INTRODUCTION

Double cropping is gaining attention in viticulture as a strategy to face climate change, offering the potential to boost yields and improve fruit composition in temperate regions (Lavado Rodas et al., 2023; Poni et al., 2021). This technique exploits the capacity of dormant buds to burst within the same growing season through a process known as "bud forcing," which involves the removal of primary shoot apices and lateral shoots. Critical to the success of this method is the proper timing, which must align with the completion of bud induction to ensure a viable forced crop.

Bud dormancy in grapevines comprises endodormancy, ecodormancy, and paradormancy. Paradormancy is regulated by hormonal interactions between vegetative organs, notably involving auxins (AUX) and cytokinins (CK) (Lavee and May, 1997). While previous studies have demonstrated the necessity of fully trimming shoots and eliminating laterals to release paradormancy and bud growth (Martínez-Moreno et al., 2019), the role of internal biochemical limitations remains less understood.

In subtropical and tropical viticulture, hydrogen cyanamide is commonly used to synchronize budburst, with studies confirming its effect in increasing CK levels and enhancing bud outgrowth (Leonel et al., 2015). The influence of AUX and CK on bud development has also been documented in woody species, with AUX generally inhibiting and CK promoting bud release and meristem activity. Mechanical manipulation of apical dominance through topping or bending disrupts AUX transport and facilitates bud activation (Cline, 1991), suggesting that exogenous hormonal treatments might further modulate this balance.

Limited studies has explored the impact of exogenous hormones on paradormancy release in *Vitis vinifera* L., especially in relation to the success of forced secondary crops. Studies in laboratory have identified gene expression differences related to hormonal control, but exclude the whole-plant physiological interactions (Pérez and Noriega, 2018).

RESEARCH OBJECTIVES

This research aims to assess the effectiveness of exogenous applications of 6-Benzyladenine (BA), a synthetic cytokinin and Naphthaleneacetic acid (NAA), a synthetic auxin, in enhancing budburst from dormant buds when forcing is applied at the groat-size stage in *Vitis vinifera* L. The objectives are (1) to determine whether the two growth regulators can influence dormant bud growth; and (2) to assess the vegetative and productive performances of both the primary and forced canopies following treatment. These insights are expected to contribute to optimizing double cropping protocols in temperate viticultural systems.

MATERIAL AND METHODS

Experimental design

The study was conducted in a 7-year-old vineyard planted with *Vitis vinifera* L. cv. 'Ortrugo' at the Università Cattolica del Sacro Cuore, Piacenza, Italy. Vines were trained to a spur-pruned cordon system with six two-node spurs per vine.

The study followed a randomized block design, and five treatments were tested: (1) untreated and unforced control (UC); (2) unforced vines treated with 6-Benzyladenine (CBA); (3) forced vines without growth regulator (FR); (4) forced vines treated with cytokinin (FBA); and (5) forced vines treated with Naphthaleneacetic acid (FNAA). Forcing was applied on 6 June 2024 at the groat size (BBCH 73) phenological stage. Forcing involved trimming all shoots above the eighth node and removing all growing laterals.

Growth regulators were applied immediately after forcing. Cytokinin treatment consisted of 6-Benzyladenine (BA) at 50 mg/L (Exilis®, SAFAPAC Ltd., UK), while auxin treatment involved α-Naphthaleneacetic acid (NAA) at 100 mg/L (Germon®, Diachem, Italy). In CBA vines, only the basal eight nodes were sprayed, and laterals were removed in the same canopy section.

Growth and physiology

Before treatment application, 30 basal, 30 apical, and 30 lateral leaves were sampled from extra vines and processed with a LI-3050A area meter (LI-COR, USA) to determine average leaf area. At forcing, all removed leaves (primary and lateral) were counted. Total removed leaf area was estimated by multiplying the number of removed leaves by the respective average leaf area. The remaining leaf area on the eight basal nodes was then calculated. Final total leaf area per vine was obtained at the end of the season by counting all primary, lateral, and forced nodes and multiplying them by their corresponding average leaf blade areas.

Phenological development was monitored in both the primary and forced canopies based on the BBCH scale. At harvest, leaf samples from primary, lateral, and forced shoots were remeasured to assess final surface areas. Shoot number and shoot fruitfulness (defined as clusters per shoot) were recorded for both the primary and forced canopies.

Photosynthesis, transpiration and stomatal conductance were measured on 20 September 2024 using an LCi T portable gas analyzer (ADC Bioscientific Ltd., UK).

Yield components and Fruit composition

Grapes were sampled weekly to monitor ripening. Primary grapes were harvested on 28 August, while forced grapes were harvested on 14 October. Yield components per vine were assessed by weighing all clusters, counting their number, and calculating average cluster weight. Three clusters per vine were analyzed for cluster mass, rachis length, and compactness (mass-to-length ratio). All berries were counted, weighed, and sampled for chemical analysis. The concentration of total soluble solids (TSS) was measured with a digital refractometer (SMART-1, Atago, Bellevue, WA, USA), while pH values were determined using a pH meter (pH 60 VioLab, Giorgio Bormac, Carpi, MO, Italy). Titratable acidity (TA) was calculated as grams per liter of tartaric acid equivalents, using titration with 0.1 N NaOH up to a pH endpoint of 8.2, carried out with a potentiometric titrator (AT 1000 Series, Hach Company, Loveland, CO, USA). A subsample of 50 berries was frozen for later analysis of organic acids by HPLC (Agilent Technologies, USA), using a Synergy 4u Hydro-RP80 A column and UV detection at 210 nm. Potassium (K^+) concentration was assessed by ion-selective electrode.

Data analysis

Statistical analysis was conducted using one-way ANOVA and post hoc Student Newman Keuls (SNK) test at $p \leq 0.05$. Comparisons between primary and forced canopies were performed with the Student's t-test. Analyses were conducted using IBM SPSS Statistics v29.0.

RESULTS

Phenology

Budburst occurred seven days after forcing (F) application and was completed within two weeks. In the primary crop, 61 days elapsed from budburst to flowering phase, while 33 days in the forced crop. Primary clusters were harvested on 28 August 2024, aligning with the veraison of the forced crop, which was harvested 47 days later, on 14 October 2024.

Vegetative Growth and Physiological Status

At treatment application, an average of 1.4 m² of leaf area was removed per vine in F, FBA, and FNAA. Forced shoot emergence averaged 15 in F, 17 in FBA, and only 3 in FNAA, with FBA showing the highest forced to primary shoot ratio (106%). In contrast, FNAA showed low shoot emergence (21%), and no forced buds developed in CBA vines.

Final leaf area (LA) was comparable between FBA and UC, whereas FNAA had the lowest. Forced leaves displayed significantly higher net assimilation rates (11.09 μmol m^{-2} s^{-1}) than primary leaves (7.51 μmol m^{-2} s^{-1}), along with increased transpiration and stomatal conductance.

Vine Yield and Fruit Composition

FNAA failed to produce forced grapes due to low bud outgrowth. Conversely, FR and FBA produced 11 and 16 forced clusters per vine, respectively, which were smaller and less compact than primary clusters, but with higher fruitfulness per shoot.

Total yield was highest in FBA (4.78 kg/vine), followed by FR (3.62 kg/vine), significantly higher than other treatments which relied solely on primary crop yield. The LA to yield ratio (LA/Y) exceeded 1.6 m²/kg in UC and CBA, while it approached unity in FR and FBA (0.99 m²/kg). After primary harvest, LA/Y for the forced crop rose to 2.8 m²/kg in FR and FBA.

Forcing significantly reduced total soluble solids (TSS) in primary grapes compared to untreated vines. Forced grapes showed the highest TSS (21–22 °Brix) and organic acid content was also higher in forced grapes (FR, FBA), driven

by increases in both tartaric and malic acids. Consequently, the TSS/TA ratio in forced grapes was reduced compared to primary grapes. Potassium content and must pH were significantly lower in forced grapes.

CONCLUSION

Shoot tip removal combined with BA application significantly increased budburst and forced yield without compromising fruit composition compared to FR. The use of NAA inhibited the development of a forced canopy, with only a few buds breaking dormancy. These outcomes suggest that endogenous CK dynamics may enhance double cropping efficiency. Future research should evaluate the role of endogenous CKs and test exogenous CK applications across different cultivars and growing conditions to optimize the double cropping technique in temperate viticulture and ease the early ripening and heat stresses caused by global warming.

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