

Deliverable 2.3
Scientific publications in journals
and Conferences



Development of innovative priming technologies
safeguarding yield security in soft fruit crops through a
cutting-edge technological approach



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Document Summary

Deliverable number & title: D2.3-Scientific publications in journals and Conferences

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☒ CUT (Coordinator)

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Project coordinator: CUT



1. Introduction

Deliverable 2.3 is linked with Task 2.3 entitled 'Dissemination in journals & Conferences'.

The overarching objective of Deliverable 2.3 was to produce a considerable number of joint publications in highly refereed journals, including a position/review paper in the commercial prospects of applying priming agents in high value-added horticultural commodities. A full list of all research papers will be delivered by the end of the project [M36] with the second edition of the current deliverable. According to Horizon policy, we published open access and the EU funding was acknowledged by the inclusion of the following text: "This study has received funding from the European's Union Horizon Europe programme with acronym PRIMESOFT, entitled 'Development of innovative priming technologies safeguarding yield security in soft fruit crops through a cutting-edge interdisciplinary approach' [Grant Agreement 101079119]".

This report is comprised of the following sections:

1. Publications in refereed journals (*Section 2*)
2. Publications in Conference proceedings (*Section 3*)
3. Oral presentations in Scientific Conferences (*Section 4*)



The current report is edition 2 (out of 2) of Deliverable 2.3

Appendix provides the full texts of all published works.



2. Publications in refereed journals

2.1 Publication I_Trends in Plant Science

Title: Next generation chemical priming: with a little help from our nanocarrier friends

Contributors: G. Gohari, M. Jiang, G.A. Manganaris, J. Zhou, V. Fotopoulos

Link: <https://www.sciencedirect.com/science/article/pii/S1360138523003837>

Acknowledgements: Cyprus University Open Access Author Fund

Brief description: Plants are exposed to multiple threats linked to climate change which can cause critical yield losses. Therefore, designing novel crop management tools is crucial. Chemical priming has recently emerged as an effective technology for improving tolerance to stress factors. Several compounds such as phytohormones, reactive species, and synthetic chimeras have been identified as promising priming agents. Following remarkable developments in nanotechnology, several unique nanocarriers (NCs) have been engineered that can act as smart delivery systems. These provide an eco-friendly, next-generation method for chemical priming, leading to increased efficiency and reduced overall chemical usage. We review novel engineered NCs (NENCs) as vehicles for chemical agents in advanced priming strategies, and address challenges and opportunities to be met towards achieving sustainable agriculture.

Highlights

- Nanocarriers (NCs) functionalized with chemical agents represent a novel approach for improved priming efficiency through targeted delivery.
- Multifunctional priming through the combined use of different agents in novel engineered nanocarriers (NENCs) has the potential to achieve multiple benefits in plants.
- The application of NENCs as seed coatings has the potential to improve crop yields, while achieving maximum cost-effectiveness compared with application at the plant level as it requires less time and labor.
- Gene-editing techniques can be used to modify the expression of targeted genes involved in plant priming as identified by transcriptomic approaches, and can enhance the ability of plants to respond to priming treatments and improve their overall performance.



2.2 Publication II_Current Plant Biology

Title: Pre-harvest application of sodium alginate functionalized with melatonin enhances secondary metabolism in strawberry fruit

Contributors: Georgiadou EC, García CJ, Taliadorou AM, Gedeon S, Valanides N, Varaldo A,, Gohari G, Balsells-Llauradó M, Alcázar R, Hertog MLATM, Tomás-Barberán FA, Manganaris GA, Fotopoulos V.

Link: <https://www.sciencedirect.com/science/article/pii/S2214662825000830?via%3Dihub>

Brief description: The application of priming agents is a promising strategy to enhance the nutritional content of fruits and overall fruit quality. The current study aimed to assess the effect of the pre-harvest application of various priming agents [melatonin (Mel), sodium alginate (NaA), sodium alginate/melatonin conjugate (Mel-NaA), and putrescine dihydrochloride (Put)] on fruit quality attributes and secondary metabolite profile of a strawberry cultivar (*Fragaria x ananassa* Duchesne cv. 'Felicity Q3'). The priming agents were directly applied on fruit at three successive developmental stages, namely large green (LG), small white (SW) and large white (LW). The use of Mel-NaA and Put showed promising results in improving fruit quality indicators (i.e. firmness, color), while Mel-NaA and putrescine-treated fruit were characterized by increased total flavonoid content. HPLC-DAD-ESI-MS/MS data showed variable regulation of flavan-3-ols, hydroxycinnamic acids, and conjugates contents by the different treatments, while ellagitannins and ellagic acid derivatives were significantly enhanced following Mel-NaA pre-treatment. Priming treatments did not result in the differential regulation of volatile organic compounds (VOCs) in comparison with controls, suggesting that primed fruit retain their aroma quality with no aroma profile 'penalty'. In addition, molecular analysis revealed that fruit pre-treatment with the priming agents resulted in variable transcriptional regulation of known strawberry allergenic proteins, with the Mel-NaA treatment showing no significant effect. This 'green' approach holds promise for advancing our understanding of the effects of NaA as a smart delivery mechanism of chemical priming agents and its potential impact on the sustainable improvement of the physicochemical attributes of strawberries during the pre-harvest stage.

Highlights

- Pre-harvest application of Mel, Mel-NaA and Put improved strawberry fruit quality indicators.
- Mel-NaA and Put-treated fruit were characterized by increased total flavonoid content.
- Ellagitannins and ellagic acid derivatives were significantly enhanced following Mel-NaA pre-treatment.
- Mel-NaA pre-treated fruit did not show increased transcript levels of known strawberry allergenic proteins.



2.3 Publication III_LWT Food Science & Technology

Title: The postharvest application of biodegradable polymers and a priming agent as a potential tool to enhance phytochemical content, aroma profile and market life of strawberry fruit.

Contributors: Georgiadou EC, Garcia Hernandez Gil CJ, Taliadorou AM, Myrtsi ED, Gohari G, Varaldo A, Torrado S, Gasperini AM, Tomás-Barberán F, Hertog, MLATM, Fotopoulos V, Manganaris GA.

Link: <https://www.sciencedirect.com/science/article/pii/S0023643825005614?via%3Dihub>

Brief description: Strawberry (*Fragaria x ananassa* Duch.) is a highly perishable crop with limited market life. The aim of our work was to dissect the efficacy of an array of molecules with potential priming effect on postharvest performance, antioxidant potential and aroma profile of strawberry fruit. Freshly harvested fruit (cv. 'Savana') of uniform size and ripening stage (commercial ripeness >80 % of the surface red color) were subjected to the following postharvest dip treatments: control (untreated), hydro-primed, NOSH-A, chitosan (CTS) and sodium alginate (NaA). NOSH-A is a proprietary priming agent that acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently. CTS is a biobased, biologically safe and biodegradable polymer that has been exploited as a nanocarrier to efficiently deliver an array of compounds, while NaA is another biodegradable polymer applied in smart nano-delivery systems. Treated fruit were subjected to 4, 8 and 12 d of cold storage (CS, 4 °C, 90 % RH) and additional maintenance at room temperature for 1 d to simulate short, medium and extended refrigerated storage, respectively. Quality attributes and fungal incidence and severity were determined, without any striking differences among the treatments applied. Polyphenolic compounds analysis by HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical compounds such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, and catechin, particularly after 8 days CS compared to freshly harvested fruit. Such changes were more evident when the priming agent NOSH-A was applied, being more pronounced in the case of pedunculagin 2 isomer that registered a significant increment. HS-SPME-GC analysis identified 140 unique volatile organic compounds (VOCs) with chitosan-treated strawberries showing the most distinct VOC profile after extended cold storage with higher contents of methyl hexanoate. Results reported herein shed light in the efficacy of an array of agents on parameters linked to secondary metabolism of strawberry fruit at postharvest level.

Highlights

- The efficacy of biodegradable polymers and a priming agent on strawberry postharvest performance was tested.
- HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical compounds after cold storage compared to harvested fruit.
- HS-SPME-GC analysis identified 140 unique volatile organic compounds (VOCs).
- Among ellagitannins, pedunculagin 2 isomer registered a significant increment in NOSH-A-treated strawberries.
- Chitosan-treated strawberries showed a distinct VOC profile after extended cold storage with higher contents of methyl hexanoate.



2.4 Publication IV_Cleaner & Circular Bioeconomy

Title: A comprehensive assessment of life cycle environmental impact and economic feasibility for different red raspberry (*Rubus idaeus* L) cultivation systems.

Contributors: Xyderou Malefaki A, Valanides N, Manganaris GA, Wasko DeVetter L, Papadaki S, Krokida M, Vyrkou A, Angelis-Dimakis A

Link: <https://www.sciencedirect.com/science/article/pii/S277280132500017X>

Brief description: Red raspberry is considered a knowledge- and capital-intensive crop that targets a niche market globally; its quality attributes and enhanced health-promoting properties are highly appreciated by the consumers. In the context of the exponential growth in demand for this specialty crop that suffers from limited shelf life, it is imperative to expand raspberry cultivation by employing sustainably-sourced production models. In the current study, we used Cyprus as a case study that is characterised by increased production costs and lack of year-round production despite the fact that the latter is feasible under different production systems and cultivation methods in different altitude-related meso-climates. Towards that goal, the current study assessed the life cycle environmental impact and life cycle costs of two different cultivation methods - open-field production that took place from May to November 2022 and protected cultivation in high-tunnels, from August 2023 to April 2024, using in both cases the same cultivar (Kwanza®) and plant type. The results indicated that protected cultivation has better environmental performance (3.7 mPt - milli eco-points - per kg of raspberry produced compared to 7.4 mPt for open-field production). Noteworthy, production cost is excessive and substantially higher compared to other countries; open-field production has a life cycle cost of 22.5 €/kg, while protected cultivation achieved a lower life cycle cost, equal to 14.0 €/kg yet still high. From an output perspective, a key observation is the increased yield of raspberries in protected cultivation as well as the enhanced water use efficiency of the crop, due to a reduction of the water footprint by 76 %. It is also important to highlight the increased harvest efficiency of the crop under high tunnel, with 500 g per plant compared to 350 g on open field cultivation. Hence, it is safe to conclude that despite the increased start-up costs and knowledge-intensive practices, the productivity of the crop is increased during the off-season months, that can be sold for a premium. The results highlight the environmental and economic impact of the two cultivation methods and will be useful for producers and crop advisors seeking to expand the raspberry cultivation in climates that resembles south-eastern Europe and are characterised as vulnerable to adverse climate change scenarios.

Highlights

- Raspberry protected cultivation has shown higher yield than open-field production.
- This leads to increased productivity increased during the off-season months.
- Raspberry protected cultivation has better ecological performance than open-field.
- Raspberry protected cultivation has almost 40 % lower life cycle cost than open-field.



2.5 Publication V_Scientia Horticulturae

Title: Application of priming agents in red raspberries prior to transplantation and at pre-flowering stages results in improved yield efficiency and enhanced secondary metabolism.

Contributors Valanides N, Georgiadou EC, Myrtsi ED, Garcia CJ, Taliadorou A, Torrado S, Hertog MLATM, Tomás-Barberán F, Fotopoulos V, Manganaris GA

Brief description: Red raspberry (*Rubus idaeus* L) fruit has high nutritional value and there is an increasing demand in its global cultivation, highlighting the need for sustainable practices to improve fruit quality as well as plant productivity. Chemical priming has recently gained attention as a sustainable horticultural crop management approach to enhance plant performance. In the current study, the effects of multiple chemical priming agents were investigated on their potential to improve yield efficiency, enhance antioxidant potential and fruit quality attributes, with special reference to aroma of 'Vica Abril' raspberry plants. Treatments included: (1) NOSH-aspirin (NOSH-A, 100 μ M), (2) melatonin (Mel, 100 μ M), (3) sodium alginate (NaA, 0.5 % w/v), (4) sodium alginate-melatonin conjugate (NaA/Melatonin, 100 μ M/0.5 % w/v), and (5) glycine-betaine (GB, 10 mM). Additional, control treatments included application of water (hydro-primed) and DMSO (0.1% v/v) (solvent control for NOSH-A). Treatment application was initially performed pre-planting at the root zone and subsequently at 27, 46 and 74 days after planting (DAP). Melatonin treatment significantly enhanced fruit yield, particularly during the early harvests, while NOSH-A significantly enhanced sucrose and ascorbic acid content and all priming agents increased total flavonoid content. The analysis of volatile organic compounds (VOCs) in raspberry fruits identified a total of 98 distinct compounds. Treatments with NaA alone or in conjugated form with Mel led to a considerable increment of kaempferol, several anthocyanins and ellagic acid derivatives, among the 13 polyphenolic compounds identified. Results reported herein highlight the potential of different priming agents as a viable preharvest strategy towards increased crop productivity and/or enhanced raspberry fruit quality.

Highlights

- Melatonin treatment significantly enhanced fruit yield efficiency on raspberry plant
- NOSH-A significantly enhanced sucrose and ascorbic acid content
- HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical compounds on raspberry fruits treated with sodium alginate, alone or in conjugated form with melatonin
- Significant improvements due to priming application were observed in caffeoylglucose, cyanidin 3-O-sophoroside, cyanidin 3,5-diglucoside, kaempferol derivative, cyanidin-3-O-glucoside, ellagic acid pentoside and ellagic acid acetyl pentoside



2.6 Publication V_Scientific Reports

Title: Impact of chemical priming on yield efficiency and on physiological and biochemical properties of field-grown strawberry plants grown under a deficit irrigation regime

Contributors Georgiadou EC, Taliadorou AM, Myrtsi ED, Torrado S, Valanides N, Bini L, Manganaris GA, Fotopoulos V.

Brief description: Strawberry is a high-value crop with substantial water requirements, rendering it particularly vulnerable to irrigation limitations, especially in regions like the Mediterranean that is characterized by low summer rainfall and restricted water sources. This study investigated the effects of different priming agents (PAs) on the yield efficiency and physiological and biochemical performance of strawberry (*Fragaria x ananassa* Duch.) cv. 'Red Sayma 1075' grown under deficit irrigation. The experiment was conducted under a commercial strawberry set up in Cyprus, using a randomized complete block design (RCBD) with five blocks serving as biological replicates. The treatments were hydroprimed, 100 μ M melatonin (Mel), 0.1% w/v sodium alginate (NaA), 0.1% w/v sodium alginate (NaA)/100 μ M melatonin (Mel), 2 mM proline, and 0.1% w/v sodium alginate (NaA)/2 mM proline. Three treatment applications were performed at different times, one at root level of tray plants prior to transplation and two foliar applications at 8 d and 15 d after transplantation. Plants received 50% of standard irrigation following priming applications to simulate conditions of deficit irrigation. Results demonstrated that Mel, NaA, and NaA/Proline treatments significantly enhanced cumulative yield of early harvest compared with hydroprimed controls. Mel and NaA/Mel displayed significantly higher endogenous Mel content compared with controls. In addition, an array of anlysis were conducted ar leaf level at 2, 15 and 29 d after imposition of deficit irrigation. Elevated proline concentrations were observed during early stress stages (2 days), particularly in NaA and NaA/proline treatments. Proline levels were suppressed after 15 d, indicating an adaptive metabolic response in NaA, NaA/Melatonin, proline as well as NaA/Proline primed plants. Significant downregulation of enzymatic antioxidants (SOD, CAT and H₂O₂) was also observed in all treatments in the initial stages of stress application and were maintained at low levels for the duration of the experiment until 29 days. Thereafter, most biochemical markers returned to baseline levels, indicating stabilization of physiological responses under sustained deficit irrigation. The findings highlight the potential of specific priming strategies, particularly those involving Mel, NaA, and NaA/proline to mitigate drought stress, sustain yield, and improve physiological resilience in strawberry cultivation under water deficit conditions.



3. Projects under evaluation for publication in refereed journals

3.1 Project I_ Journal of Agriculture and Food Research

Title: Comparative environmental impacts of strawberry cultivation in Southeastern Europe: A study across open-field and protected cultivation systems towards sustainably-sourced production models

Contributors: Frakolaki G, Parcharidou M, Boukouvalas C, Papadaki S, Panagiotou N, Valanides N, Kokkini A, Georgiadou EC, Milivojević J, Tsormpatsidis E, Raffaelli D, Mezzetti B, Manganaris GA, Krokida M.

Brief description: The current study evaluated the environmental impacts of three different strawberry cultivation systems (open-field, protected soil-based, and protected soilless cultivation) across four Southeastern European countries: Italy, Serbia, Greece and Cyprus. To this aim, a Life Cycle Assessment (LCA) was employed in accordance with ISO 14040/14044 standards and ReCiPe2016 (H) was chosen as the impact assessment methodology. The objectives of the study were twofold: first, to develop a life cycle inventory (LCI) encompassing all flows and processes related to strawberry production, and second, to identify the practices and processes contributing the most significant environmental burden. The scope of the study was focused on crop production; thus, the boundaries of the system were set as cradle-to-farmgate and the assessment covered all in-field processes, by taking into account the land preparation, the planting, the fertilization and pesticides' use, the irrigation and the harvesting of the strawberries. Post-harvest activities were assumed to be identical across the different systems and thus fall outside the scope of this study. Results indicated significant differences across both the countries examined and the cultivation systems tested. Cyprus exhibited the highest global warming potential, primarily due to excessive fertilizer use in soilless cultivation, while Serbia showed the lowest impact, reflecting more sustainably-sourced agricultural practices. Freshwater eutrophication was lower in Serbia and Italy, indicating efficient nutrient management. Cyprus' open-field systems are facing constraints in terms of soil acidification and toxicity, while Serbia is benefited from appropriate fertilizer and pesticide use. Fossil resource scarcity increased from open-field to soilless cultivation due to the higher reliance on plastics. Among the different countries and cultivation systems examined, the open field cultivation in Cyprus and protected soilless cultivation in Serbia exhibited the highest water consumption, driven by climate conditions and plant density differences. These findings underscore the importance of adopting region-specific strategies to optimize sustainability in capital-intensive strawberry production systems.



3.2 Project III_Horticultural Research/Journal of Experimental Botany

Title: Implementation of novel priming agents as a stress alleviation strategy in strawberry production and their role on improving yield and fruit quality (*tentative title*)

Contributors: Taliadorou A, Georgiadou EC, Myrtsi E, Torrado S, Valanides N, Tomás-Barberán F, Garcia CJ, Manganaris GA, Fotopoulos V.

Brief description: Strawberry (*Fragaria × ananassa* Duch.) is a high value fruit, popular for its sweet taste and antioxidant properties. However, as a salt sensitive crop, its productivity is threatened by high soil salinity, especially in arid and semiarid regions. The present study evaluated the effectiveness of different priming agents as well as their combination with bio-polymer based carriers, in mitigating the adverse effects of salinity, focusing on strawberry yield and fruit quality. Experiment was performed in a net-house in a factorial split-plot design, using three salinity levels (0, 40, and 75 mM NaCl) and six priming treatment applications: hydroprimed (control), NaA, melatonin, NaA + melatonin, proline, and NaA + proline. Cumulative fruit yield as well as fruit antioxidant content were significantly improved by priming under both control and saline conditions.



3.3 Project V_Plant Nano Biology / Plant Stress

Title: Exploring encapsulated priming agents and functionalized hydrogel prototypes towards mitigation of salt stress in strawberry plants (*tentative title*)

Contributors Georgiadou EC, Taliadorou AM, Myrtzi ED, Torrado S, Soteriou A, Manganaris GA, Fotopoulos V.

Brief description: Soil salinity is one of the major abiotic stresses limiting crop productivity worldwide, particularly in arid and semi-arid regions. Present study explored the potential of salicylic acid (SA) encapsulated in zein and sodium alginate functionalized with graphene oxide (GO) as innovative priming agents to mitigate salinity stress in strawberry (*Fragaria × ananassa*) plants. The experiment was conducted between April and June 2025 where fresh rooted strawberry plants were planted in 1.4 L pots in peat-perlite substrate (3:2 ratio) under controlled conditions and fertilized with a mixture of minerals. Different concentrations of salicylic acid (SA), zein, and their encapsulated formulations, as well as graphene oxide (GO) and GO-functionalized sodium alginate (NaA-GO), were evaluated to assess their effectiveness against salinity stress. Three foliar treatment applications were performed at different time-points prior to flowering, followed by salinity stress initiation (100 mM NaCl) 2 days after the last priming application. Physiological and biochemical parameters were evaluated to assess plant performance and oxidative stress responses in response to the different treatments. Physiological measurements and leaf sampling were performed at 2, 4, 6 and 8 days post-treatment under salinity stress. Biochemical markers including malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, and nitric oxide (NO) were quantified. Results demonstrated that all treatments effectively mitigate the negative impacts of salinity stress on strawberry plants. At early stages of stress induction, Zein and SA alone significantly reduce MDA content while encapsulated formulations showed consistent reductions in MDA content across all time points, with 50 mg/L Zein-SA exhibiting the strongest effect. Similarly, GO and GO-functionalized sodium alginate treatments significantly decreased MDA levels while hydrogen peroxide content was more variably affected. However, encapsulated formulations and GO treatments at 0.02–0.04 mg/mL markedly reduced H_2O_2 accumulation, suggesting effective ROS detoxification. Proline content was significantly reduced in all SA, Zein and encapsulated treatments reflecting the strong treatment impact on osmotic balance. Functionalized NaA-GO combinations displayed strong performance in decreasing proline content across all time points, confirming their role in enhancing stress tolerance. Nitric oxide levels also decreased significantly, particularly under intermediate concentrations of SA and GO. Encapsulation and nanocarrier systems provide a promising approach for the mitigation of salinity-induced oxidative stress. The observed reductions in biochemical stress markers, suggest that these treatments can effectively limit oxidative damage under abiotic stress conditions. The study highlights the synergistic effect of bioactive compounds with nanocarrier systems in the mitigation of oxidative damage induced under saline environments.



4. Publications in Conference proceedings and technical magazines

4.1 Publication I_Acta Horticulturae

Title: Exploring the potential of priming agents towards enhanced performance of *Rubus* species

Contributors: G.A. Manganaris, N. Valanides, R. Gohari, J. Milivojevic, L.W. DeVetter, V. Fotopoulos

Journal: Acta Horticulturae

Cite as: Manganaris G.A., Valanides N., Gohari R., Milivojevic J., DeVetter L.W., Fotopoulos V. Exploring the potential of priming agents towards enhanced performance of *Rubus* species. Acta Horticulturae 2024, 1388, 7-16.

Brief description: Plants are exposed to multiple threats linked to climate change which can cause critical yield losses. Therefore, designing novel crop management tools is crucial. Chemical priming has recently emerged as an effective technology for improving tolerance to stress factors. Several compounds such as phytohormones, reactive species, and synthetic chimeras have been identified as promising priming agents. Following remarkable developments in nanotechnology, several unique nanocarriers (NCs) have been engineered that can act as smart delivery systems. These provide an eco-friendly, next-generation method for chemical priming, leading to increased efficiency and reduced overall chemical usage. We review novel engineered NCs (NENCs) as vehicles for chemical agents in advanced priming strategies, and address challenges and opportunities to be met towards achieving sustainable agriculture. The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of fruit crops is relatively novel. The process of priming involves prior exposure to biotic or abiotic stress factors rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: (i) chemicals (i.e., hormones, Reactive Oxygen Nitrogen and Sulphur Species (RONSS), and small organic molecules), (ii) microorganisms [i.e., arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR)], and (iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops that have high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. To our knowledge, the concept of priming in soft fruits is relatively new with scarce information available. The aim of the current report is dual. Initially, this report provides information regarding the prospects of priming agents as a novel agricultural and technological approach to improve stress tolerance for a range of *Rubus* species, namely red raspberry, blackberry, boysenberry, cloudberry, loganberry and black raspberry. Additionally, it describes the challenges and constraints of raspberry production within a global context, providing examples and case studies from the United States and Europe, two industries with striking differences in their production models.



4.2 Publication II_ ISFC Magazine

Title: Are priming agents a step forward to enhance soft fruit yield efficiency?

Contributors: G.A. Manganaris, V. Fotopoulos

Cite as: Manganaris G.A. Are priming agents a step forward to enhance soft fruit yield efficiency? ISFC Magazine 2024, pp. 72-73.

Brief description:

Introduction: The development of non-toxic synthetic and natural priming agents (PAs) towards sustainably-sourced and environmentally sound products towards a resource-efficient circular economy is an R&D activity that recently has received considerable attention. However, the effort of the Lead Market Initiative (LMI) Advisory Group to trigger a market prospective for innovative products, remains still, to a large extent, unimplemented. PRIMESOFT is a European-funded project (www.prime-soft.eu) and its overarching objective is to explore innovations in the application of PAs in soft fruits from a range of perspectives to take advantage of the EU-driven effort for product innovation, development and marketization.

Defining the term 'priming agent': The process of priming involves prior exposure to abiotic stress factors rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: (i) chemicals (i.e., hormones, Reactive Oxygen Nitrogen and Sulphur Species (RONSS), and small organic molecules), (ii) microorganisms [i.e., arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR)], and (iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Recent research on chemical priming has provided further knowledge of the complex mode of action of specific signalling molecules involved in the process and enhanced plant tolerance against individual abiotic stresses. PAs additionally offers an attractive alternative to commonly employed methodologies for enhancing tolerance to stresses, as some are particularly time-consuming (i.e. conventional breeding) and others are currently unacceptable in EU (i.e. plant genetic modification).

The concept and the novelty: Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops that have high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of soft fruit crops is entirely novel. The presentation will provide information regarding the prospects of priming agents as a novel agricultural and technological approach to improve stress tolerance, giving special reference to strawberry cultivation. To our knowledge, existing technologies representing major competition are limited to relatively few formulations/biostimulants based on silicon nutrition/supplementation and which do not always provide cross-protection against multiple abiotic stress factors, such as drought, salinity and heat. The novelty of our scientific



strategy lays on the fact that it encompasses (1) the exploration of both a naturally derived priming agent (PA) in the form of melatonin as well as a synthetic PA recently co-patented by the HO (use of NOSH/NOSH-A in plants; WO/2015/123273) and (2) the employment of both advanced nanomaterial engineering and encapsulation techniques through electro-hydrodynamic processes to enhance PA's efficiency towards increment of yield, enhancement of health-promoting properties and additionally ameliorate plant damage under climate change-related abiotic stress conditions in added-value soft fruit crops, namely strawberry and raspberry.

Conclusion: Priming agents (PAs) have gathered unprecedented attention, with many companies investing in R&D initiatives for their development, as the Europe's new bioeconomy strategy and action plan aspires at driving towards an increased and sustainable use of renewable resources. We aim to bridge the gap between chemical and nanomaterial priming research and agricultural practice in order to bring the inventions (i.e. beneficial effects of advanced nanomaterials conjugated with natural metabolite-based PAs) closer to application and commercialization when it comes to agricultural practice and more broadly to sustainable or "green" technologies.



5. Presentations in Scientific Conferences

5.1 V ISHS Balkan Symposium on Fruit Growing

Presenter: George Manganaris

Dates: June 18-21, 2023

Organization: International Society for Horticultural Science (ISHS)

Place: Zagreb (Croatia)

Type of presentation: invited talk

Title: The efficacy of priming agents on qualitative attributes and phytochemical properties of strawberry fruits

Contributors: S. Gedeon, E.C. Georgiadou, CJ Garcia, N. Valanides, A.M. Taliadorou, M. Balsells-Llauradó, G. Gohari, A. Assiotis, F.A. Tomás-Barberán, V. Fotopoulos, GA Manganaris

Abstract: Strawberry (*Fragaria x ananassa* Duch.) is one of the most popular fruit in the world due both to its taste and health-promoting properties. The fruit's organoleptic quality characteristics include aroma, sweetness, acidity and fruit firmness. Numerous studies propose the application of chemical agents to improve fruit quality. The use of priming agents (PAs) is a promising strategy that is being widely expanded over the recent years to enhance the nutritional quality of fruits. To this end, our study aimed to evaluate the effect of the pre-harvest application of an array of priming agents (melatonin, sodium alginate, their combination and putrescine) on the qualitative attributes and phytochemical properties of an early-harvested strawberry cultivar. The priming agents were applied at three successive developmental stages, namely large green (LG), small white (SW) and large white (LW). Fully-ripe strawberries without visible damage or disease symptoms were harvested and an array of quality attributes [(fresh fruit weight, volume, color, flesh firmness, soluble solids content (SSC) and titratable acidity (TA)] were determined. In addition, an array of phytochemical properties were assessed [anthocyanins (pelargonidin-3-glucoside (Pg-3-gluc), cyanidin-3-glucoside (Cy-3-gluc) and pelargonidin-3-rutinoside (Rg-3-rut), flavonols (quercetin, kaempferol, myricetin and other flavonol glycosides), flavan-3-ols (proanthocyanidins and catechins), hydroxybenzoic and hydroxycinnamic acid derivatives (caffeic, ferulic and p-coumaric derivatives, gallic, and other hydroxybenzoic derivatives, ellagic acid, and ellagitannins (ellagic acid conjugates; hydrolysable tannins such as sanguin H6 sanguinarin, lambertianin, etc).]. The potential beneficial effect through the application of such priming agents on qualitative and phytochemical properties of strawberries is discussed.



5.2 XIII ISHS International *Rubus* and *Ribes* Symposium

Presenter: George Manganaris

Organization: International Society for Horticultural Science (ISHS)

Dates: July 14-21, 2023

Place: Portland (United States of America)

Type of presentation: keynote talk

Title: The employment of priming agents as elicitors towards enhanced performance of raspberry plants

Contributors: G.A. Manganaris, N. Valanides, R. Gohari, J. Milivojevic, L.W. DeVetter, V. Fotopoulos

Abstract: The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of fruit crops is relatively novel. The process of priming involves prior exposure to biotic or abiotic stress factors rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: (i) chemicals (i.e., hormones, Reactive Oxygen Nitrogen and Sulphur Species (RONSS), and small organic molecules), (ii) microorganisms [i.e., arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR)], and (iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops that have high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. To our knowledge, the concept of priming in soft fruits is relatively new with scarce information available. The aim of the current report is dual. Initially, this report provides information regarding the prospects of priming agents as a novel agricultural and technological approach to improve stress tolerance for a range of *Rubus* species, namely red raspberry, blackberry, boysenberry, cloudberry, loganberry and black raspberry. Additionally, it describes the challenges and constraints of raspberry production within a global context, providing examples and case studies from the United States and Europe, two industries with striking differences in their production models.

Highlights

- Expanding global production of soft fruits, including *Rubus* species, is challenged by biotic and abiotic stress factors. Climate change and extremes in weather necessitate new tools that can rapidly and economically reduce crop loss due to abiotic stress and should complement other breeding and horticultural research efforts.
- In the context of the high demand for fresh and processed raspberries in the world market, there is a great need to increase the profitability of cultivation through the use of innovative, low-cost technologies, such as the application of priming agents.
- Research and use of priming agents should be prioritized, particularly considering the adverse stress conditions experienced due to climate change. It is also important to understand and apply the potential benefit from the postharvest use of priming agents towards enhanced cold chain management from field to consumer.
- As ongoing research continues to unravel the specific mechanisms behind these priming agents' effects on raspberry physiology and fruit quality, the potential for optimizing raspberry production and providing consumers with high-quality, nutritious berries becomes increasingly evident.



5.3 International Soft Fruit Conference

Presenter: George Manganaris

Organization: Delphy

Dates: January 10-11, 2024

Place: Den Bosch (The Netherlands)

Type of presentation: invited talk

Title: Are priming agents a step forward to enhance soft fruit yield efficiency?

Contributors: G.A. Manganaris, V. Fotopoulos

Brief description

Introduction: The development of non-toxic synthetic and natural priming agents (PAs) towards sustainably-sourced and environmentally sound products towards a resource-efficient circular economy is an R&D activity that recently has received considerable attention. However, the effort of the Lead Market Initiative (LMI) Advisory Group to trigger a market prospective for innovative products, remains still, to a large extent, unimplemented. PRIMESOFT is a European-funded project (www.prime-soft.eu) and its overarching objective is to explore innovations in the application of PAs in soft fruits from a range of perspectives to take advantage of the EU-driven effort for product innovation, development and marketization.

Defining the term 'priming agent': The process of priming involves prior exposure to abiotic stress factors rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: (i) chemicals (i.e., hormones, Reactive Oxygen Nitrogen and Sulphur Species (RONSS), and small organic molecules), (ii) microorganisms [i.e., arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR)], and (iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Recent research on chemical priming has provided further knowledge of the complex mode of action of specific signalling molecules involved in the process and enhanced plant tolerance against individual abiotic stresses. PAs additionally offers an attractive alternative to commonly employed methodologies for enhancing tolerance to stresses, as some are particularly time-consuming (i.e. conventional breeding) and others are currently unacceptable in EU (i.e. plant genetic modification).

The concept and the novelty: Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops that have high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of soft fruit crops is entirely novel. The presentation will provide information regarding the prospects of priming agents as a novel agricultural and technological approach to improve stress tolerance, giving special reference to strawberry cultivation. To our knowledge, existing technologies representing major competition are limited to relatively few formulations/biostimulants based on silicon nutrition/supplementation and which do not always provide cross-protection against multiple abiotic stress factors, such as drought, salinity and heat. The novelty of our scientific



strategy lays on the fact that it encompasses (1) the exploration of both a naturally derived priming agent (PA) in the form of melatonin as well as a synthetic PA recently co-patented by the HO (use of NOSH/NOSH-A in plants; WO/2015/123273) and (2) the employment of both advanced nanomaterial engineering and encapsulation techniques through electro-hydrodynamic processes to enhance PA's efficiency towards increment of yield, enhancement of health-promoting properties and additionally ameliorate plant damage under climate change-related abiotic stress conditions in added-value soft fruit crops, namely strawberry and raspberry.

Conclusion: Priming agents (PAs) have gathered unprecedented attention, with many companies investing in R&D initiatives for their development, as the Europe's new bioeconomy strategy and action plan aspires at driving towards an increased and sustainable use of renewable resources. We aim to bridge the gap between chemical and nanomaterial priming research and agricultural practice in order to bring the inventions (i.e. beneficial effects of advanced nanomaterials conjugated with natural metabolite-based PAs) closer to application and commercialization when it comes to agricultural practice and more broadly to sustainable or "green" technologies.



5.4 European Horticulture Congress

Presenter: Egli Georgiadou

Dates: March 12-16, 2024

Organization: International Society for Horticultural Science (ISHS)

Place: Bucharest (Romania)

Type of presentation: oral talk

Title: NOSH-A, a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S) and aspirin, as a promising tool to enhance phytochemical content of strawberry fruits

Contributors: GA Manganaris, E.C. Georgiadou, A.M. Taliadorou, V. Fotopoulos, CJ Garcia, F.A. Tomás-Barberán, M.I. Gil

Abstract: Strawberry (*Fragaria x ananassa* Duch.) is one of the most popular fruit due to its superior taste and its health-promoting properties. At the same time, it is a highly perishable crop with limited market life. The working hypothesis of our experimental approach was to dissect the efficacy of an array of molecules with potential 'priming' effect on postharvest performance and antioxidant potential of strawberry fruits after 4,8 and 12 days of cold storage (4°C, 90% R.H.) and additional maintenance at room temperature for 1 day. Strawberry fruits (cv. 'Savana') of uniform size and ripening stage (commercial ripeness >80% of the surface red color), were hand-harvested and immediately transferred to the laboratory. After removal of defective fruits, they were separated to 24 lots of 60 fruits each. Each three lots were subjected to immersion with the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A, (4) chitosan (CTS), (5) CTS-NOSH-A, (6) sodium alginate (Alg), (7) alginate-NOSH-A and (8) CTS-Alg-NOSH-A. NOSH-A (patent WO/2015/123273) acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently. Chitosan is a biobased, biologically safe and biodegradable polymer that has been exploited as a nanocarrier to deliver efficiently an array of compounds, while alginate (sodium-based in our case) is another biodegradable polymer applied in nano smart delivery systems. Quality attributes (fresh fruit weight, volume, color, flesh firmness, soluble solids content (SSC) and titratable acidity (TA)) were determined, without however any striking differences among treatments. Interestingly, a notable increase in an array of phytochemical compounds were monitored in fruits treated with NOSH-A such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, catechin. Notably the increment in such compounds was more pronounced after 8 days cold storage with a consequential decrease after 12 days of cold storage.



5.5 X ISHS Strawberry Symposium

Presenter: Vasileios Fotopoulos

Dates: March 16-21, 2025

Organization: International Society for Horticultural Science (ISHS)

Place: Yancheng (China)

Type of presentation: oral talk

Title: The spraying efficacy of an array of compounds with potential priming effect on secondary metabolism of strawberry fruits

Contributors: E.C. Georgiadou, J Garcia, Tomás-Barberán, M. Hertog, V. Fotopoulos, GA. Manganaris

Abstract: The application of priming agents is a promising strategy that is being widely expanded over the recent years to enhance quantitative and qualitative attributes for an array of horticultural crops. Strawberry (*Fragaria x ananassa* Duch.) is a delicate fruit crop with exponential growth in terms of production volumes and consumption. However, there are certain periods during the calendar year with limited availability. In the current study, the effect of melatonin, sodium alginate, their combination and putrescine) on the qualitative attributes and phytochemical properties of an early-harvested strawberry cultivar that mainly produces off-season was tested. In particular, melatonin (N-acetyl-5-methoxytryptamine, Mel), is a well-conserved hormone that acts as a growth regulator, being present both in animals and plants and has received accumulating interest over the recent years. The priming agents were applied on three successive developmental stages, namely large green (LG), small white (SW) and large white (LW). Fully-ripe strawberries without visible damage or disease symptoms were harvested and an array of quality attributes [(fresh fruit weight, volume, color, flesh firmness, soluble solids content (SSC) and titratable acidity (TA)] phytochemical properties were assessed [anthocyanins, flavonols, flavan-3-ols, hydroxybenzoic and hydroxycinnamic acid derivatives and other hydroxybenzoic derivatives, ellagic acid, and ellagitannins] and volatile organic compounds were assessed. We hypothesize that the exogenous pre-harvest application of priming agents (Mel, NaA, their combination in a conjugated form (Mel +NaA), and Put) on strawberry fruit undergoing ripening processes on-bush -under non-stressful conditions- will result in improved physicochemical properties and qualitative attributes. The potential beneficial effect through the application of such priming agents on qualitative and phytochemical properties and aromatic profile of strawberries is discussed.



Presenter: Elena Myrtsi

Dates: March 16-21, 2025

Organization: International Society for Horticultural Science (ISHS)

Place: Yancheng (China)

Type of presentation: oral talk

Title: The efficacy of a novel priming agent on postharvest performance and phytochemical profile of strawberry fruits

Contributors: E.C. Georgiadou, E. Myrtsi, C.J. Garcia, Tomás-Barberán, V. Fotopoulos, G.A. Manganaris

Abstract: The Strawberry (*Fragaria x ananassa* Duch.) is one of the most popular fruit crops worldwide with a considerable growth in terms of production volumes, mainly attributed to its appealing appearance and high nutritional and phytochemical content. Strawberry fruits (cv. 'Savana') of uniform size and ripening stage (commercial ripeness >80% of the surface red color), were hand-harvested and separated to 24 lots of 60 fruits each. Each three lots were subjected to immersion with the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A, (4) chitosan (CTS), (5) sodium alginate (Alg). Subsequently, such fruit were analyzed after 4, 8 and 12 days of cold storage (4°C, 90% R.H.) and additional maintenance at room temperature for 1 day. NOSH-A (patent WO/2015/123273) acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently. Chitosan is a biobased, biologically safe and biodegradable polymer that has been exploited as a nanocarrier to deliver efficiently an array of compounds, while alginate (sodium-based in our case) is another biodegradable polymer applied in nano smart delivery systems. Interestingly, results indicated a notable increase in an array of phytochemical compounds were monitored in fruits treated with NOSH-A such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, catechin. This increase was more pronounced after 8 days cold storage and reduced thereafter concomitant with quality losses. Results reported herein indicates that compounds with priming activity in order to combat abiotic stress conditions at preharvest level can be additionally exploited at postharvest level on fresh produce with enhanced qualitative or phytochemical properties or both.



Presenter: Egli Georgiadou

Dates: March 16-21, 2025

Organization: International Society for Horticultural Science (ISHS)

Place: Yancheng (China)

Type of presentation: oral talk

Title: The efficacy of a compounds with potential priming effect on alleviation of stress conditions due to salinity in strawberry plants and fruits' phytochemical content

Contributors: E.C. Georgiadou, N. Valanides, A. Taliadorou, E. Myrtsi, S. Torrado, V. Fotopoulos, GA Manganaris

Abstract: Strawberry plants (cv. "Red Cayma 10-75"), received as fresh rooted tray plants, were cultivated during the period October 2023 – February 2024 under greenhouse conditions (Nicosia, Cyprus, 35°8'0"N 33°2'0"E) and used for the needs of the current study. Fresh tray plants were transplanted in 6.5 L pots in the greenhouse where they were arranged in randomized complete block design, consisting in five blocks and two different factors (different treatment application within blocks) and different levels of salinity stress implementation. The following treatments were applied: (1) Untreated, (2) hydroprimed, (3) sodium alginate (0.1 % w/v), (4) melatonin (100 µM), (5) melatonin conjugated with alginate, (6) proline (2 mM) and (7) proline conjugated with alginate. To ensure the binding of the compounds on leaf surface, 0.1% w/v Tween-20 surfactant was added in each solution. Treatment application was performed at different timepoints between transplantation and plant growth. In order to evaluate the efficiency of the compounds applied as stress alleviation strategies, salinity stress was imposed 4-days after the last priming application at two concentrations (40 mM and 75 mM) along with the control (no stress implementation). Noteworthy, salinity treatments led to fruit with higher phytochemical content. This study will discuss the effect of the compounds applied and its conjugates with potential priming effect on yield efficiency, as well as physiological and biochemical indices.





5.6 International Symposium on Biotechnological Tools

Presenter: Egli Georgiadou

Dates: May 5-8, 2025

Organization: International Society for Horticultural Science (ISHS)

Place: Rimini (Italy)

Type of presentation: flash oral talk

Title: The application of priming agents as stress alleviation strategy to combat abiotic conditions in strawberry plants: a genomic approach

Contributors: EC Georgiadou, A Taliadorou, E Myrtsi, S Torrado, N Valanides, CJ Garcia, F Tomas-Barberan, GA Manganaris, V Fotopoulos

Abstract: Strawberry cultivation and production is currently affected by multiple challenges that threaten both yield efficiency and fruit quality parameters. Considering that strawberry plant is a salt sensitive crop, this study aimed to dissect the efficacy of such stress conditions under different regimes of salt conditions. The trial was implemented as a factorial scheme with three levels of salinity stress and application of five priming agents (100 μ M Melatonin, 0.1 % w/v sodium alginate (NaA)/100 μ M melatonin, 2mM proline, 0.1% w/v sodium alginate (NaA)/2 mM proline.) with two controls (Untreated and Hydro-primed), in split-plot design with five replicates (five blocks). In order to prevent excess salt accumulation in the growth medium, pots were irrigated with fresh water at five days intervals along the experimental period for 24h and then continue with the irrigation using saline water. Based in the physicochemical and biochemical analysis conducted, a set of 81 samples were selected for further RNA sequencing analysis. This study will report the main findings regarding the genes involved and/or governing the efficacy of priming agents in the alleviation of stress conditions as well as how salinity may affect the qualitative properties and phytochemical profile of strawberry fruits.



5.7 Annual meeting of RECROP COST action

Presenter: George Manganaris

Dates: May 14-16, 2025

Organization: COST (European Cooperation in Science and Technology) /RECROP: reproductive enhancement of CROP resilience to extreme climate

Place: Thessaloniki (Greece)

Type of presentation: oral talk

Title: The application of priming agents as a stress alleviation strategy to combat abiotic conditions in soft fruit crops

Contributors: EC Georgiadou, N Valanides, A Taliadorou, E Myrtsi, S Torrado, V Fotopoulos, GA Manganaris

Abstract: Soft fruits comprise a diverse group of crops that belongs to different genus (Fragaria, Ribes, Rubus, Vaccinium) and thus are being characterized by different properties and requirements. The current presentation aims to shed light in the efficacy of priming agents to strawberry and raspberry plants grown both under conventional conditions and or after application of stress factors, namely salinity. Special reference will be given to strawberry cultivation and production that is currently affected by multiple challenges that threaten both yield efficiency and fruit quality parameters. The trial was implemented as a factorial scheme with three levels of salinity stress and application of five priming agents (100 μ M Melatonin, 0.1% w/v sodium alginate (NaA)/100 μ M melatonin, 2 mM proline, 0.1% w/v sodium alginate (NaA)/2 mM proline) with two controls (Untreated and Hydro-primed), in split-plot design with five replicates (five blocks). Considering raspberry plants, melatonin (Mel), glycine betaine (GB), NOSH, sodium alginate (SA), combination of Mel and SA, DMSO and Water (Control), were applied in 4 successive growth stages of cv. 'Vica Abril'. Overall, this study will report the main findings regarding the genes involved and/or governing the efficacy of priming agents in the alleviation of stress conditions as well as how salinity may affect the qualitative properties and phytochemical profile of strawberry fruits.



5.8 IV International Strawberry Congress

Presenter: George Manganaris

Dates: September 17-19, 2025

Organization: Coöperatie Hoogstraten

Place: Antwerp (Belgium)

Type of presentation: oral talk

Title: The application of priming agents as an alleviation strategy to combat salinity stress in strawberry plants: a physiological, biochemical and transcriptomic approach

Contributors: EC Georgiadou, S Torrado, A Taliadorou, V Fotopoulos, GA Manganaris

Abstract: Strawberry cultivation and production is affected by multiple challenges that threaten both yield efficiency and fruit quality parameters. The current study aims to shed light in the efficacy of priming agents to improve plant productivity and fruit quality of strawberry plants grown under conventional and salt stress conditions through a physiological, biochemical and transcriptomic approaches. The trial was conducted as a factorial design with three levels of salinity stress (0 mM, 40 mM and 75 mM) and application of five priming agents (100 μ M Melatonin, 0.1% w/v sodium alginate (NaA)/100 μ M melatonin, 2 mM proline, 0.1% (w/v) sodium alginate (NaA)/2 mM proline) with hydro-prime as control, in a split-plot manner with five blocks. This study will report the main findings regarding the genes involved and/or governing the efficacy of priming agents (PAs) in the alleviation of stress conditions as well as how salinity may affect the qualitative properties and phytochemical profile of strawberry fruits.



Presenter: Egli Georgiadou

Dates: September 17-19, 2025

Organization: Coöperatie Hoogstraten

Place: Antwerp (Belgium)

Type of presentation: poster

Title: The efficacy of postharvest application of coatings and priming agents on quality attributes, volatilome fingerprinting and antioxidant potential of strawberry fruits

Contributors: EC Georgiadou, E Myrtsi, A Taliadorou, S Torrado, V Fotopoulos, GA Manganaris

Abstract: Strawberry (*Fragaria x ananassa* Duch.) is a highly perishable crop with limited market life. Several coatings have been reported to have a beneficial effect on its storage potential yet, to our knowledge, few of them have been adopted at commercial scale. The concept of applying compounds with priming effect at plant level to combat stress conditions (i.e. salinity, drought, heat) is quite novel, while scarce information exist regarding the efficacy of such compounds under storage conditions (where cold temperature maintenance can be considered as a stress factor). To this aim, the efficacy of biodegradable polymers and a priming agent on strawberry postharvest performance was tested in the current study. In particular, we applied in freshly harvested strawberry fruits (cv. 'Savana') of uniform size and ripening stage the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A, (4) chitosan (CTS), (5) sodium alginate. NOSH-A (patent WO/2015/123273) acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently. Chitosan is a biobased, biologically safe and biodegradable polymer that has been exploited as a nanocarrier to deliver efficiently an array of compounds, while alginate (sodium-based in our case) is another biodegradable polymer applied in nano smart delivery systems. Quality attributes were determined, without however any striking differences among treatments. Interestingly, a notable increase in an array of phytochemical compounds were monitored in fruits treated with NOSH-A such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, catechin. Notably the increment in such compounds was more pronounced after 8 days cold storage with a consequential decrease after 12 days of cold storage. HS-SPME-GC analysis identified 140 unique volatile organic compounds (VOCs). Chitosan-treated strawberries showed the most distinct VOC profile after extended cold storage with higher contents of methyl hexanoate.



Presenter: Sofia Torrado

Dates: September 17-19, 2025

Organization: Coöperatie Hoogstraten

Place: Antwerp (Belgium)

Type of presentation: poster

Title: The impact of chemical priming on the physiology and productivity of strawberries under deficit irrigation

Contributors: S Torrado, E Georgiadou, E Myrtsi, A Taliadorou, V Fotopoulos, GA Manganaris

Abstract: The experiment was conducted from October 2023 to February 2024 in a strawberry field in Astromeritis village, Nicosia district (160 m (520 ft) above sea level, latitude: 35°8'0"N, longitude: 33°2'0"E), using fresh rooted strawberry "Red Cayma 10-75" plants. Fresh tray plants were transplanted into 6.5L pots in the net house. The trial was carried out using a factorial design with 50% deficit irrigation and the application of five priming agents (PAs) with two controls (untreated and hydro-primed), in a split-plot design with five replicates (five blocks). This study used five priming agents (PAs) and two controls (hydro-primed and untreated) allocated in each block: untreated, water-spray, 0.1% w/v sodium alginate (NaA), 100µM melatonin, 0.1% w/v sodium alginate (NaA)/100µM melatonin, 2mM proline, and 0.1% w/v sodium alginate (NaA)/2mM proline. To ensure that the PAs bind to the leaf surface, each solution contained 0.1% w/v Tween-20 surfactant. The first application was performed as a root application two days before transplantation. In the net-house, two additional foliar treatments were applied prior to flowering at 8 and 15 days after transplantation. This study will provide insights to what extent compounds tested affected yield efficiency and earliness of production. A set of biochemical, physiological factors analysed will be additionally discussed.



5.9 Conference of the Hellenic Society for Horticultural Science

Presenter: Egli Georgiadou

Dates: October 20-23, 2025

Organization: Hellenic Society for Horticultural Science

Place: Volos (Greece)

Type of presentation: oral

Title: Η εφαρμογή παραγόντων έναυσης ως στρατηγική για τη διαχείριση συνθηκών αβιοτικής καταπόνησης λόγω αλατότητας σε φυτά φράουλας

Contributors: ΑΧ Γεωργιάδου, Α Ταλιαδώρου, Ε Μύρτση, S Torrado, Ν Βαλανίδης, F Tomás-Barberán, C J Garcia, ΓΑ Μαγγανάρης, Β Φωτόπουλος

Abstract: Τα τελευταία χρόνια παρατηρείται συνεχής αύξηση των ακραίων καιρικών φαινομένων όπως ξηρασία, ζέστη, ψύχος και αλατότητα. Επομένως, είναι σημαντική η έρευνα νέων τεχνολογιών για τη βελτίωση της ανθεκτικότητας των φυτών σε παράγοντες αβιοτικής καταπόνησης. Η παρούσα μελέτη στοχεύει στην αξιολόγηση της επίδρασης διαφορετικών παραγόντων έναυσης και των συνδυασμών τους με αλγινικό νάτριο (NaA) έναντι της εφαρμογής δύο επιπέδων αλατότητας και της επίδρασής τους στην παραγωγικότητα και την ποιότητα των καρπών της φράουλας. Τα φυτά φράουλας (ποικιλία "Red Sayama 1075"), παραλήφθηκαν ως φρέσκα ριζωμένα φυτά σε δίσκους και μεταφυτεύτηκαν κάτω από δίχτυ σε κτήμα καλλιέργειας φράουλας (Λευκωσία, Κύπρος, 35°8'0"N 33°2'0"E). Το πείραμα σχεδιάστηκε ως παραγοντικό σχέδιο με δύο διαφορετικές συγκεντρώσεις αλατιού και με πέντε επαναλήψεις και εφαρμογές παραγόντων έναυσης. Οι επεμβάσεις κατανεμήθηκαν τυχαία στην κύριο κομμάτι γης, ενώ τα επίπεδα καταπόνησης κατανεμήθηκαν σε υπό κομμάτια γης. Οι ακόλουθες μεταχειρίσεις εφαρμόστηκαν: 1) νερό, 2) μελατονίνη, 3) NaA, 4) NaA/μελατονίνη, 5) προλίνη, 6) NaA/προλίνη. Παρατηρήσαμε ότι η εφαρμογή παραγόντων έναυσης αυξάνει σημαντικά την παραγωγικότητα των φυτών κατά περισσότερο από 50% σε σύγκριση με τα φυτά που ψεκάστηκαν μόνο με νερό, όταν δεν υπάρχει καταπόνηση. Υπό συνθήκες ήπιας αλατότητας, η NaA/μελατονίνη και η προλίνη δείχνουν σημαντική αύξηση στην παραγωγή. Σημαντική βελτίωση στην περιεκτικότητα ασκορβικού οξέος στους καρπούς φράουλας έδειξε η έναυση με προλίνη, μελατονίνη, και NaA σε σύγκριση με τα φυτά που ψεκάστηκαν μόνο με νερό υπό ήπιες συνθήκες αλατότητας. Σημαντικές μεταβολές παρατηρήθηκαν υπό έντονη αλατότητα, όπου η έναυση με NaA και προλίνη είχε σημαντικά θετική επίδραση στην περιεκτικότητα ανθοκυανινών. Ακολούθως, η μεταχειρίσεις NaA, προλίνη και οι συνδυασμοί τους επηρέασαν σε μεγάλο βαθμό την ολική φαινολική περιεκτικότητα των καρπών φράουλας υπό συνθήκες έντονης καταπόνησης, σε σύγκριση με τα φυτά που ψεκάστηκαν μόνο με νερό. Η μεταχείριση NaA/προλίνη στην ολική φαινολική περιεκτικότητα ήταν σημαντική ακόμη και όταν δεν υπήρχε καταπόνηση. Τέλος, στα φλαβονοειδή υπό ήπια ή καθόλου καταπόνηση οι μεταχειρίσεις με μελατονίνη και NaA/μελατονίνη αυξήθηκαν, ενώ υπό έντονη καταπόνηση αυξήθηκε η ουσία κερκετίνη-3-γλυκουρονίδιο στις μεταχειρίσεις με μελατονίνη και με NaA/προλίνη.



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Title: Βελτιστοποίηση παραγωγής και ποιότητας σμέουρων (*Rubus idaeus* L.) μέσω εφαρμογής παραγόντων έναυσης

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Abstract: Τα σμέουρα (*Rubus idaeus*) αποτελούν φρούτα υψηλής διατροφικής αξίας, καθώς είναι πλούσια σε αντιοξειδωτικά και συμβάλλουν θετικά στην πρόληψη χρόνιων ασθενειών. Το αυξανόμενο ενδιαφέρον για την κατανάλωση και την καλλιέργειά τους έχει οδηγήσει σε σημαντική παγκόσμια αύξηση της παραγωγής τους. Στην παρούσα μελέτη διερευνήθηκε η επίδραση πέντε παραγόντων έναυσης στην παραγωγή και στα ποιοτικά χαρακτηριστικά των καρπών (ποικ. 'Vica Abril'). Στη μελέτη αυτή εφαρμόστηκαν οι εξής μεταχειρίσεις: (i) μελατονίνη (Mel, 100 μ M), (ii) γλυκίνη-βεταΐνη (GB, 10 mM), (iii) αλγινικό νάτριο (NaA, 0,5% w/v), (iv) σύμπλοκο NaA με Mel (NaA/Mel, 0,5% (w/v)/100 μ M) και (v) NOSH-Ασπιρίνη (NOSH-A, 100 μ M), ενώ χρησιμοποιήθηκαν ως μάρτυρες το νερό (μάρτυρας) και το διμεθυλοσουλφοξείδιο (DMSO). Το NOSH-A αποτελεί ένα καινοτόμο προϊόν που μπορεί να δρα ως ταυτόχρονος δότης μονοξειδίου του αζώτου (NO), υδρόθειου (H₂S) και ακετυλοσαλικυλικού οξέος. Η πρώτη εφαρμογή πραγματοποιήθηκε με ριζοπότισμα στα νεαρά φυτάρια πριν τη μεταφύτευσή τους, και στη συνέχεια με ψεκασμό σε τέσσερα χρονικά σημεία: 3, 30, 60 και 90 ημέρες μετά τη φύτευση. Σκοπός της εργασίας ήταν να αξιολογηθεί η επίδραση των μεταχειρίσεων στην απόδοση και την ποιότητα των καρπών, καθώς και να μελετηθούν οι πιθανές εφαρμογές τους για τη βελτίωση της καλλιέργειας σμέουρων. Τα αποτελέσματα της μελέτης έδειξαν ότι το NOSH-A και η Mel αποτελούν υποσχόμενες μεταχειρίσεις, με το πρώτο να ενισχύει την ποιότητα των φρούτων αποδίδοντας σμέουρα πλούσια σε βιταμίνη C και φλαβονοειδή, ενώ το δεύτερο ενισχύει την απόδοση της παραγωγής κατά 13%. Επιπλέον, στη μεταχείριση με Mel παρατηρείται ένα αρωματικό προφίλ με ενισχυμένα χαρακτηριστικά εσπεριδοειδών, όπως αποτυπώνεται από τη σημαντικά αυξημένη συγκέντρωση λεμονενίου, η οποία ανέρχεται στο 8,19%. Επιπρόσθετα η μεταχείριση των καρπών με μελατονίνη οδήγησε σε σημαντική μείωση του ποσοστού των χλωδών δυσάρεστων οσμών.



6. Appendix

- I. Gohari G, Jiang M, Manganaris GA, Zhou J, Fotopoulos V. Next generation chemical priming: with a little help from our nanocarrier friends. *Trends in Plant Science* **2024**, 29, 150-166. ([pdf file](#))
- II. Georgiadou EC, Garcia CJ, Taliadorou AM, Gedeon S, Valanides N, Varaldo A, Gohari G, Balsells-Llauradó M, Alcázar R, Hertog MLATM, Tomás-Barberán FA, Manganaris GA, Fotopoulos V. Pre-harvest application of melatonin, putrescine and sodium alginate functionalized with melatonin as potential enhancers of secondary metabolism in strawberry fruit. *Current Plant Biology* **2025**, 43:100515. ([pdf file](#))
- III. Xyderou Malefaki A, Valanides N, Manganaris GA, Wasko DeVetter L, Papadaki S, Krokida M, Vyrkou A, Angelis-Dimakis A. A comprehensive assessment of life cycle environmental impact and economic feasibility for different red raspberry (*Rubus idaeus* L) cultivation systems. *Cleaner & Circular Bioeconomy* **2025**, 11100150. ([pdf file](#))
- IV. Georgiadou EC, Garcia Hernandez Gil C, Taliadorou AM, Myrtsi E, Gohari G, Varaldo A, Torrado S, Marcon Gasperini A, Tomás-Barberán F, Hertog MLATM, Fotopoulos V, Manganaris GA. The postharvest application of biodegradable polymers and a priming agent as a potential tool to enhance phytochemical content, aroma profile and market life of strawberry fruit. *LWT- Food Science & Technology* **2025**, 227:117877. ([pdf file](#))
- V. Valanides N, Georgiadou EC, Myrtsi ED, Garcia CJ, Taliadorou A, Torrado S, Hertog MLATM, Tomás-Barberán F, Fotopoulos V, Manganaris GA. Application of priming agents in red raspberries prior to transplantation and at pre-flowering stages results in improved yield efficiency and enhanced secondary metabolism. *Scientia Horticulturae* **2025**, 114465. ([pdf file](#))
- VI. Manganaris GA, Valanides N, Gohari R, Milivojevic J, DeVetter LW, Fotopoulos V. Exploring the potential of priming agents towards enhanced performance of *Rubus* species. *Acta Horticulture* 2024, in press ([pdf file](#))
- VII. Manganaris G.A. Are priming agents a step forward to enhance soft fruit yield efficiency? *ISFC Magazine* 2024, pp. 72-73. ([pdf file](#))
- VIII. Georgiadou EC, Taliadorou AM, Myrtsi ED, Torrado S, Valanides N, Bini L, Manganaris GA, Fotopoulos V. Impact of chemical priming on yield efficiency and on physiological and biochemical properties of field-grown strawberry plants grown under a deficit irrigation regime. *Scientific Reports* **2025**, in press.



- IX. Frakolaki G, Parcharidou M, Boukouvalas C, Papadaki S, Panagiotou N, Valanides N, Kokkini A, Georgiadou EC, Milivojević J, Tsormpatsidis E, Raffaelli D, Mezzetti B, Manganaris GA, Krokida M. Comparative Environmental Impacts of Strawberry Cultivation in Southeastern Europe: A study across open-field and protected cultivation systems towards sustainably-sourced production models. *Journal of Agriculture and Food Research* **2025**, under evaluation.
- X. Taliadorou A, Georgiadou EC, Myrtsi E, Torrado S, Valanides N, Tomás-Barberán F, Garcia CJ, Manganaris GA, Fotopoulos V. Implementation of novel priming agents as a stress alleviation strategy in strawberry production and their role on improving yield and fruit quality (tentative title). *in preparation*
- XI. Georgiadou EC, Taliadorou AM, Myrtsi ED, Torrado S, Soteriou A, Manganaris GA, Fotopoulos V. Exploring encapsulated priming agents and functionalized hydrogel prototypes towards mitigation of salt stress in strawberry plants (tentative title) *in preparation*



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Next generation chemical priming: with a little help from our nanocarrier friends

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Plants are exposed to multiple threats linked to climate change which can cause critical yield losses. Therefore, designing novel crop management tools is crucial. Chemical priming has recently emerged as an effective technology for improving tolerance to stress factors. Several compounds such as phytohormones, reactive species, and synthetic chimeras have been identified as promising priming agents. Following remarkable developments in nanotechnology, several unique nanocarriers (NCs) have been engineered that can act as smart delivery systems. These provide an eco-friendly, next-generation method for chemical priming, leading to increased efficiency and reduced overall chemical usage. We review novel engineered NCs (NENCs) as vehicles for chemical agents in advanced priming strategies, and address challenges and opportunities to be met towards achieving sustainable agriculture.

Essentials of plant chemical priming in a nutshell

Due to their sessile lifestyle, plants, including both crop and non-crop species, are continuously challenged by multiple types of biotic and abiotic stresses throughout their life cycle. Plants may be exposed to stress episodes sequentially or simultaneously. Crucially, a combination of biotic or abiotic stresses may exacerbate the devastating effects on crop productivity compared with the individual effects of the stressors. Recent advances in plant stress physiology have focused the questions of plant biologists on how plants prepare themselves for the possible recurrence of a stress that has passed, and on the type of responses that are generated following recurrence of the same stress factor. Another critical concern has arisen about the responses of plants exposed to different stresses at different stages of their life cycle.

Among the approaches used to address environmental constraints, seed and seedling priming has been receiving an increasing degree of attention, as evidenced by ~1957 documents in the agricultural and biological sciences according to the SCOPUS database, and more than half of these works have been published in the past 5 years. The main philosophy of priming is to enhance the tolerance of plants to stress factors by using priming agents, and this is achieved by activating multiple defense-related pathways. Plants can be primed to better tolerate the stressors through modifications in primary and secondary metabolism. The remarkable effects of priming have been demonstrated across a range of crop and non-crop species. Priming can be initiated naturally following exposure to an environmental stress (also known as physiological priming or hardening), and it can also be achieved by exogenous treatment with biotic (organismal) and abiotic (nonorganismal) priming agents [1]. The latter most commonly involve chemical agents such as natural metabolites or synthetic chemical compounds, and present exciting opportunities for more effective use of plant priming in crop stress management [2].

Changes in osmoregulation, detoxification of reactive oxygen species (ROS), and protein and ion homeostasis mediated by chemical priming agents have been associated with acquired tolerance

Highlights

-Nanocarriers (NCs) functionalized with chemical agents represent a novel approach for improved priming efficiency through targeted delivery.

Multifunctional priming through the combined use of different agents in novel engineered nanocarriers (NENCs) has the potential to achieve multiple benefits in plants.

The application of NENCs as seed coatings has the potential to improve crop yields, while achieving maximum cost-effectiveness compared with application at the plant level as it requires less time and labor.

Gene-editing techniques can be used to modify the expression of targeted genes involved in plant priming as identified by transcriptomic approaches, and can enhance the ability of plants to respond to priming treatments and improve their overall performance.

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in plants. Morphogenetic responses and the release of biologically active compounds are the result of such metabolic changes. Among the priming agents used, sodium nitroprusside [3,4], hydrogen peroxide [5], sodium hydrosulfide [6,7], melatonin [8,9], polyamines [10,11], amino acids [12], and volatile organic compounds [13,14] have been shown to confer remarkable increases in stress tolerance. Recently, Antoniou *et al.* [15] demonstrated the potential of a newly synthesized aspirin variant (**NOSH-A**, see [Glossary](#)) as a highly promising priming agent. NOSH-A acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently, and NOSH specifically donates NO and H₂S. The simultaneous donation of multiple signal/hormonal molecules by NOSH compounds makes them highly attractive candidates for priming agents through the concept of multifunctional priming whereby multiple benefits can be achieved through the synergistic activity of different agents.

Mode of action

Priming results in a modified physiological state that causes the plants to respond in a more robust way after they have been exposed to a stressor. Compared with nonprimed plants, such an acquired primed state manifests itself as faster and/or more efficient defense responses to the stressors, leading to improved plant phenotypes. To survive and reproduce, plants rely on highly sophisticated and elaborate systems to defend themselves. Priming triggers an array of complex biochemical and molecular changes via molecular signaling [2,16]. ROS, which act as second messengers in plant signaling pathways, accumulate during eustress and/or distress [16]. The acquisition of tolerance to stressors also depends on signaling by calcium ions which act as a second messengers. Ca²⁺ is a key cellular component that plays a role in coordinating cellular signaling responses to environmental stimuli [17]. Such signaling molecules can then trigger the induction of defense-related genes. Furthermore, enhancement of plant tolerance through the exogenous application of NO, H₂S, and H₂O₂ involves the regulation of various defense-related genes. The expression of these genes is not only influenced before the imposition of abiotic stress but is also increased during stress conditions. These include not only genes encoding proteins directly engaged in safeguarding plants from stress but also genes related to gene regulation, such as transcription factor (TF) genes, and those involved in signal transduction pathways [18,19]. For instance, ethylene priming increased the expression levels of antioxidant enzymes and ethylene biosynthesis genes, resulting in tolerance to waterlogging stress [20]. Sucrose-mediated priming can induce the expression of specific genes involved in the synthesis of pathogenesis-related proteins (i.e., *PRs*) [21]. In addition to second messenger signaling and gene regulation, hormonal signaling is also a critical player involved in the primed state [22]. Fang *et al.* [22] reported that priming with salicylic acid (SA) increased the concentration of hormones such as SA, abscisic acid, and dihydrozeatin, while it decreased the levels of gibberellins GA₄ and GA₇. Such changes in hormonal status may act as signals to activate defense pathways and modulate subsequent responses of plants to stressors. Furthermore, induced epigenetic modifications are major contributors to maintaining the primed state in subsequent plant generations, and thus contribute to the concept of priming memory, discussed later.

Priming memory

Priming memory can be simply defined as the phenomenon whereby plants, including seeds and seedlings, are exposed to a mild stress event which in turn boosts their ability to cope with future stressors [23,24]. In other words, the ability to retain the information/experience of past stressors is referred to as the memory of primed seeds or seedlings. Both the memory of initial (priming) stress and the retrieval of stored information are essential when faced with subsequent stress, particularly when no stress occurs between the two stressful events [25]. This acquired ability primes plants to respond more quickly or strongly to recurrent stressors [26]. Such acquired information in the primed state can prime plants in the same generation and pass on the 'information' to the next generation [27].

Glossary

Nanocarriers (NCs): small, practical transporters that can change their physical properties such as charge and shape to deliver relevant chemicals to plant tissues.

Nanoparticles (NPs): particles that range in size from 1 to 100 nm and feature two or three exterior dimensions or internal surface structures. In contrast to their molecular counterparts, NPs exhibit unique physicochemical characteristics such as a high surface-to-volume ratio, an unusual surface structure, and increased reactivity. These characteristics are a result of NP cohesiveness, chemical composition, stability, surface structure, shape, and minor size. The capabilities of NPs can be time-controlled, target-specific, self-regulated, programmable, and multifunctional.

Nanoparticle-based smart delivery systems (NSDS): systems that communicate with various organs, tissues, cells, or chemicals as they move through plant tissues. Nanoparticle-biological (nano-bio) interactions relate to the interaction between man-made nanomaterials and a biological system.

Nanotubes (NTs): cylinder-shaped objects with sizes ranging from 1 to 100 nm.

NOSH-A: also known as NBS-1120, NOSH-A is a novel nitric oxide- and hydrogen sulfide-releasing hybrid, which was initially formulated as an anticancer drug but also displays protective effects against abiotic stress conditions in plants.

Novel engineered nanocarriers (NENCs): to effectively convey the substance, loaded NENCs are delivered to the target plant tissues. NENCs provide the opportunity for surface functionalization with targeting ligands. Consequently, NENCs may be made to release the materials they are laden with in a regulated manner to maintain the level of delivery to target areas for a longer time.

Facilitated quicker and stronger responses may be associated with changes in chromatin structure owing to DNA methylation and/or histone modifications [28,29]. As has been well reviewed by Rapp and Wendel [30], the term 'epigenetics' refers to a class of heritable molecular changes that do not involve alteration in the sequence of the DNA [31]. These epigenetic modifications generally cause critical changes in chromatin structure. They can contribute to stress memory and plant resistance to stressors by influencing the expression levels of relevant genes [29]. For example, Laura *et al.* [28] demonstrated epigenetic control of defense genes during methyl jasmonate (MeJA)-induced priming in rice (*Oryza sativa*). In this work the expression levels of *OsBBPI* and *OsPOX*-like defense-related genes were upregulated by MeJA priming in response to wounding. Histone modifications (H3K9ac and H3K4me3) and DNA methylation-like epigenetic markers were also used to link gene upregulation to epigenetic regulation. The authors demonstrated a correlation between chromatin modifications and the level of expression of the *OsBBPI* gene. Following MeJA priming, acetylation and trimethylation of lysine residues in the N-terminus of histone H3 in the promoter region of the *OsBBPI* gene were observed in rice leaves in response to wounding. In addition, critical changes in genome-wide DNA methylation were modulated by MeJA priming upon wounding. Similarly, Kim *et al.* [32] reported that exogenously applied acetic acid promoted *de novo* jasmonic acid (JA) synthesis and histone H4 acetylation which primes the JA signaling pathway toward increased tolerance to drought stress. The priming process initiates a phase of stress memory. This involves a modified transcriptional regulatory event in which the priming stimulus induces lasting alterations in gene expression or a changed transcriptional reaction to a subsequent stimulus – indicative of memory. The discovery of the role of chromatin changes in stress priming can be traced back to a study investigating the impact of a secondary exposure to bacterial pathogens in relation to systemic acquired resistance (SAR) responses. This priming is linked to enduring alterations in histone modifications at various loci, demonstrating priming-dependent transcriptional memory after a delay of several days [33]. Epigenetic processes, including DNA methylation and histone modifications, are currently under examination as crucial elements in promoting broad-spectrum resistance to both abiotic and biotic stresses. These mechanisms are being investigated for their potential roles as carriers of stress memory that are capable of activating immune responses [34].

Regarding transcriptional priming, Holness *et al.* [35] recently investigated H3K4me3 as a potential priming/memory epigenetic mark in arabidopsis (*Arabidopsis thaliana*) plants subjected to high light stress followed by drought stress. In this report it was shown that there is a memory that enables the plants to store and apply the acquired information at a later time in cases where they have been subjected to priming. In comparison to the plants that were not primed or subjected to drought stress, H3K4me3 enrichment was observed in plants subjected to drought and high light stress, suggesting that this mark may be a target for stress memory in plants [35].

Priming stands out as an effective strategy to fortify plant resistance against biotic stresses and pathogens. During this process, plants implement defensive measures against potential threats, and concurrently ready their defense systems for swifter and/or more robust responses in the future. Notably, the effectiveness of priming goes beyond pathogens, and extends to resistance against arthropods [36]. Chemical priming, specifically the induction of SAR, can be achieved by the direct application of substances such as SA and 2,6-dichloroisonicotinic acid (NHP), or by the use of artificial compounds such as the SA structural analog benzothiadiazole (BTH). For instance, activation of SAR by priming compounds such as BTH is often associated with a primed state that allows plants to 'recall' prior infections or stress exposures. Treating arabidopsis with BTH resulted in priming marked by the accumulation of mRNAs and inactive proteins of mitogen-activated protein kinase 3 (MPK3) and MPK6 [26]. Primed plants through SAR equip

themselves with pattern recognition receptors (PRRs) and pathogen-responsive MPKs, and these are activated upon a second infection by elicitors such as the flagellin-derived peptide flg22 through pathways dependent on NPR1. Chemical priming agents utilizing SAR were found to boost the transcription of genes encoding PRRs and accumulate MPK3 and MPK6 in the form of corresponding mRNAs and inactive proteins that can be activated during subsequent stress. Furthermore, epigenetic changes facilitate the rapid activation of stress response-related TFs, enabling plants to enhance and expedite their response to pathogens [26,37].

Nanotechnology-assisted improvement of priming approaches

In addition to the use of natural metabolites and synthetic chemical compounds, recent advances in the field of plant priming include the employment of nanotechnology and its tools as innovative solutions. For example, **nanoparticles (NPs)** have been shown to play a significant role in the protection of plants against adverse environmental conditions, and NPs have been shown to scavenge ROS [38] and improve photosynthetic efficiency by attenuating osmotic and oxidative stress [39–41]. This is largely linked with their nano-size (1–100 nm in at least one dimension), thus giving them diverse physicochemical properties such as higher solubility, reactivity, and biochemical activity depending on their high surface-to-volume ratio and high surface energy [42]. However, these properties also make them ideal candidates for acting as smart **nanocarriers (NCs)** for chemical priming agents, thus achieving targeted delivery and optimal priming activity while lowering their potential environmental impact [43]. This review summarizes current information on advanced NC systems that could be functionalized with chemical agents for enhanced priming efficiency, and concludes that further research will be necessary to address environmental impact and health and safety concerns (Box 1) to achieve optimal usage and exploitation of this technology in crop stress management.

Smart phyto-nanotechnology

More unexpected dangers are posing a threat to agricultural systems all around the world. The sophisticated agronomic use of nanotechnology in plants, known as phyto-nanotechnology, is crucial for preserving food supply and sustainable agriculture, and even economic stability

Box 1. Environmental and/or health and safety concerns

The public perception and acceptance of nanotechnology in food and agriculture also pose a challenge. Concerns about the unknown health effects of nanomaterials may influence consumer attitudes. Transparent communication and education about the safety assessments and benefits of nanomaterial applications are essential to build trust. Nanomaterials, like any other substances, must be handled safely and sustainably to contribute positively to society. They fall under the EU regulatory frameworks of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, the Classification, Labeling and Packaging (CLP) legislation, and the EU Observatory for Nanomaterials (EUON). Manufacturers, importers, and downstream users are obliged under REACH to ensure that nanomaterials do not pose risks to human health or the environment. In 2018, the European Commission made amendments to the REACH Annexes that enhance registration requirements for nanomaterials. Starting in 2020, REACH registration dossiers are required to provide more detailed information about nanomaterials to further ensure safety and compliance.

Assessment of the fate and transport of nanomaterials in the environment is important as they can enter soil, water, and air through various routes. It is critical to assess the potential adverse effects of nanomaterials on non-target organisms such as soil microorganisms, aquatic organisms, and beneficial insects. Understanding how nanomaterials can accumulate in organisms and move through food chains is vital for evaluating their long-term impacts. Regarding health and safety concerns, individuals involved in the production, handling, and application of nanomaterials should be aware of potential occupational exposure risks and take appropriate safety measures.

Health risks can arise from inhalation of NPs or direct contact with the skin, especially if NPs can penetrate biological barriers or cause toxic effects. Assessing the potential toxicity of nanomaterials to humans, including their effects on cells, genes, and specific organs, is crucial for understanding their safety profiles. Comprehensive risk assessments and the implementation of appropriate risk management strategies are essential to address these concerns. These strategies may include the use of safe-by-design approaches in the synthesis of nanomaterials, the application of protective measures during handling and use, and consideration of relevant regulations and guidelines related to nanomaterials.

[44,45]. The use of phyto-nanotechnology (such as new materials, novel methods, or advanced technologies) provides a wide range of potential applications and research areas, such as agrochemicals, nutrients, plant genetic breeding, and biosensors, compared with the production materials utilized in conventional agricultural methods.

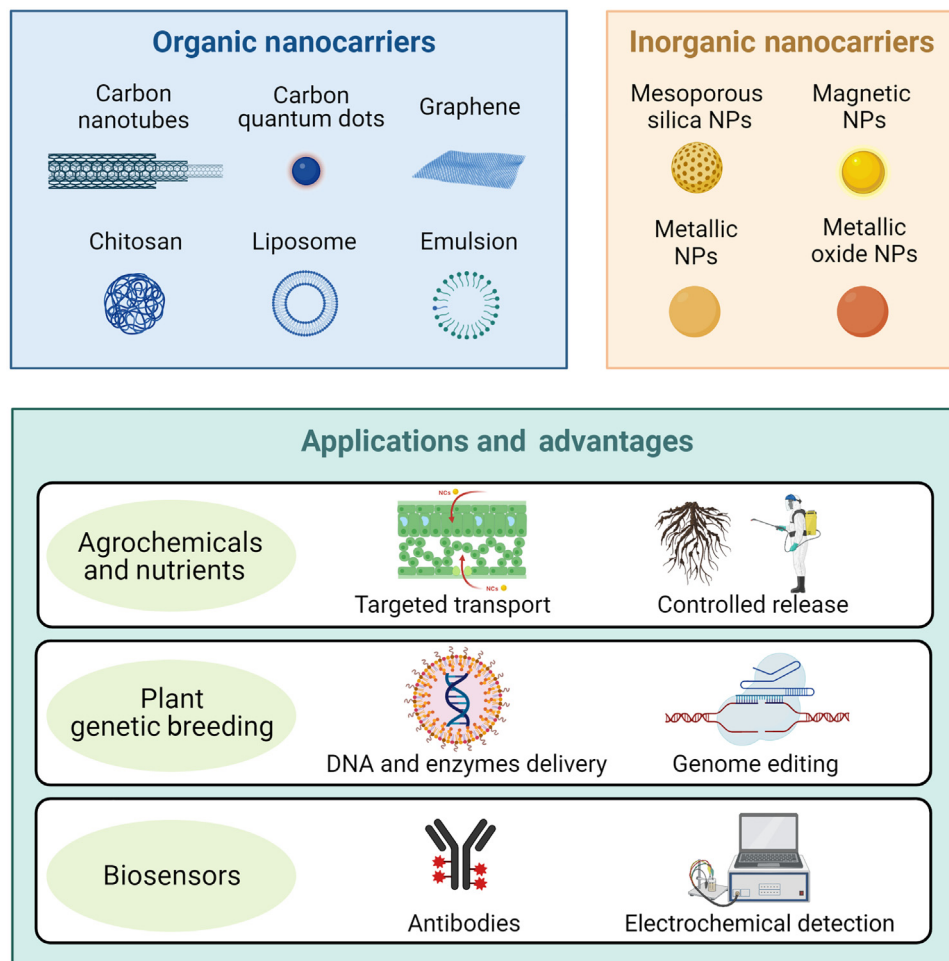
Phyto-nanotechnology has the potential to change current agriculture practices by enabling the targeted transport of nutrients (such as proteins or nucleotides) and the planned release of agrochemicals (such as pesticides or fertilizers) [46,47]. By maximizing nutrient uptake and increasing the tolerance for environmental challenges, a revised consideration of the communications between crops and nanomaterials can increase agricultural productivity [48]. A high transformation efficiency for delivering genes using phyto-nanotechnology can be attained in plant cells without the use of external chemical and physical methods, demonstrating its remarkable applicability in crop breeding, especially plant genetic engineering [49,50]. Notably, phyto-nanotechnology can use nanomaterials with high tensile strength, high throughput, different charges, and small sizes to make it more accurate and effective [51]. Agriculture, biotechnology, and even the food industry have all employed phyto-nanotechnology to create biosensors or to act as 'sensing materials' [52,53]. Diverse categories of nanomaterials such as antibody nanosensors, carbon-based electrochemical nanosensors, nanowire nanosensors, plasmonic nanosensors, and fluorescence resonance energy transfer (FRET)-based nanosensors have been reported as instruments for measuring and detecting fungal pathogens, viruses, bacteria, plant metabolic flux, and residual of pesticides in food [54]. The use of smart phyto-nanotechnology in agricultural systems has supported traditional agricultural methods and practices by providing improved management and resource-efficient advanced 'smart' cropping system.

NP-based smart delivery systems: applications and advantages

Nanoparticle-based smart delivery systems (NSDS) comprise a series of nanomaterials with passive or active targeted transport, or physical and chemical targeted transport, which may achieve targeted release by monitoring endogenous stimuli (e.g., redox and pH value variations) or exogenous stimuli (e.g., electric pulses, light, magnetic fields, temperature variations), thereby improving the entire plant life cycle including seed germination and seedling establishment [55]. Compared with all other priming techniques, NSDS are markedly more efficient. The most important properties of NSDS in priming are to promote electron exchange and increase surface response capabilities related to different components of tissues and cells in plants [56].

NCs are nano-sized carriers based on the concept of NSDS. NCs can promote smart delivery and have favorable impacts on crops, including boosting mineral absorption, enhancing photosynthesis, inducing seed germination, improving crop yield and quality, and expediting crop breeding [57]. The contribution of NCs to plant growth and development is principally influenced by the size, composition, concentration, and physical and chemical properties of NCs [58]. The use of NCs as biosensors, agrochemicals, and nutrients for the protection or production of crops under regulated circumstances is presently a major topic in plant science (Figure 1). Although novel nanotechnology methods addressing technical issues in plant genetic breeding or genome-editing techniques in high demand, new developments in siRNA/miRNA/DNA delivery have only recently found applicability in plants.

NCs can be divided into two categories according to their material properties: organic NCs (ONCs) and inorganic NCs (INCs) (Figure 1). For instance, Santana *et al.* [59] developed targeted carbon-based nanomaterials, TP- β -CDs, for transporting chemical cargoes, and TP-pATV1-SWCNTs for plasmid DNA delivery to chloroplasts, and employed innovative plant biorecognition techniques. Evaluating the impact on cell viability, plants treated with TP- β -CDs (20 mg.l⁻¹) and



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Figure 1. Nanoparticle (NP)-based smart delivery systems: applications and advantages. Organic nanocarriers (ONCs) have been extensively utilized in phyto-nanotechnology, including carbon-based ONCs [carbon nanotubes (CNTs), carbon quantum dots (CQDs), and graphene] and polymeric ONCs (chitosan, liposome, and emulsion). Inorganic nanocarriers (INCs) have been broadly applied in phyto-nanotechnology, such as mesoporous silica NCs (MSNCs), magnetic NCs (MNCs), metallic NCs, and metallic oxide NCs. The applications and advantages of nanoparticle-based smart delivery systems can be divided into three aspects: agrochemicals and nutrients (targeted transport, controlled release), plant genetic breeding (DNA and enzyme delivery, genome editing), and biosensors (antibodies, electrochemical detection).

TP-pATV1-SWCNTs (2 mg.l^{-1}) displayed no significant differences in the percentage of dead cells compared with the control group. This research highlights the heightened efficiency in delivering chemical and plasmid DNA cargoes into chloroplasts through the topical application of carbon nanomaterials engineered with targeting peptides.

Organic nanocarriers

ONCs are created in the context of agricultural systems from carbohydrates, proteins, lipids, and other organic molecules up to a specified size, for example, a radius of $<100 \text{ nm}$ [60]. Based on the fundamental laws of materials science, physical chemistry, and polymer sciences, as well as the physicochemical characteristics of the source materials, ONCs can be produced using top-down and bottom-up methods, or a mixture of both. To explore their applications and the structure–function correlations, ONCs are characterized by their biological properties,

dimensions, internal structures, morphology, and surface properties. ONCs have been extensively applied in phyto-nanotechnology, for example, carbon-based ONCs [**nanotubes (NTs) such as** carbon nanotubes (CNTs), carbon quantum dots (CQDs), and graphene] and polymeric ONCs (chitosan NCs, liposomes, and emulsions).

Carbon-based ONCs

Carbon allotropes known as CNTs feature cylinder-shaped nanostructures with diameters of 1–50 nm [61]. They are characterized as multiwalled nanotubes (MWNTs) or single-walled nanotubes (SWNTs). CNTs are regarded as cutting-edge fertilizers that act as either growth promoters or slow-release fertilizers [62]. CQDs are semiconductor nanocrystals with diameters of 2–10 nm [63]. CQDs are able to enhance the effects of quantum yield or light coverage, and can act as light converters for photosynthesis in plants [64]. A nano-graphene coating made of carbon can lengthen the duration of KNO_3 release while minimizing loss from runoff and leaching [65].

Polymeric ONCs

By altering their physical or chemical properties, ONCs such as chitosan NCs help NCs to more easily enter the epidermal cells of plant leaves, enhance their stability, and reduce their tendency to aggregate [66]. The effects of chitosan NCs and their modified forms on defense-associated systems in crops under abiotic stress have recently been revealed in pertinent research [67]. siRNA transport systems with chitosan NCs incorporated have provided a new approach to crop improvement by allowing the target pest to dominate in a specific way because chitosan has the capacity to permeate cell membranes and interact with RNA [68]. Nano-liposomes help nutrient absorption and transport in a variety of plants because NCs enable the assimilation and transportation of nutrients [69]. Owing to their tiny size and increased surface contact area, nano-liposomes have been approved for the targeted delivery of vitamins, minerals, nutraceuticals, and antibacterial agents [70]. Nonspecific receptors encourage the introduction of negatively charged nano-liposomes into plant cells, which is then followed by adhesion, particle recognition, and eventually endocytosis [71]. A surfactant (such as proteins, lipids, or modified starches) with an average droplet size of 20–200 nm stabilizes a combination of several immiscible liquids to form nano-emulsions [72]. Because of their unique properties, nano-emulsions are ideal intermediates for the transport of nutraceuticals, hydrophobic medicines, and bioactive compounds, and can also encapsulate hydrophobic antioxidant components [73].

Inorganic nanocarriers

INCs such as mesoporous silica NCs (MSNCs), magnetic NCs (MNCs), metallic NCs, and metallic oxide NCs have been broadly applied in phyto-nanotechnology. MSNCs have porous structures resembling honeycombs, and have an adjustable pore size or outside particle diameter in the nm range [74]. They have a large number of unfilled channels that can enclose and absorb various agricultural chemicals and bioactive compounds. MSNCs have been used to transport plasmids carrying the *GFP* gene into plant cells for gene expression. Biochemical analysis and genome modifications in plants can take advantage of proteins and enzymes loaded into MSNCs [75]. By incorporating the transgene into the genome, this method prevents the transmission of the corrected traits to the next generation.

Diverse magnetic materials such as iron (Fe), cobalt (Co), and nickel (Ni), as well as the derived chemical compounds, can be included in MNCs. They can be classified as carbon-coated MNCs [76], magnetic virus-like NCs (VNCs) [77], and other magnetic NCs. For targeted delivery, they can be controlled by magnetic field gradients.

Metallic NCs such as Au and Ag NCs, and metallic oxide NCs such as titanium dioxide (TiO₂), copper oxide (CuO), and zinc oxide (ZnO) NCs, have been widely used as delivery carriers in plant systems because of their superior catalytic, electrical, and light-absorbing properties and high efficiency in the delivery of biomolecules to plants [78,79]. Many metallic oxides and metallic NCs have been utilized in various crop management practices including crop protection and fertilization [48]. Metallic oxides and metallic NCs can improve plant development and growth from the initial stage of germination through to senescence and death in many plant species [45].

Novel engineered nanocarriers

Promising **novel engineered nanocarriers (NENCs)** for chemical priming agents have been the subject of significant research and development, as illustrated in Figure 1. These carriers can be broadly categorized into two main types: organic and inorganic carriers. Within this diverse landscape, various reports have highlighted their versatile applications in delivering a range of essential elements to plants [80]. These elements include nutrients, phytohormones, plant osmolytes, polyamines, and amino acids, all of which play crucial roles in enhancing plant growth and stress tolerance and the quality of agricultural crops.

Among the array of NCs, chitosan stands out as one of the most effective and sustainable options. Chitosan is a biobased, biologically safe, and biodegradable polymer that holds immense potential for delivering various compounds such as SA [81], melatonin [82], putrescine [83], selenium [84], and gibberellic acid [85]. Moreover, chitosan-coated fertilizers can be considered to be an efficient means of delivering micronutrients to plants, particularly elements such as Fe [86]. This approach has proved to be effective in enhancing nutrient uptake by plants. Furthermore, chitosan holds significant promise as a carrier for postharvest treatments aimed at enhancing the quality and extending the shelf life of various agricultural crops. This versatile compound has demonstrated its effectiveness in delivering substances such as polyamines and amino acids, benefiting fruits like grapes [87], plums [88,89], strawberries [90], and persimmons [91].

Among carbon-based NCs, graphene oxide (GO) emerges as a promising candidate for delivering specific compounds to crops such as grapevine [92] and sweet basil [93] under salinity stress conditions. Furthermore, CQDs, characterized by their small size (<10 nm), have shown potential to transport biomolecules into grapevine leaves following foliar application under salinity stress. Notably, functionalized CQDs have been found to bolster enzymatic and nonenzymatic antioxidant systems, thus aiding plants in combating abiotic stresses such as salinity [41,94] and heavy metal stress [95]. In addition, the autofluorescent nature of CQDs enables real-time monitoring of carrier translocation within plants, facilitating precise tracking [96].

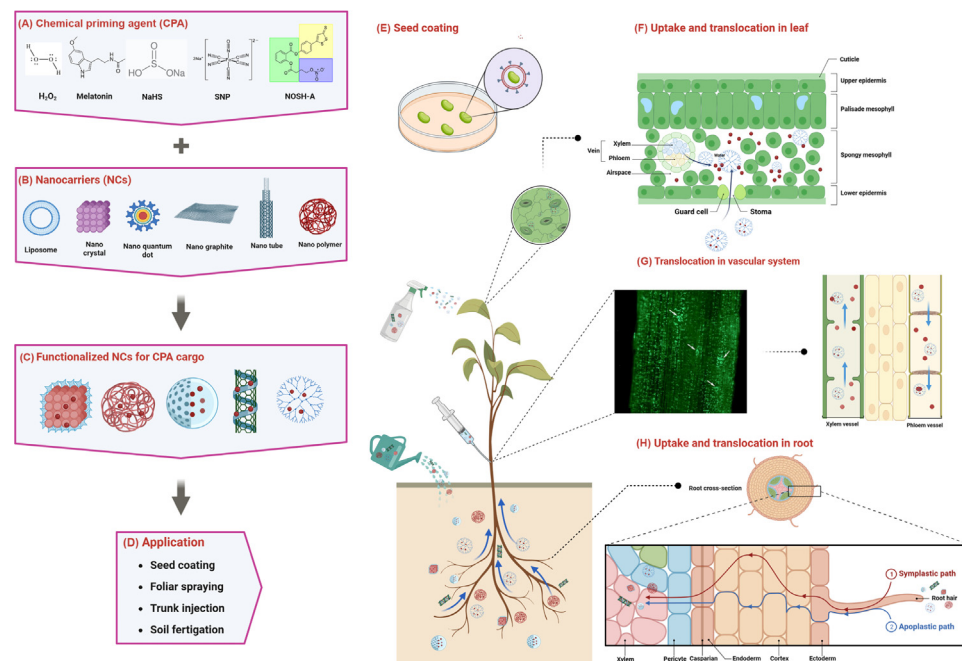
Recently, clay-based carriers have also gained attention as promising vehicles for delivering various beneficial compounds to plants. For instance, Masoudniaragh *et al.* [97] reported that the application of functionalized halloysite clay with proline not only improved agronomic parameters but also significantly increased the production of essential oil compounds such as germacrene D and methyl chavicol in sweet basil under salinity stress. This underscores the potential of clay-based carriers to enhance crop quality and yield through controlled compound delivery. The field of NCs for chemical priming agents is in constant evolution, and they present unique advantages through organic, inorganic, and carbon-based carriers. These have the potential to revolutionize agriculture by improving nutrient delivery, stress tolerance, and overall crop health. Future research will likely unveil even more innovative applications, further contributing to sustainable and efficient agricultural practices.

Controlled release mechanisms of NENCs

In agriculture there is growing interest in the use of nanotechnology to deliver biologically active ingredients via NCs because of its potential to address the challenges posed by adverse environmental conditions and the increasing demand for biologically active ingredients in food [42]. Previous reports have shown that excess application of traditional agrochemical application methods has reduced bioavailability and consequently has had a negative impact on the environment. These findings highlight the need for controlled delivery systems to improve efficacy [98]. Controlled release mechanisms using NCs have therefore been explored as part of crop improvement for the delivery of pesticides/biopesticides and slow-release fertilizers/biofertilizers, as well as for micronutrient encapsulation, stabilization of plant growth regulators, and targeted delivery of genetic material [99]. In addition, the use of nanomaterials to encapsulate agrochemicals allows improved storage and controlled release directly at the site of application, thus improving efficacy and reducing environmental impact. Several nano-delivery systems have been developed for agricultural applications, including emulsions, hydrogels, vesicular carriers (liposomes, noisomes, and transferosomes), and polymeric carriers (chitosan, chitin, polyhydroxybutyrate, cellulose, and starch), which offer advantages such as high surface area, increased activity, and rapid mass transfer [100].

From a chemical point of view, several different strategies can be used to achieve controlled release (Figure 2). Nanomaterials can be produced in several ways, including coating them with thin polymer films, encapsulating them in nanoporous materials, or creating nanoemulsions [101]. By using these approaches, the encapsulated compounds can be protected from loss through evaporation and leaching, thus ensuring gradual release of the encapsulated compounds. NCs are selected based on several factors, including the physicochemical properties of the chemical to be delivered, the desired release kinetics, and the biology of the target plant [102]. Carriers can be designed to provide controlled release profiles and protect compounds from degradation. They can also improve solubility and enhance cellular uptake [103]. To achieve controlled and slow release of compounds, NCs use various mechanisms. NCs can release compounds by diffusion, where the payload molecules move from regions of high concentration within the carrier to regions of lower concentration in the surrounding environment. By modifying the composition of the carrier, its surface properties, and the size of the carrier itself, the release rate can be controlled. In addition, these small vehicles can be designed to degrade over time and release the encapsulated compounds. Environmental factors such as temperature and pH, and the presence of enzymes such as proteases, phospholipases, and oxidoreductases, can trigger degradation [104]. The release of compounds can be tailored to specific requirements by controlling the degradation rate. NCs can be designed to respond to external stimuli such as light, temperature, pH, or magnetic fields. These stimuli can lead to the release of the encapsulated compounds by inducing changes in the structure or properties of the carrier [105]. External magnetic fields can also be used to guide and localize magnetic NCs. The release of compounds can be targeted to specific areas by directing the carriers to the desired location. This improves their efficiency and reduces the amount of compound required [105].

By providing a new delivery system for phytohormones, chemical priming agents, nutrients, and pesticides, nanomaterials can improve the efficiency of nutrient use. Various nanomaterials have the potential to effectively deliver plant growth-promoting compounds via roots, leaves, and seed coats, and these include carbon-based nanomaterials, mineral NPs, metal materials, and metal oxide materials. This delivery system can enhance uptake and translocation within the plant. This improves yields and reduces environmental impact [83,106]. In addition, nanomaterials have the potential to improve soil health and manipulate rhizosphere interactions, including root–soil–microbiome interactions, which can improve crop yields [106]. Smart delivery of



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Figure 2. Schematic pathway of utilizing novel engineered nanocarriers (NENCs) for delivery of chemical priming agents in plants. (A) Various chemical priming agents (CPAs) have been explored for enhancing plant stress tolerance. Notable agents include sodium nitroprusside, hydrogen peroxide, sodium hydrosulfide, melatonin, and polyamines. In addition, a novel aspirin (acetylsalicylic acid) variant, NOSH-A, has shown promise by releasing nitric oxide (NO), hydrogen sulfide (H_2S), and aspirin simultaneously, offering potential benefits in stress mitigation. (B) Nanocarriers (NCs) used in plant applications encompass a diverse range of materials, including liposomes, nanocrystals, nanotubes, graphene oxide, nanopolymers, nanosheets, and more. (C) These nanoparticles (NPs) undergo surface modifications to introduce specific functional groups or molecules, thereby enhancing their properties. When coupled with priming agents, these functionalized NCs give rise to novel engineered NPs. (D) Several modes of application of functionalized nanomaterials in plants can take place, such as through seed coating, foliar application, trunk injection, and soil fertigation. (E) Seed coating with NPs has emerged as a promising technique in seed priming technology. This innovative approach holds great potential for enhancing seed germination, promoting robust seedling growth, and ultimately improving overall plant performance, making it an increasingly important tool in modern agriculture. (F) Some NPs can also penetrate leaf tissues, facilitated by the relatively large size of stomatal openings. NPs <5 nm in size can directly penetrate the leaf cuticle. (G) One key process is the apoplastic transport of NPs through the endodermis, facilitating their movement towards the aerial parts of the plant. Within the plant vascular system, notably the xylem, NPs find a significant conduit for distribution and translocation. (H) NPs smaller than the pore size of root epidermal cell walls, typically in the range of 5–20 nm, can traverse these pores and enter the root tissues, illustrating the significance of the apoplastic pathway in root uptake. Figure created with BioRender.

nanomaterials in low doses to crops can alter their responses to biotic and abiotic stresses, thereby improving their resilience to drought, extreme heat, heavy metals, and salinity [42]. It remains a challenge to efficiently and effectively deliver nanomaterials to plant roots. Targeted approaches such as foliar application and seed coatings are promising, although soil application of nanomaterials is not energy-efficient [107]. Foliar applications face challenges such as degradation, low uptake, and weathering, although they can be applied using existing infrastructure. The development of nanomaterials with improved leaf adhesion and uptake will be necessary to overcome these challenges.

NENC uptake, translocation, and biological impact in plants

Although the mechanisms by which NENCs are taken up and transported in plants remain poorly understood, it is generally agreed that these processes depend on various factors such as the type of NP, its physicochemical properties, the plant species, and the plant substrate (soil,

hydroponics, or culture medium). In general, active transport mechanisms including signaling, recycling, and plasma membrane regulation are involved in the uptake of NENCs by plants [108,109]. Although endocytic pathways are well characterized for the uptake of NPs in animals, plant cells also follow endocytic pathways to take up engineered NPs [108]. Only selected particles can pass through the pores of the plant cell wall, which acts as a semipermeable barrier [109,110].

By forming complexes with membrane transporter proteins or root exudates, plants translocate NPs [111,112]. The interaction between NPs and plants is influenced by NP properties including size, porosity, hydrophobicity, and surface characteristics [43]. There are several ways in which plants can absorb NPs. First, small NPs (<10 nm) can be taken up by roots through pores in the root epidermal cell walls, known as the apoplastic pathway, but larger particles are prevented from entering [111]. The diffusion of small NPs through the apoplast and into the endodermis can be driven by osmotic pressure and capillary forces [109]. Another way in which NPs can be taken up by plants is by crossing the inner side of the plasma membrane via the symplastic pathway [109]. NPs can cross the porous matrix of the cell wall by binding to protein carriers, passing through aquaporins and ion channels, or by penetrating the membrane and creating new channels [108,109]. NPs can also migrate to neighboring cells through plasmodesmata, which are channels 20–50 nm in diameter [111,112]. Stomatal pores are another route for NPs to enter plants [109]. In addition to entering the leaves, NPs can also be transported to the plant roots [113]. Recent advances have improved our understanding of the uptake, translocation, and agglomeration kinetics of NPs, including their dependence on shape, size, and composition [109,111]. Plants take up NPs through their cell walls and the cell membrane of the root epidermis, and then undergo a series of complex events that enable them to be translocated from the roots to the leaves via the vascular bundle (xylem). The uptake of nanomaterials is size-specific because NPs need to pass through pores on the cell membrane to cross intact cell membranes. Before they reach the xylem, the NPs must be passively integrated through the apoplast of the endodermis. The xylem plays a crucial role in the distribution and translocation of NPs. The cell wall is made up of a porous network of polysaccharide fibers which allows the cells to take up water molecules and other dissolved substances [108,109].

Foliar application dynamics

Entry of NPs through stomatal pores on the leaf surface is the main mechanism of NP uptake in plants following foliar application (Figure 2). Some NPs can penetrate the leaf tissue because of the relatively large size of the stomatal openings. Several studies have shown that NPs taken up by leaves are translocated to other parts of the plant [114,115]. Translocation of TiO₂ NPs to the roots via foliar uptake was observed in soybean plants by Hong *et al.* [115]. Furthermore, Gohari *et al.* [39] reported that TiO₂ NPs could penetrate from leaves and translocate in the xylem in sweet basil (*Ocimum basilicum* L.). Several factors such as NP size, surface properties, and leaf characteristics influence the foliar uptake of NPs. The size of the NPs is critical as smaller particles are more likely to enter the stomata. Hydrophilic NPs were shown to be able to pass through stomatal pores when their size was ~40 nm [116]. The cuticle acts as the main natural barrier against NPs entering plant tissues, and protects leaves from water loss and uncontrolled solute exchange. There are two pathways for solute uptake: diffusion and permeation for non-polar solutes via the lipophilic pathway, and passage of polar solutes through polar aqueous pores via the hydrophilic pathway. NPs <4.8 nm can penetrate directly into the cuticle, and larger NPs (>5 nm) can accumulate in leaves through an unclear mechanism [108]. In addition to size, the surface properties of the NPs and the characteristics of the leaf surface also play an important role in foliar uptake. Surface modifications or coatings on NPs can affect their interaction with the leaf surface and subsequent uptake. Moreover, as widely known, the inclusion of surfactants such as Tween during the spraying of nanomaterials can enhance the efficacy of foliar uptake. This improvement

is realized by increasing cuticular and subcuticular penetration, thereby facilitating the transport of nanomaterials following foliar spraying. It is worth highlighting that the incorporation of Tween 20 or Tween 80 can be beneficial for the foliar delivery of NCs [39–41]. The concentration of NPs is a critical factor to consider in their relocation and aggregation when applied by foliar spraying. This factor is evident in the application of multiwall carbon nanotubes (MWCNTs) to sweet basil plants, where a dose-dependent relationship was observed. Lower doses of MWCNTs (25 and 50 mg.l⁻¹) exhibited positive effects by alleviating salt stress-induced damage. These effects included enhancements of antioxidant enzymatic activities, phenolic compounds, physiological parameters such as chlorophyll and carotenoid content, nonenzymatic (i.e., phenolic content) and enzymatic antioxidant components [i.e., ascorbate peroxidase (APX), catalase (CAT), and guaiacol peroxidase (GP) activities], and essential oil content and composition. However, higher concentrations (100 mg.l⁻¹) of MWCNTs-COOH resulted in aggregation within plant tissues, leading to toxicity symptoms [117]. Furthermore, increasing the concentration of TiO₂ and ZnO NPs from 20 mg.l⁻¹ to 40 mg.l⁻¹ resulted in a significant reduction in germination of both onion and fennel seeds [45]. Therefore, the concentration of NPs plays a crucial role in determining their effectiveness and potential adverse effects on plants following foliar application.

Soil application dynamics

The uptake mechanism of NPs in plants by soil application involves the interaction of NPs with the roots and their subsequent uptake into vascular system of the plants (Figure 2). NPs come into contact with the root surface when they are applied to the soil. The uptake mechanisms and behaviors of NPs in plant roots have been investigated in several studies [118]. Among the mechanisms, the apoplastic pathway is one of the major routes of NP uptake. NPs smaller than the pore size of root epidermal cell walls (typically ~5–20 nm) can enter the roots through these pores [112]. However, particles larger than the pore size are typically blocked and cannot enter. Once NPs enter the root, they may undergo several processes to reach the plant vascular system. These include diffusion through the apoplast, where NPs move through the spaces between cells, and eventually reach the endodermis [119]. NPs must passively integrate into the apoplast to cross the endodermis and enter the stele [119]. An important step in the translocation of NPs to the aerial parts of the plant is the apoplastic transport of NPs through the endodermis. The vascular system of plants, particularly the xylem, plays a significant role in the distribution and translocation of NPs within the plant. Xylem vessels serve as the main pathway for the uptake of water and solutes, including NPs, from roots to shoots [120]. Wang *et al.* [121] demonstrated the uptake, translocation, and distribution of CuO NPs in maize plants through the xylem. The uptake of NPs by plant roots is influenced by several factors, including NP properties, root structure, and environmental conditions. The interaction with the root surface and subsequent uptake can be modulated by NP properties such as size, shape, and surface characteristics [38]. NP uptake can also be influenced by root characteristics such as root exudates, surface charge, and root hair density [110].

Seed priming dynamics

The mechanism of uptake of NPs into plants by seed coatings involves the application of NPs to the surface of seeds, which allows their penetration into the seed and subsequent transport to various plant tissues during germination and seedling development. Seed coating with NPs has gained interest in recent years, particularly in seed priming technology, because of its potential benefits in improving seed germination, seedling growth, and overall plant performance [55]. NPs can adhere to the seed surface or penetrate the seed tissue when applied as a seed coating. The mechanism of NP penetration into seeds is still not fully understood and may vary depending on the specific NP and seed characteristics. However, insights into the possible pathways and mechanisms involved have been provided by several studies. The seed coat, which consists of

several layers that form a physical barrier, is one possible mechanism for NP penetration. NPs can interact with the seed coat and penetrate through microcracks, pores, or gaps on the surface of the seed coat [122,123]. These apertures may be the result of natural imperfections in the seed coat, mechanical damage, or chemical treatments applied during the processing of the seed. In the study by Yu *et al.* [123], machine learning was employed to assess NP uptake during seed priming. The findings revealed that the concentration of NPs and ionic strength played a crucial role in influencing shoot fresh weight, primarily by regulating NP uptake. Notably, the uptake of NPs experienced a significant deceleration when the NP concentration surpassed 50 mg.l⁻¹. Although factors including zeta potential and hydrodynamic diameter did not demonstrate noticeable effects on NP uptake, their biological impacts should not be disregarded. Natural openings such as stomata or lenticels in some seed coats are another mechanism for NP uptake in seeds. Stomata are tiny pores that are mainly found on the surface of leaves, but they can also be found on the seed coats of some species of plants. Through these openings, which provide direct access to the inside of the seed, NPs can enter the seed [124]. Seed priming involves the pre-soaking or pre-germination treatment of seeds in various solutions (e.g., 'NP suspensions') and is a very promising approach for improving seed performance. During the seed-priming process, NPs can penetrate into the tissues of the seed through the process of imbibition in which water and solutes are absorbed by the seed [2]. The NPs contained in the priming solution can be absorbed by water, allowing them to enter the seed [125]. Once inside the seed, NPs can interact with the seed components such as proteins, lipids, and cell membranes, and affect seed metabolism and germination processes.

Bioimaging and detection of NENCs in plant tissues

To understand the uptake, distribution, and potential effects of NENCs, bioimaging and detection of NENCs in plant tissues is crucial. Electron microscopy techniques, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM), have been instrumental in visualizing the internalization and localization of NENCs in plant tissues at high resolution. The SEM provides a detailed view of surface morphology, whereas the TEM enables the study of subcellular structures. Recent studies have used these techniques to investigate the interactions between NENCs and plant tissues, including uptake pathways and cellular localization [126,127]. For example, SEM and TEM were employed to visualize the accumulation of silver NPs in various plant tissues, revealing their internalization and localization within root cells [128]. Furthermore, electron microscopy techniques have aided in determining the size, shape, and aggregation state of NENCs within plant tissues, providing valuable insights into their behavior and potential impacts [128]. For example, a study by Proença *et al.* [129] investigated the uptake and distribution of TiO₂ NPs in lettuce leaves using SEM and TEM. The results showed that the TiO₂ NPs were mainly aggregated on the leaf surface in the form of larger agglomerates. TEM analysis revealed the presence of internalized NPs within the cells of the leaf, suggesting the possibility of their translocation through the tissues of the plant. To visualize and track NENCs in plant tissues, fluorescence microscopy has emerged as a valuable tool. Fluorescent labels attached to the NENCs allow real-time monitoring and localization. This technique enables the visualization of NENCs within plant cells and tissues, thus providing insights into their distribution patterns and cellular interactions. Recent advances in fluorescence microscopy, such as the use of quantum dots and other fluorophores, have improved the sensitivity and specificity of NENC detection in plants [128]. For example, fluorescence microscopy coupled with confocal imaging was used to study the intracellular fate of silica-based NCs in arabidopsis, and enabled the visualization of their uptake and distribution within different plant cell compartments [130].

Compared with conventional fluorescence microscopy, confocal laser scanning microscopy offers improved resolution and depth penetration. It enables 3D imaging of NENCs in plant tissues,

making it easier to analyze how NENCs distribute at different depths in the tissue. The internalization and subcellular trafficking of NENCs in different plant species was visualized using confocal microscopy [114,129]. For example, a study by Sun *et al.* [130] used confocal laser scanning microscopy to investigate the uptake and intracellular localization of gold NPs in arabidopsis root cells. This revealed endocytic internalization of NPs and their accumulation within endosomal compartments, shedding light on the interactions between cells and the possible mechanisms of uptake. Super-resolution microscopy techniques, such as stimulated emission depletion (STED) microscopy and structured illumination microscopy (SIM), have revolutionized bioimaging by breaking the diffraction barrier. Detailed insights into the subcellular localization and interactions of NENCs are provided by these techniques. Super-resolution microscopy has been used to study the localization and movement of quantum dot-labeled NCs within plant cells, allowing visualization at the nm scale [129]. Wang *et al.* [131] used STED microscopy to visualize the uptake and intracellular trafficking of polymer-coated magnetic NPs in maize root cells. The results demonstrated the ability of STED microscopy to resolve the subcellular distribution of NENCs and their interactions with specific organelles.

Concluding remarks and future perspectives

In the past decade, nanotechnology has made great strides in the design, production, and use of NCs in agriculture. NCs for crops cannot be broadly applied in agricultural operations or activities because of high costs or other issues. By promoting interdisciplinary approaches to the design and synthesis of intelligent NCs, the challenges of phyto-nanotechnology can be overcome. To achieve this, a combined collaborative project combining the complementary professional strengths of geneticists, engineers, botanists, biochemists, and chemists may open up a new frontier in phyto-nanotechnology. Current applications suggest that further research is required in this area of plant development or growth to improve the sustainability of agricultural systems. Identifying the underlying mechanism of the influence of NCs on plants may further benefit from future studies utilizing open-field experiments.

Plant cell walls have a porosity of only 15 nm [132], indicating the need to manage the size of NCs for effective translocation. In addition, the effectiveness of NCs in entering the chloroplast membrane is strongly correlated with their zeta potential values [133]. Together with the information in the previous section, it is important to characterize or describe the cultivation method, concentration, size, and zeta potential of the NCs in a study so that the researchers can more easily follow the research and understand how the NCs improve plant growth and development.

Despite all the positive outcomes, design safety considerations must be taken into consideration to help the community to deal with the potential negative consequences of new NCs on the environment (e.g., NCs in a daily necessity product) [134]. These NCs must be properly formulated for usage in the agricultural system, taking the treatment techniques (foliar and soil) into account, to have a significant impact and guarantee superior crop quality. While using these NCs in plant systems, additional precautions must be taken because their excessive usage may harm the environment. However, it cannot be denied that the favorable effects of NCs have made significant contributions to many areas of agricultural systems from germination to postharvest (see [Outstanding questions](#)).

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Outstanding questions

Is nano-priming more effective than traditional chemical priming in promoting plant growth and development under control and stress conditions? If so, what are the underlying mechanisms?

Which molecular pathways regulate the enhancement of plant growth, development, and stress tolerance by nano-priming? Which genes play a vital role in these regulatory processes?

What are the most effective NCs, and how can we improve the innovative methods for the controlled release of biomolecules in crop plants?

How can nano-priming with NPs affect the next generation of plants?

In terms of transgenerational changes in plants, are there any differences in seed priming or seedling priming approaches?

Once plants have been primed with NPs, what epigenetic mechanisms are involved? Is there a common epigenetic response to different stressors?

How will nano-primed plants respond when they are exposed to recurring stressors? Will there be any significant differences in comparison to nonprimed plants?

Declaration of interests

V.F. is a coinventor of patent WO/2015/123273 dealing with the use of NOSH-A in plants. The remaining authors declare no conflicts of interest.

References

- Westman, S.M. *et al.* (2019) Defence priming in *Arabidopsis* – a meta-analysis. *Sci. Rep.* 9, 13309
- Savvides, A. *et al.* (2016) Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends Plant Sci.* 21, 329–340
- Tanou, G. *et al.* (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *Plant J.* 72, 585–599
- Mohammadi, M. *et al.* (2023) Stress memory in seedlings of primed seed chickpea (*Cicer arietinum* L.) using sodium nitroprusside under cold stress. *Plant Stress* 8, 100163
- Gohari, G. *et al.* (2020) Interaction between hydrogen peroxide and sodium nitroprusside following chemical priming of *Ocimum basilicum* L. against salt stress. *Physiol. Plant.* 168, 361–373
- Christou, A. *et al.* (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defense pathways. *J. Exp. Bot.* 64, 1953–1966
- Ocvirk, D. *et al.* (2021) The effects of seed priming with sodium hydrosulphide on drought tolerance of sunflower (*Helianthus annuus* L.) in germination and early growth. *Ann. Appl. Biol.* 178, 400–413
- Antoniou, C. *et al.* (2017) Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. *J. Pineal Res.* 62, e12401
- Zhang, Y. *et al.* (2021) Seed priming with melatonin improves salt tolerance in cotton through regulating photosynthesis, scavenging reactive oxygen species and coordinating with phytohormone signal pathways. *Ind. Crop. Prod.* 169, 113671
- Tanou, G. *et al.* (2014) Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. *Plant Cell Environ.* 37, 864–885
- Alcázar, R. *et al.* (2020) Polyamines: small amines with large effects on plant abiotic stress tolerance. *Cells* 9, 2373
- Cai, J. and Aharoni, A. (2022) Amino acids and their derivatives mediating defense priming and growth tradeoff. *Curr. Opin. Plant Biol.* 69, 102288
- Tian, S. *et al.* (2019) Priming with the green leaf volatile (Z)-3-hexenyl-1-yl acetate enhances salinity stress tolerance in peanut (*Arachis hypogaea* L.) seedlings. *Front. Plant Sci.* 10, 785
- Li, X. *et al.* (2022) Priming with the green leaf volatile (Z)-3-hexenyl-1-yl acetate enhances drought resistance in wheat seedlings. *Plant Growth Regul.* 98, 477–490
- Antoniou, C. *et al.* (2020) Exploring the potential of nitric oxide and hydrogen sulfide (NOSH)-releasing synthetic compounds as novel priming agents against drought stress in *Medicago sativa* plants. *Biomolecules* 10, 120
- Dildabek, A. *et al.* (2020) Crosstolerant effect of salt priming and viral infection on *Nicotiana benthamiana*. *Eurasian J. Appl. Biotechnol.* 2020, UDC578.24
- Tuteja, N. and Sopory, S.K. (2008) Chemical signaling under abiotic stress environment in plants. *Plant Signal. Behav.* 3, 525–536
- Antoniou, C. *et al.* (2016) Unravelling chemical priming machinery in plants: the role of reactive oxygen–nitrogen–sulfur species in abiotic stress tolerance enhancement. *Curr. Opin. Plant Biol.* 33, 101–107
- Kerchev, P. *et al.* (2020) Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnol. Adv.* 40, 107503
- Wwioko, E. *et al.* (2017) Comparative physiological, biochemical, and genetic responses to prolonged waterlogging stress in okra and maize given exogenous ethylene priming. *Front. Physiol.* 8, 632
- Gómez-Ariza, J. *et al.* (2007) Sucrose-mediated priming of plant defense responses and broad-spectrum disease resistance by overexpression of the maize pathogenesis-related PRms protein in rice plants. *Mol. Plant-Microbe Interact.* 20, 832–842
- Fang, S. *et al.* (2018) Chemical priming of seed alters cotton floral bud differentiation by inducing changes in hormones, metabolites and gene expression. *Plant Physiol. Biochem.* 130, 633–640
- Hake, K. and Romeis, T. (2019) Protein kinase-mediated signaling in priming: Immune signal initiation, propagation, and establishment of long-term pathogen resistance in plants. *Plant Cell Environ.* 42, 904–917
- Avramova, Z. (2019) Defence-related priming and responses to recurring drought: Two manifestations of plant transcriptional memory mediated by the ABA and JA signalling pathways. *Plant Cell Environ.* 42, 983–997
- Hilker, M. and Schmölling, T. (2019) Stress priming, memory, and signalling in plants. *Plant Cell Environ.* 42, 753–761
- Hönl, M. *et al.* (2023) Chemical priming of plant defense responses to pathogen attacks. *Front. Plant Sci.* 14, 1146577
- Wang, X. *et al.* (2017) Priming: a promising strategy for crop production in response to future climate. *J. Integr. Agric.* 16, 2709–2716
- Laura, B. *et al.* (2018) Epigenetic control of defense genes following MeJA-induced priming in rice (*O. sativa*). *J. Plant Physiol.* 228, 166–177
- Nguyen, H.M. *et al.* (2020) Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. *Front. Plant Sci.* 11, 494
- Rapp, R.A. and Wendel, J.F. (2005) Epigenetics and plant evolution. *New Phytol.* 168, 81–91
- Bonasio, R. *et al.* (2010) Molecular signals of epigenetic states. *Science* 330, 612–616
- Kim, J.M. *et al.* (2017) Acetate-mediated novel survival strategy against drought in plants. *Nat. Plants* 3, 17097
- Oberkofler, V. *et al.* (2021) Epigenetic regulation of abiotic stress memory: Maintaining the good things while they last. *Curr. Opin. Plant Biol.* 61, 102007
- Mladenov, V. *et al.* (2021) Deciphering the epigenetic alphabet involved in transgenerational stress memory in crops. *Int. J. Mol. Sci.* 22, 7118
- Holness, S. *et al.* (2023) Highlight induced transcriptional priming against a subsequent drought stress in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* 24, 6608
- Mauch-Mani, B. *et al.* (2017) Defense priming: an adaptive part of induced resistance. *Annu. Rev. Plant Biol.* 68, 485–512
- Gully, K. *et al.* (2019) Biotic stress-induced priming and de-priming of transcriptional memory in *Arabidopsis* and apple. *Epigenomes* 3, 3
- Rico, C.M. *et al.* (2013) Effect of cerium oxide nanoparticles on rice: a study involving the antioxidant defense system and in vivo fluorescence imaging. *Environ. Sci. Technol.* 47, 5635–5642
- Gohari, G. *et al.* (2020) Titanium dioxide nanoparticles (TiO₂ NPs) promote growth and ameliorate salinity stress effects on essential oil profile and biochemical attributes of *Dracocephalum moldavica*. *Sci. Rep.* 10, 912
- Mohammadi, M.H.Z. *et al.* (2021) Cerium oxide nanoparticles (CeO₂ NPs) improve growth parameters and antioxidant defense system in Moldavian balm (*Dracocephalum moldavica* L.) under salinity stress. *Plant Stress* 1, 100006
- Gohari, G. *et al.* (2021) Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticles effectively prime grapevine (*Vitis vinifera* cv. 'Sultana') against salt stress. *BMC Plant Biol.* 21, 120
- Ioannou, A. *et al.* (2020) Advanced nanomaterials in agriculture under a changing climate: the way to the future? *Environ. Exp. Bot.* 176, 104048

43. Khan, I. *et al.* (2019) Nanoparticles: properties, applications and toxicities. *Arab. J. Chem.* 12, 908–931
44. Shang, Y. *et al.* (2019) Applications of nanotechnology in plant growth and crop protection: a review. *Molecules* 24, 2558
45. Jiang, M. *et al.* (2021) Phytonanotechnology applications in modern agriculture. *J. Nanobiotechnol.* 19, 430
46. Acharya, A. and Pal, P.K. (2020) Agriculture nanotechnology: translating research outcome to field applications by influencing environmental sustainability. *NanoImpact* 19, 100232
47. Salama, D.M. *et al.* (2021) Applications of nanotechnology on vegetable crops. *Chemosphere* 266, 129026
48. Gogos, A. *et al.* (2012) Nanomaterials in plant protection and fertilization: current state, foreseen applications, and research priorities. *J. Agric. Food Chem.* 60, 9781–9792
49. Yan, Y. *et al.* (2022) Nanotechnology strategies for plant genetic engineering. *Adv. Mater.* 34, 2106945
50. Agrawal, S. *et al.* (2022) Plant development and crop protection using phytonanotechnology: a new window for sustainable agriculture. *Chemosphere* 299, 134465
51. Demirel, G.S. *et al.* (2021) Nanotechnology to advance CRISPR–Cas genetic engineering of plants. *Nat. Nanotechnol.* 16, 243–250
52. Duhan, J.S. *et al.* (2017) Nanotechnology: the new perspective in precision agriculture. *Biotechnol. Rep.* 15, 11–23
53. Sanzari, I. *et al.* (2019) Nanotechnology in plant science: to make a long story short. *Front. Bioengin. Biotechnol.* 7, 120
54. Chaudhry, N. *et al.* (2018) Bio-inspired nanomaterials in agriculture and food: Current status, foreseen applications and challenges. *Microb. Pathog.* 123, 196–200
55. do Espírito Santo Pereira, A. *et al.* (2021) Nanotechnology potential in seed priming for sustainable agriculture. *Nanomaterials* 11, 267
56. Nile, S.H. *et al.* (2022) Nano-priming as emerging seed priming technology for sustainable agriculture – recent developments and future perspectives. *J. Nanobiotechnol.* 20, 254
57. Nair, R. (2016) Effects of nanoparticles on plant growth and development. *Plant Nanotechnol.* 5, 95–118
58. Siddiqi, K.S. and Husen, A. (2017) Plant response to engineered metal oxide nanoparticles. *Nanoscale Res. Lett.* 12, 92
59. Santana, I. *et al.* (2022) Targeted carbon nanostructures for chemical and gene delivery to plant chloroplasts. *ACS Nano* 16, 12156–12173
60. Pan, K. and Zhong, Q. (2016) Organic nanoparticles in foods: fabrication, characterization, and utilization. *Annu. Rev. Food Sci. Technol.* 7, 245–266
61. Chang, X. *et al.* (2020) Effects of carbon nanotubes on growth of wheat seedlings and Cd uptake. *Chemosphere* 240, 124931
62. Safdar, M. *et al.* (2022) Engineering plants with carbon nanotubes: a sustainable agriculture approach. *J. Nanobiotechnol.* 20, 275
63. Kargozer, S. *et al.* (2020) Quantum dots: a review from concept to clinic. *Biotechnol. J.* 15, 2000117
64. Li, Y. *et al.* (2021) Carbon dots as light converter for plant photosynthesis: augmenting light coverage and quantum yield effect. *J. Hazard. Mater.* 410, 124534
65. Her, S.C. and Liang, Y.M. (2022) Carbon-based nanomaterials thin film deposited on a flexible substrate for strain sensing application. *Sensors* 22, 5039
66. Nadendla, S.R. *et al.* (2018) HarpinPss encapsulation in chitosan nanoparticles for improved bioavailability and disease resistance in tomato. *Carbohydr. Polym.* 199, 11–19
67. Riseh, R.S. *et al.* (2022) Nano/microencapsulation of plant biocontrol agents by chitosan, alginate, and other important biopolymers as a novel strategy for alleviating plant biotic stresses. *Int. J. Biol. Macromol.* 222, 1589–1604
68. Zhang, X. *et al.* (2010) Chitosan/double-stranded RNA nanoparticle-mediated RNA interference to silence chitin synthase genes through larval feeding in the African malaria mosquito (*Anopheles gambiae*). *Insect Mol. Biol.* 19, 683–693
69. Karny, A. *et al.* (2018) Therapeutic nanoparticles penetrate leaves and deliver nutrients to agricultural crops. *Sci. Rep.* 8, 7589
70. Zou, L. *et al.* (2015) A novel delivery system dextran sulfate coated amphiphilic chitosan derivatives-based nanoliposome: capacity to improve in vitro digestion stability of (–)-epigallocatechin gallate. *Food Res. Int.* 69, 114–120
71. Taherkhani, S. *et al.* (2014) Covalent binding of nanoliposomes to the surface of magnetotactic bacteria for the synthesis of self-propelled therapeutic agents. *ACS Nano* 8, 5049–5060
72. Gupta, A. *et al.* (2016) Nanoemulsions: formation, properties and applications. *Soft Matter* 12, 2826–2841
73. Salvia-Trujillo, L. *et al.* (2014) Impact of microfluidization or ultrasound processing on the antimicrobial activity against *Escherichia coli* of lemongrass oil-loaded nanoemulsions. *Food Control* 37, 292–297
74. Torney, F. *et al.* (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat. Nanotechnol.* 2, 295–300
75. Martin-Ortigosa, S. *et al.* (2014) Mesoporous silica nanoparticle-mediated intracellular Cre protein delivery for maize genome editing via loxP site excision. *Plant Physiol.* 164, 537–547
76. Corredor, E. *et al.* (2009) Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. *BMC Plant Biol.* 9, 45
77. Huang, X. *et al.* (2011) Magnetic virus-like nanoparticles in *N. benthamiana* plants: a new paradigm for environmental and agronomic biotechnological research. *ACS Nano* 5, 4037–4045
78. Zhao, L. *et al.* (2020) Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. *J. Agric. Food Chem.* 68, 1935–1947
79. Yan, X. *et al.* (2022) AgNPs-triggered seed metabolic and transcriptional reprogramming enhanced rice salt tolerance and blast resistance. *ACS Nano* 17, 492–504
80. Campos, E.V. *et al.* (2023) Encapsulated plant growth regulators and associative microorganisms: Nature-based solutions to mitigate the effects of climate change on plants. *Plant Sci.* 111688
81. Aazami, M.A. *et al.* (2023) Protective effects of chitosan based salicylic acid nanocomposite (CS-SA NCs) in grape (*Vitis vinifera* cv. 'Sultana') under salinity stress. *Sci. Rep.* 13, 883
82. Gohari, G. *et al.* (2023) Mitigation of salinity impact in spearmint plants through the application of engineered chitosan-melatonin nanoparticles. *Int. J. Biol. Macromol.* 224, 893–907
83. Panahirad, S. *et al.* (2023) Foliar application of chitosan-putrescine nanoparticles (CTS-Put NPs) alleviates cadmium toxicity in grapevine (*Vitis vinifera* L.) cv. Sultana: modulation of antioxidant and photosynthetic status. *BMC Plant Biol.* 23, 411
84. Sheikhalipour, M. *et al.* (2023) Seedling nanoprimer with selenium-chitosan nanoparticles mitigates the adverse effects of salt stress by inducing multiple defence pathways in bitter melon plants. *Int. J. Biol. Macromol.* 124923
85. dos Santos Guaraldo, M. *et al.* (2023) Priming with sodium nitroprusside and hydrogen peroxide increases cotton seed tolerance to salinity and water deficit during seed germination and seedling development. *Environ. Exp. Bot.* 209, 105294
86. Giglou, M.T. *et al.* (2022) A new method in mitigation of drought stress by chitosan-coated iron oxide nanoparticles and growth stimulant in peppermint. *Ind. Crop. Prod.* 187, 115286
87. Khalili, N. *et al.* (2022) Chitosan-enriched salicylic acid nanoparticles enhanced anthocyanin content in grape (*Vitis vinifera* L. cv. Red Sultana) berries. *Polymers* 14, 3349
88. Mahmoudi, R. *et al.* (2022) Application of glycine betaine coated chitosan nanoparticles alleviate chilling injury and maintain quality of plum (*Prunus domestica* L.) fruit. *Int. J. Biol. Macromol.* 207, 965–977
89. Mahmoudi, R. *et al.* (2022) Postharvest chitosan-arginine nanoparticles application ameliorates chilling injury in plum fruit during cold storage by enhancing ROS scavenging system activity. *BMC Plant Biol.* 22, 555
90. Bahmani, R. *et al.* (2022) Evaluation of proline-coated chitosan nanoparticles on decay control and quality preservation of strawberry fruit (cv. Camarosa) during cold storage. *Horticulturae* 8, 648
91. Nasr, F. *et al.* (2021) Chitosan-phenylalanine nanoparticles (Cs-Phe Nps) extend the postharvest life of persimmon (*Diospyros kaki*) fruits under chilling stress. *Coatings* 11, 819
92. Zahedi, S.M. *et al.* (2023) Proline-functionalized graphene oxide nanoparticles (GO-pro NPs): A new engineered nanoparticle to

- ameliorate salinity stress on grape (*Vitis vinifera* L. cv sultana). *Plant Stress* 7, 100128
93. Ganjavi, A.S. *et al.* (2021) Glycine betaine functionalized graphene oxide as a new engineering nanoparticle lessens salt stress impacts in sweet basil (*Ocimum basilicum* L.). *Plant Physiol. Biochem.* 162, 14–26
 94. Gohari, G. *et al.* (2020) Putrescine-functionalized carbon quantum dot nanoparticles (Put-CQD) effectively prime grape (*Vitis vinifera* cv. Sultana) against salt stress. *BMC Plant Biol.* 21, 120
 95. Panahirad, S. *et al.* (2023) Putrescine-functionalized carbon quantum dot (put-CQD) nanoparticle: A promising stress-protecting agent against cadmium stress in grapevine (*Vitis vinifera* cv. Sultana). *Plant Physiol. Biochem.* 197, 107653
 96. Gade, A. *et al.* (2022) Carbon-based nanomaterials for sustainable agriculture: their application as light converters, nanosensors, and delivery tools. *Plants* 11, 511
 97. Masoudniaragh, A. *et al.* (2021) Using halloysite nanotubes as carrier for proline to alleviate salt stress effects in sweet basil (*Ocimum basilicum* L.). *Sci. Hortic.* 285, 110202
 98. Shelar, A. *et al.* (2023) Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: challenges, risk assessment, and future perspectives. *Nano-Micro Lett.* 15, 54
 99. Gade, A. *et al.* (2023) Nanofertilizers: the next generation of agrochemicals for long-term impact on sustainability in farming systems. *Agrochemicals* 2, 257–278
 100. Xin, X. *et al.* (2020) Nano-enabled agriculture: from nanoparticles to smart nanodelivery systems. *Environ. Chem.* 17, 413–425
 101. Patra, J.K. *et al.* (2018) Nano based drug delivery systems: recent developments and future prospects. *J. Nanobiotechnol.* 16, 71
 102. Vega-Vásquez, P. *et al.* (2020) Nanoscale drug delivery systems: from medicine to agriculture. *Front. Bioeng. Biotechnol.* 8, 79
 103. Idumah, C.I. (2023) Design, development, and drug delivery applications of graphene polymeric nanocomposites and bionanocomposites. *Emergent Mater.* 6, 777–807
 104. Xu, C. *et al.* (2019) Biodegradable nanoparticles of polyacrylic acid-stabilized amorphous CaCO₃ for tunable pH-responsive drug delivery and enhanced tumor inhibition. *Adv. Funct. Mater.* 29, 1808146
 105. Li, C. *et al.* (2023) Recent progress in nanotechnology-based drug carriers for resveratrol delivery. *Drug Deliv.* 30, 2174206
 106. Venugopalan, V.K. *et al.* (2022) Smart fertilizers – a way ahead for sustainable agriculture. *J. Plant Nutr.* 45, 2068–2076
 107. Lowry, G.V. *et al.* (2019) Opportunities and challenges for nanotechnology in the agri-tech revolution. *Nat. Nanotechnol.* 14, 517–522
 108. Lv, J. *et al.* (2019) Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges. *Environ. Sci. Nano* 6, 41–59
 109. Husted, S. *et al.* (2022) What is missing to advance foliar fertilization using nanotechnology? *Trends Plant Sci.* 28, 90–105
 110. Schwab, F. *et al.* (2016) Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants – critical review. *Nanotoxicology* 10, 257–278
 111. Pacheco, I. and Buzzea, C. (2018) Nanoparticle uptake by plants: beneficial or detrimental? In *Phytotoxicity of Nanoparticles* (Faisal, M. *et al.*, eds), pp. 1–61, Springer
 112. Deng, C. *et al.* (2020) Bok choy (*Brassica rapa*) grown in copper oxide nanoparticles-amended soils exhibits toxicity in a phenotype-dependent manner: Translocation, biodistribution and nutritional disturbance. *J. Hazard. Mater.* 398, 122978
 113. Zhao, L. *et al.* (2016) Metabolomics to detect response of lettuce (*Lactuca sativa*) to Cu(OH)₂ nanopesticides: oxidative stress response and detoxification mechanisms. *Environ. Sci. Technol.* 50, 9697–9707
 114. Ghafariyan, M.H. *et al.* (2013) Effects of magnetite nanoparticles on soybean chlorophyll. *Environ. Sci. Technol.* 47, 10645–10652
 115. Hong, J. *et al.* (2014) Evidence of translocation and physiological impacts of foliar applied CeO₂ nanoparticles on cucumber (*Cucumis sativus*) plants. *Environ. Sci. Technol.* 48, 4376–4385
 116. Eichert, T. and Goldbach, H.E. (2008) Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces – further evidence for a stomatal pathway. *Physiol. Plant.* 132, 491–502
 117. Gohari, G. *et al.* (2020) Modified multiwall carbon nanotubes display either phytotoxic or growth promoting and stress protecting activity in *Ocimum basilicum* L. in a concentration-dependent manner. *Chemosphere* 249, 126171
 118. Ahmed, B. *et al.* (2021) Nanoparticles in the soil-plant system: a review. *Environ. Chem. Lett.* 19, 1545–1609
 119. Peng, C. *et al.* (2020) Bioavailability and translocation of metal oxide nanoparticles in the soil-rice plant system. *Sci. Total Environ.* 713, 136662
 120. Aslani, F. *et al.* (2014) Effects of engineered nanomaterials on plants growth: an overview. *Sci. World J.* 2014, 641759
 121. Wang, W.N. *et al.* (2013) Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *J. Nanopart. Res.* 15, 1417
 122. Khodakovskaya, M. *et al.* (2009) Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano* 3, 3221–3227
 123. Yu, H. *et al.* (2022) Integrating machine learning interpretation methods for investigating nanoparticle uptake during seed priming and its biological effects. *Nanoscale* 14, 15305–15315
 124. Raliya, R. *et al.* (2016) Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using ZnO nanoparticles synthesized by soil fungi. *J. Agric. Food Chem.* 64, 3111–3118
 125. Wang, P. *et al.* (2016) Nanotechnology: a new opportunity in plant sciences. *Trends Plant Sci.* 21, 699–712
 126. Dong, R. *et al.* (2019) Recent developments in luminescent nanoparticles for plant imaging and photosynthesis. *J. Rare Earths* 37, 903–915
 127. Demirer, G.S. (2023) Detecting and quantifying nanoparticle-mediated biomolecule delivery in plants. *Nat. Rev. Methods Primers* 3, 16
 128. Detection methods of nanoparticles in plant tissues. In *New Visions in Plant Science* (Yan, A. *et al.*, eds), 74101, InTechOpen
 129. Proença, P.L. *et al.* (2022) Fluorescent labeling as a strategy to evaluate uptake and transport of polymeric nanoparticles in plants. *Adv. Colloid Interf. Sci.* 305, 102695
 130. Sun, D. *et al.* (2014) Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. *Plant Cell Rep.* 33, 1389–1402
 131. Wang, H. *et al.* (2019) Deep learning enables cross-modality super-resolution in fluorescence microscopy. *Nat. Methods* 16, 103–110
 132. Wu, H. *et al.* (2017) Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. *ACS Nano* 11, 11283–11297
 133. Wong, M.H. *et al.* (2016) Lipid exchange envelope penetration (LEEP) of nanoparticles for plant engineering: a universal localization mechanism. *Nano Lett.* 16, 1161–1172
 134. Scherlinger, M. (2008) Nanoecotoxicology: environmental risks of nanomaterials. *Nat. Nanotechnol.* 3, 322–323



Pre-harvest application of sodium alginate functionalized with melatonin enhances secondary metabolism in strawberry fruit

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ABSTRACT

The application of priming agents is a promising strategy to enhance the nutritional content of fruits and overall fruit quality. The current study aimed to assess the effect of the pre-harvest application of various priming agents [melatonin (Mel), sodium alginate (NaA), sodium alginate/melatonin conjugate (Mel-NaA), and putrescine dihydrochloride (Put)] on fruit quality attributes and secondary metabolite profile of a strawberry cultivar (*Fragaria x ananassa* Duchesne cv. 'Felicity Q3'). The priming agents were directly applied on fruit at three successive developmental stages, namely large green (LG), small white (SW) and large white (LW). The use of Mel-NaA and Put showed promising results in improving fruit quality indicators (i.e. firmness, color), while Mel-NaA and putrescine-treated fruit were characterized by increased total flavonoid content. HPLC-DAD-ESI-MS/MS data showed variable regulation of flavan-3-ols, hydroxycinnamic acids, and conjugates contents by the different treatments, while ellagitannins and ellagic acid derivatives were significantly enhanced following Mel-NaA pre-treatment. Priming treatments did not result in the differential regulation of volatile organic compounds (VOCs) in comparison with controls, suggesting that primed fruit retain their aroma quality with no aroma profile 'penalty'. In addition, molecular analysis revealed that fruit pre-treatment with the priming agents resulted in variable transcriptional regulation of known strawberry allergenic proteins, with the Mel-NaA treatment showing no significant effect. This 'green' approach holds promise for advancing our understanding of the effects of NaA as a smart delivery mechanism of chemical priming agents and its potential impact on the sustainable improvement of the physicochemical attributes of strawberries during the pre-harvest stage.

1. Introduction

Strawberry (*Fragaria x ananassa*) is one of the most important fleshy fruit commercially, mainly due to its desirable aroma, juicy texture, bright red color, and sweetness [37]. It is a significant source of vitamins and health-promoting bioactive compounds with high antioxidant capacity (reviewed in [37,44]) and has shown considerable increase in production over the recent years, with China and the United States being

the leading producers (UN Food and Agriculture Organization, Corporate Statistical Database, [65]).

Agrochemicals are usually incorporated into the cultivation practices to increase crop production, enhance fruit quality or extend shelf-life of strawberry fruits. However, concerns regarding the environmental impact and human health of such chemicals has shifted the interest into the discovery of sustainable alternatives [11]. Interestingly, the exogenous application of chemical priming agents, both at the pre- and

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post-harvest level, is being considered as a highly-promising technological approach towards alleviation of plant stress conditions and enhanced yield efficiency [19,57]. Priming agents are natural metabolites, synthetic compounds and/or nanomaterials that are generally known to enhance cell tolerance and amelioration of stress-induced plant growth inhibition [57] that can subsequently lead to higher yield and improved fruit quality attributes [3]. Notably, the use of nanocarriers (including biopolymers and nanoparticles) as smart delivery vehicles for chemical agents towards improved growth and stress protection in plants is nowadays being extensively explored (reviewed in [19]).

Precisely, nutritional and organoleptic quality of fruits could be improved by exogenous application of hormones, which play a vital role in physiological, biochemical and molecular control of ripening [50]. Melatonin (N-acetyl-5-methoxytryptamine, Mel), is a well-conserved hormone that acts as a growth regulator, being present both in animals and plants [4]. Mel is a small indolamine with amphiphilic structure that has a high antioxidant capacity. Application of agents like melatonin have been proven to significantly improve plant responses against abiotic stresses. A key trait of melatonin that makes it an ideal molecule for priming is its ability to easily diffuse through the cell membrane and into the cytoplasm, thus leading to fast activation when plant cells are exposed to stressful conditions [75]. For instance, the application of higher concentrations of Mel to strawberries through injection at the green stage accelerated ripening, which was accompanied by a higher activity of PAL enzyme, increased accumulation of total phenolics and anthocyanins, as well as increased scavenging capacity [39]. Okatan et al., [48] found that the pre-harvest foliar application of Mel on different strawberry cultivars positively affected fruit yield, quality (fructose and titratable acidity), and antioxidants (phenolics and ascorbic acid). However, the mechanistic effect of Mel at pre-harvest level in strawberry fruit has been scarcely examined.

As fruits ripen, the presence of reactive oxygen species (ROS) leads to oxidative stress and cell membrane disruption, contributing to fruit ripening [27]. Polyamines (PAs) are essential components of the antioxidative system and play a crucial role in safeguarding membranes from oxidative injury caused by ROS [29,58]. Furthermore, PAs are key elements in numerous biological processes and stress responses in plants, including plant growth, morphogenesis, and fruit development [16], while they are also widely applied to prolong the shelf-life of perishable horticultural crops [49]. Exogenous application of putrescine (Put) on strawberry either alone [30] or in combination with chitosan [6], has been reported to maintain strawberry quality attributes leading to extended shelf-life. However, such studies do not provide supporting evidence regarding the mechanistic action of polyamines on fruit physiology.

Natural polysaccharides and oligosaccharides have also been reported to prolong the shelf-life and to enhance fruit quality attributes. Such polysaccharides could be used as moisture and gas semi-permeable edible barriers, thus affecting the fruit internal atmosphere [8]. Alginate is a natural polysaccharide derived from brown marine algae (Phaeophyceae), with strong affinity for water and is a biopolymer that has a coating function [1]. NaA is the most common salt of alginate [74] and is prepared by the neutralization of purified alginic acid with appropriate pH control agents (FDA, TITLE 21). Recent studies have presented compelling evidence supporting the use of NaA for preserving fruit firmness and prolonging the shelf life of strawberries [2,7], without however any study on the effect of alginate salts when applied at pre-harvest level.

While there is currently limited research on the pre-harvest application of NaA on strawberries, given the valuable carrier properties attributed to alginate, it becomes imperative to explore its potential as a biodegradable biopolymer for smart and sustainable delivery of priming agents, such as Mel, to enhance the quality of strawberry fruits. We hypothesized that the exogenous pre-harvest application of priming agents (Mel, NaA, their conjugated form Mel-NaA, and Put) on

strawberry fruit undergoing ripening on-bush under non-stressful conditions results in improved physicochemical properties and fruit quality attributes. To this end, our study aimed to assess the effect of the exogenous pre-harvest application of the aforementioned priming treatments on the fruit quality attributes, polyphenolic profile, and gene expression levels of an early-harvested strawberry cultivar.

2. Material and methods

2.1. Plant material and treatment application

Strawberry plants (*Fragaria x ananassa* Duchesne cv. 'Felicity Q3', a cultivar that is amenable to early production in the Mediterranean basin during winter period), were planted in clay soil. To avoid water, raised beds of 1 m width and 0.6 m space between the beds (total 1.6 m) were used in an experimental farmer's field in Ayios Ioannis Malountas village (35°04'48"N 33°10'40"E, at 313.45 m altitude) at Nicosia district, Cyprus. The raised beds were mulched with silver plastic, with 12 plants planted per linear meter, spaced in three rows per raised bed, whereas each plant was spaced 0.30 m from each other. Plants were covered with low plastic tunnels (0.9 m) with aeration holes and fertilized with 50 kg/ha of 16–8–24–6Ca+TE (ULTRASOL SQM) using double drip irrigation on each raised bed (1.6 L/h spaced every 0.22 m). Experiment was set up as a completely randomized design with five treatments (described below). Prior to treatment application, 100 fruits per treatment were labelled at the large green (LG) stage (Fig. 1); fruit of similar developmental stage were selected in order to be synchronized during ripening. Experimental applications were targeted exclusively onto labelled fruit with different treatments being applied to fruits growing in different plants, and included the following five treatments: (T0) Water-sprayed (control), (T1) NaA (0.5 % w/v), (T2) Mel (100 µM), (T3) Mel-NaA (100µM-0.5 % w/v) and (T4) Put (1 mM). All plants were root-watered according to standard cultivation practices. Treatments were applied at weekly intervals starting on 15 December, corresponding to the following successive developmental stages: green receptacle with enlarged achenes (LG), small white receptacle and green achenes (SW), and large white receptacle with brown achenes (LW), as described by Symons et al. [62]. To ensure fruit full coverage and high binding of the priming agents, 0.1 % w/v Tween-20 surfactant was added in each solution, freshly prepared for every time point.

Strawberry fruits, were harvested at commercial maturity stage 1 week after the last application. Representative fruits with uniform color were assessed based on the absence of pest/disease damage such as *Botrytis* infection. Based on this selection, only 45 fruit per treatment were collected for further analysis. One lot (three 5-fruit sublots corresponding to three biological replications) was flash frozen and kept at −80 °C until needed for biological, enzymatic or molecular analyses as described below. The second lot (three 10-fruit sublots corresponding to three biological replications) was immediately used for fruit quality assessment.

2.2. Fruit quality attributes

Fruit weight, volume, color parameters and firmness were determined according to Hadjipieri et al. [24]. Color parameters were monitored, using a reflection colorimeter (CR-400, Konica Minolta, Osaka, Japan), while flesh firmness (FF) was measured using a texture analyzer (TA.XT plus, Stable Micro Systems, Surrey, U.K.).

Soluble solid content (SSC) and titratable acidity (TA) were measured in fruit juice isolated using a professional juicer according to Hadjipieri et al. [24]. SSC was quantified using a refractometer (Atago, PR-32α, Japan), while TA was determined with the use of an automatic multiple positions titrator (862 Compact Titrator, Metrohm AG, Switzerland).

Developmental phases



Fig. 1. Strawberry developmental stages and dates of the pre-harvest applications. 15/12/22: 1st spray application, 22/12/22: 2nd spray application, 29/12/22: 3rd spray application, 09/01/23: Harvest day. Abbreviations: Large green (LG), Small white (SW), Large white (LW), Red (R).

2.3. Total soluble sugars (TSS), glucose, fructose, sucrose content

Sugars [total soluble sugars (TSS), glucose, fructose, and sucrose] were extracted according to Hadjipieri et al. [23], with slight modifications. Triplicate strawberry samples (0.3 g) per treatment were extracted with 10 mL of 80 % v/v ethanol and sugars were determined spectrophotometrically as described elsewhere [13,28].

2.4. Determination of total phenolic content

Total phenolic content was extracted from the samples following the procedure of Shehata et al. [59] with slight modifications: 2 mL of 50 % (v/v) methanol was added to 0.05 g of ground frozen strawberry fruit and vortexed, and mixtures were placed at -20°C for 48 h. Samples were then centrifuged at $16000 \times g$ at 4°C for 10 min (Eppendorf Centrifuge 5415 R), and the supernatant was stored at -20°C . The total phenolic content was estimated by the method of Georgiadou et al. [17], with absorbance being measured at 765 nm (TECAN, Infinite 200[®] PRO). Results were expressed as gallic acid equivalents (GAE; mg 100 g^{-1} FW) and analysis were conducted in triplicate per treatment.

2.5. Determination of reduced ascorbic acid

The extraction for the reduced ascorbic acid was performed as described by Habibzadeh et al. [22] with some modifications. In detail, 0.2 g were vortexed with 1.5 mL 2 % (w/v) metaphosphoric acid and then centrifuged for 1 min at $16000 \times g$ at 4°C (Eppendorf Centrifuge 5415 R). The reduced ascorbic acid was estimated by the method of Georgiadou et al. [17] with modifications. Briefly, 500 μL of the diluted 2 % w/v metaphosphoric acid extract was added to 900 μL of 50 mmol L^{-1} 2,6-dichloroindophenol and absorbance was monitored at 520 nm (TECAN, Infinite 200[®] PRO). Ascorbic acid (AsA) content was quantified using a standard curve and expressed on a fresh weight base in mg 100 g^{-1} .

2.6. Determination of total anthocyanin content

Total anthocyanin content was extracted from the samples following the procedure of Bal and Ürün [6] with some modifications: 1 mL of

95 % v/v ethanol: 0.1 N HCl (85:15) was added to 0.1 g of ground frozen strawberry fruit and vortexed. Mixtures were placed at -20°C for 24 h, centrifuged at $16000 \times g$ at 4°C for 10 min (Eppendorf Centrifuge 5415 R), and supernatants stored at -20°C .

Total anthocyanin content was quantified by the pH-differential assay, according to Georgiadou et al. [17], with absorbances measured at 510 and 700 nm (TECAN, Infinite 200[®] PRO). Anthocyanin concentration was calculated as cyanidin-3-O-glucoside (CY3) equivalents and expressed on a fresh weight base as mg 100 g^{-1} .

2.7. Determination of total flavonoid content

Total flavonoid content was quantified from the samples following the procedure of Meyers et al. [43] with some modifications. Ten mL of acetone was added to 1 g of ground frozen strawberry fruit and vortexed, and mixtures were placed at -20°C for 48 h. Samples were then centrifuged at $16000 \times g$ at 4°C for 10 min (Eppendorf Centrifuge 5415 R), and supernatants stored at -20°C . Total flavonoid content was estimated by the method of Chang et al. [12] with slight modifications. The reaction mixture consisted of 0.5 mL plant extract, 1.5 mL of 95 % v/v ethanol, 0.1 mL of 10 % w/v aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm (TECAN, Infinite 200[®] PRO). The results were expressed on a fresh weight base as mg 100 g^{-1} quercetin equivalents.

2.8. Quantification of MDA and H_2O_2 content

The assessment of lipid peroxidation was carried out by quantifying the malondialdehyde (MDA) content produced through the thiobarbituric acid (TBA) reaction, as outlined by Filippou et al. [15].

The quantification of hydrogen peroxide (H_2O_2) content was performed through the reaction of H_2O_2 with potassium iodide (KI), by measuring the oxidation of iodide (I^-) to iodine (I) according to Loreto and Velikova [34].

2.9. Quantification of endogenous melatonin and polyamine content

Melatonin extraction assay was conducted following the guidelines

of the Melatonin ELISA Kit (Enzo Life Sciences, Farmingdale, NY, USA). In summary, fruit samples weighing 0.5 g were finely ground into powder using liquid nitrogen and homogenized in 125 μ L of 1 $\times$ stabilizer solution from the kit. Subsequently, 750 μ L of cold ethyl acetate was added, and the mixture was subjected to vortexing. Following 5 min incubation on ice, the solution was centrifuged at 1000 $\times$ g at 4 °C for 10 min. The resultant organic layer was transferred to a new glass tube and subjected to overnight drying. The resulting pellet was reconstituted in 200 μ L of 1 $\times$ stabilizer for melatonin quantification following the manufacturer's specified protocols. The levels of free polyamines were quantified by high-performance liquid chromatography (HPLC) separation of dansyl derivatives, as elsewhere described [40]. Analyses were performed in three biological replicates per point of analysis.

2.10. (Poly)phenolic compound analysis by HPLC-DAD-ESI-MS/MS

The analysis was achieved as previously reported by Salazar-Orbea et al. [54] and Buendía et al. [9] with modifications. Freeze-dried samples (100 mg) were extracted with 1 mL of MeOH/ H₂O/HOAc (70:29:1, v/v/v). The samples were vortexed for 1 min and then sonicated for 30 min at room temperature. They were then centrifuged for 15 min at 20000 $\times$ g at 10 °C (Thermo Scientific™ Sorvall™ ST 16, Germany). The supernatant was filtered through a 0.22 μ m PVDF filter and three replicates were analyzed. Phenolics identification and quantification were performed on an Agilent 1100 HPLC equipment coupled in series to a photodiode array detector (G1315D) and an HCT Ultra Bruker Daltonics ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface HPLC-DAD-ESI-MS/MS (Ion Trap). The chromatographic separation was completed using a Poroshell 120 EC column (3 $\times$ 100 mm, 2.7 μ m) from Agilent Technologies (Waldbronn, Germany). The mobile phases used were H₂O/HCOOH (Panreac, Barcelona, Spain) 99:1 (v/v) (A) and acetonitrile (J.T. Baker, Deventer, The Netherlands) (B). The gradient was set as indicated: 0 min, 5 % B; 7 min, 18 % B; 17 min, 28 % B; 22 min, 50 % B; 27 min, 90 % B; 29–35 min, 5 % B. The flow rate, the injection volume and the column temperature were 0.5 mL/min, 10 μ L, and 25 °C respectively. The UV spectra were recorded in the range of 200–600 nm. The ESI parameters included: nebulizer pressure 65 psi, dry gas flow 11 L/min, and dry gas temperature 350 °C. The capillary voltage was set at 4 kV, and spectra were acquired in the negative ionization mode in the range of m/z 100–1500 and target mass 700. Automatic MS/MS mode was applied with fragmentation amplitude 1 V and 3 no. of parents. Phenolics were classified by their UV spectra, retention time, molecular weight, and MS/MS fragmentation pattern. Their quantification was completed using authentic standards of castalagin (280 nm), catechin (280 nm) p-coumaric acid (320 nm), pelargonidin (520 nm), ellagic acid (360 nm) and quercetin (360 nm) to assess the content of ellagitannins, flavan-3-ols, hydroxycinnamic acids, anthocyanins, ellagic acid conjugates and flavonols respectively. The authentic standard of castalagin was supplied by Prof. Stephan Quideau (ISM, University of Bordeaux, France), while the others standards (catechin, p-coumaric acid, pelargonidin, ellagic acid, and quercetin) were purchased from Sigma-Aldrich.

2.11. VOC profiling

VOC analysis was adapted from the methodology described by Vandendriessche et al. [66]. For each treatment, three replicate strawberries were sampled. Green parts were removed from the fruit, which were then cut into small pieces. Subsequently the tissue was homogenized with 0.5 mL of 1.0 M NaCl per gram of fruit. The resulting blend was transferred to 15 mL Falcon tubes, snap-frozen in liquid nitrogen, and stored at –80 °C until VOC analysis. Prior to analysis, the samples were thawed overnight at 4 °C. A 5 g aliquot of the juice mixture was transferred to a 20 mL headspace vial (Filter Service, Belgium), flushed with filtered air, and sealed. For solid-phase microextraction (SPME),

the vials were incubated for 35 min at 40 °C on a heated tray. Volatiles were then extracted by exposing a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μ m film thickness; Supelco Inc., USA) SPME fiber to the vial's headspace for 30 min at 40 °C. The extracted aroma compounds were analyzed using an Agilent 7890 A gas chromatograph (GC) coupled with an Agilent 5975 C Network Mass Selective Detector (MS) (Agilent Technologies, USA) and a Gerstel Multi-Purpose Sampler MPS 2 (Germany). Following extraction, the compounds were thermally desorbed into the injector, which was heated to 250 °C and equipped with a SPME liner (0.75 mm i.d.; Supelco Inc., USA). Splitless injection was conducted for 0.5 min at a flow rate of 50 mL/min, with a 10-min fiber thermal conditioning. Separation of volatiles was achieved using a 30 m $\times$ 250 μ m $\times$ 0.250 μ m HP-5MS column (Agilent Technologies), with helium as the carrier gas at a flow rate of 1.2 mL/min. The GC temperature program was as follows: an initial hold at 35 °C for 5 min, followed by a ramp to 150 °C at 4 °C/min, then a second ramp to 240 °C at 50 °C/min, with a final hold at 240 °C for 5 min. The total run time was 40.55 min. The mass spectrometer scanned the m/z range from 30 to 350, with the ion source and quadrupole set to 230 °C and 150 °C, respectively. Chromatographic and spectral data were processed using MassHunter Workstation (Unknowns Analysis v10.1, Agilent Technologies). Identification of volatile compounds was carried out using the NIST 2020 database (NIST20, NIST, USA), with the compounds listed in [Supplementary Table 2](#).

2.12. RNA isolation and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted for all treatments in triplicate, using 100 mg strawberry fruit material, as reported in [25]. Total RNA was then treated with recombinant DNase I (RNase-free) (Cat. No. 2270 A, Takara Bio Inc.), in order to remove gDNA. For first-strand cDNA synthesis, 0.5 μ g of total RNA from each sample was transcribed into cDNA using the PrimeScript™ RT reagent Kit (Perfect Real Time) following the manufacturer's instructions (Takara Bio, Japan). Expression levels were analyzed in a Biorad IQ5 real-time PCR cycler (Biorad, USA). In total, three biological replicates were performed for each treatment. RT-qPCR was carried out in a final volume of 10 μ L, containing 4 μ L 5-fold diluted first-strand cDNA, 0.5 μ L each gene-specific primer (10 pmol/ μ L) and 5 μ L 2 $\times$ master mix (KAPA SYBR® FAST qPCR Kit, Kapa-Biosystems). Reaction conditions were initial denaturation 95 °C for 5 min, followed by 40 cycles (95 °C for 30 s, annealing temperature (Ta °C) for 30 s, and 72 °C for 30 s) and a final elongation stage at 72 °C for 5 min. The amplification cycle was followed by a melting curve run, with 61 cycles with 0.5 °C increments between 65 and 95 °C. The primer information related to allergen-related genes and melatonin biosynthetic genes (50 and 58 °C) are shown in [Supplementary Table 3](#). Strawberry *GAPDH* and *ACTIN* were used as housekeeping reference genes. Relative quantification and statistical analysis of gene expression levels using the pairwise fixed reallocation randomization test were carried out using the REST-XL software according to Pfaffl et al. [52]. Heatmaps were created using ClustVis 2.0 according to Metsalu and Vilo [42].

2.13. Statistical analysis

For quality attributes, statistical analysis was performed with SPSS v24.0 (SPSS Inc., Chicago, IL, United States), using one-way ANOVA analysis and then Duncan's multiple range test at significance level 5 % ($p \leq 0.05$). For biochemical analysis, one-way ANOVA analysis was performed, followed by Tukey-HSD post hoc test ($P \leq 0.05$). Figures were prepared using GraphPad version 9.4.0 (GraphPad Software, San Diego, CA, USA). For phytochemical analysis, both statistical analysis (one-way ANOVA analysis was conducted including Tukey-HSD post hoc test ($p \leq 0.05$) and plot designs were conducted using GraphPad version 10.1.0 (GraphPad Software, San Diego, CA, USA). For the VOC-data, multivariate statistical analysis, PCA, PLS and VIP analysis, was done using JMP-Pro (v17, SAS Institute Inc., Cary, NC).

3. Results

3.1. The effect of priming agents on quality attributes

As far as the color parameters are concerned, a brighter color, corresponding to higher L values, was recorded in sodium alginate-treated strawberries compared with the other treatments (Supplementary Table 1). Less intense red colour, evidenced by the lower a^* values, was recorded in strawberries treated with sodium alginate, both alone and functionalized by melatonin. Strawberries treated with the conjugate Mel-sodium alginate had lower b^* , C and h values compared with the other treatments, but higher a^*/b^* values compared with fruit treated with sodium alginate and Put (Supplementary Table 1). The pre-harvest application of different priming agents showed differences regarding FF. In particular, putrescine-treated strawberries were characterized by higher firmness values compared with the water-sprayed and melatonin applications. However, there were no significant differences in volume, weight, SSC, TA and RI (Table 1).

While no differences in SSC were determined, variations in total soluble sugars and sucrose were recorded (Fig. 2). In particular, application of Mel-NaA induced the highest concentration in TSS ($p < 0.01$) compared with the NaA treatment. However, no significant differences were observed between Mel and Mel-NaA treatment as well as between Mel and NaA. Moreover, the Put treatment also had a significant increase ($p < 0.05$) in TSS content compared with NaA. Regarding the sucrose content, Mel and Mel-NaA treatments had the highest sucrose content compared with the water-sprayed treatment. Furthermore, even though Mel and Mel-NaA induced similar levels in sucrose content, they both showed a significant ($p < 0.05$) increase of sucrose content compared with NaA. Putrescine treatment did not show any significant effect on the sucrose content (Fig. 2).

3.2. The effect of priming agents on phenolics

The pre-harvest application of the priming agents had a notable effect on strawberries' total flavonoid content; In particular, total flavonoids showed higher concentration in Mel-NaA and Put ($p < 0.01$), as well as in NaA-treated fruit ($p < 0.05$) compared with water-sprayed ones (Fig. 3D). On the other hand, no differences in the contents of total phenolics, reduced ascorbic acid, and total anthocyanins were observed (Fig. 3A,B,C). The highest total flavonoid content was observed in fruits treated with Put, compared with the control. Additionally, Mel alone did not affect flavonoid content; however, when NaA was used as a carrier for Mel in the form of functionalized alginate-melatonin treatment (Mel-NaA), a significant increase in total flavonol content was observed (Fig. 3D).

3.3. The effect of priming agents on stress markers

None of the treatments applied resulted in significant changes in malondialdehyde (MDA) and H_2O_2 contents, which are commonly used stress markers indicative of potential oxidative stress damage

(Supplementary Figure 1), thus suggesting that the priming treatments did not result in cellular damage effects in fruit.

3.4. The effect of priming agents on endogenous melatonin and polyamine contents

No significant differences were observed in free polyamine contents (Fig. 4A,B,C). However, the highest melatonin levels in fruit were observed in samples treated with Mel-NaA followed by Put. Interestingly, the application of Mel alone did not increase melatonin content within the fruit compared with the control group. However, when functionalized alginate was used as a delivery system for melatonin, there was a significant enhancement in endogenous melatonin levels in the fruit (Fig. 4D).

3.5. The effect of priming agents on individual polyphenolic compounds

The phytochemical analysis revealed that pre-harvest treatments differentially regulated the content of several polyphenolic compounds (ellagitannins, flavan-3-ols, hydroxycinnamic acids, ellagic acid and conjugates) (Fig. 5). There are two isomers of pedunculagin (isomer 1 and 2) with same mass and same structure but different stereochemistry in the hydroxyl at C-1 of glucose. NaA resulted in the lowest amount of Bis-HHDP-glucose (Pedunculagin)_isomer 1 compared with the water-sprayed treatment, whereas the rest of the treated fruit had similar Bis-HHDP-glucose (Pedunculagin)_isomer 1 content to water-sprayed fruit and NaA ones (Fig. 5A,B). Regarding the content in Bis-HHDP-glucose (Pedunculagin)_isomer 2, Mel-NaA-treated fruit registered the highest content on this compound compared with Mel, NaA and water-sprayed. However, the Put-treated fruit did not show any differences among the other treatments. Regarding the flavan-3-ols, procyanidin dimer B1 registered the highest concentration in putrescine dihydrochloride-treated fruit ($p < 0.05$) compared with water-sprayed (Fig. 5C). In addition, procyanidin dimer B2 registered the highest concentration in Mel-NaA ($p < 0.05$) compared with water-sprayed (Fig. 5D). The other treatments did not show differences when compared with the water-sprayed one. Putrescine dihydrochloride and Mel showed the highest contents on p-Coumaroyl hexose isomers 1 & 2 compared with other treatments, yet similar to water-sprayed (Fig. 5E, F). On the other hand, NaA and Mel-NaA showed lowest content in isomer 1 than water-sprayed. A similar pattern was observed in the contents of p-Coumaroyl hexose 2, yet with less pronounced differences. Several p-coumaroyl-glucose isomers are feasible; the metabolites found here with the same formula and mass spectra signals were detected in agreement with previous studies [9].

Significant differences were monitored with reference to ellagic acid and its conjugates (Fig. 5G,H). Mel-NaA had the highest content on ellagic acid rhamnoside compared with water-sprayed and the rest of the treatments. Furthermore, the same treatment positively impacted on the content of ellagic acid, compared with sodium alginate and Mel. This is also consistent with the effects of treatments on ellagitannins described above, and makes sense from the biosynthetic point of view

Table 1

The effect of pre-harvest application of NaA, Mel, Mel-NaA and Put on fruit quality attributes (Fresh fruit weight (g), volume (mL), Soluble solids content (SSC, °Brix), Titratable acidity (TA, % citric acid) and Flesh firmness, N) of strawberry fruits (cv. 'Felicity Q3').

	Weight (g)	Volume (mL)	SSC (°Brix)	TA (% citric acid)	RI (SSC/TA)	Force (N)
Water-sprayed	19.39 ± 1.32 a	22.07 ± 1.63 a	9.30 ± 0.23 b	0.99 ± 0.03 a	9.45 ± 0.5 a	2.34 ± 0.1 bc
Sodium alginate (NaA)	17.84 ± 0.93 a	20.90 ± 1.08 a	9.23 ± 0.12 b	1.00 ± 0.04 a	9.28 ± 0.25 a	2.63 ± 0.12 ab
Melatonin (Mel)	18.86 ± 1.12 a	22.33 ± 1.47 a	9.47 ± 0.09 ab	1.04 ± 0.02 a	9.13 ± 0.18 a	2.36 ± 0.1 bc
Sodium alginate/Melatonin (Mel-NaA)	17.51 ± 1.65 a	20.56 ± 1.96 a	9.33 ± 0.03 b	1.05 ± 0.04 a	8.89 ± 0.29 a	2.44 ± 0.11 abc
Putrescine dihydrochloride (Put)	17.43 ± 1.28 a	19.63 ± 1.59 a	9.50 ± 0.12 ab	1.01 ± 0.05 a	9.41 ± 0.4 a	2.72 ± 0.12 a

Each treatment measurements in a row: Water-sprayed= Water + Tween 20, NaA= Sodium Alginate + Tween 20, Mel= Melatonin + Tween 20, Mel-NaA = Melatonin + Sodium Alginate + Tween 20, Put = Put dihydrochloride + Tween 20). Data represent the mean (n = 30 fruit) ± SE. Different letters are significantly different ($p \leq 0.05$) within each column.

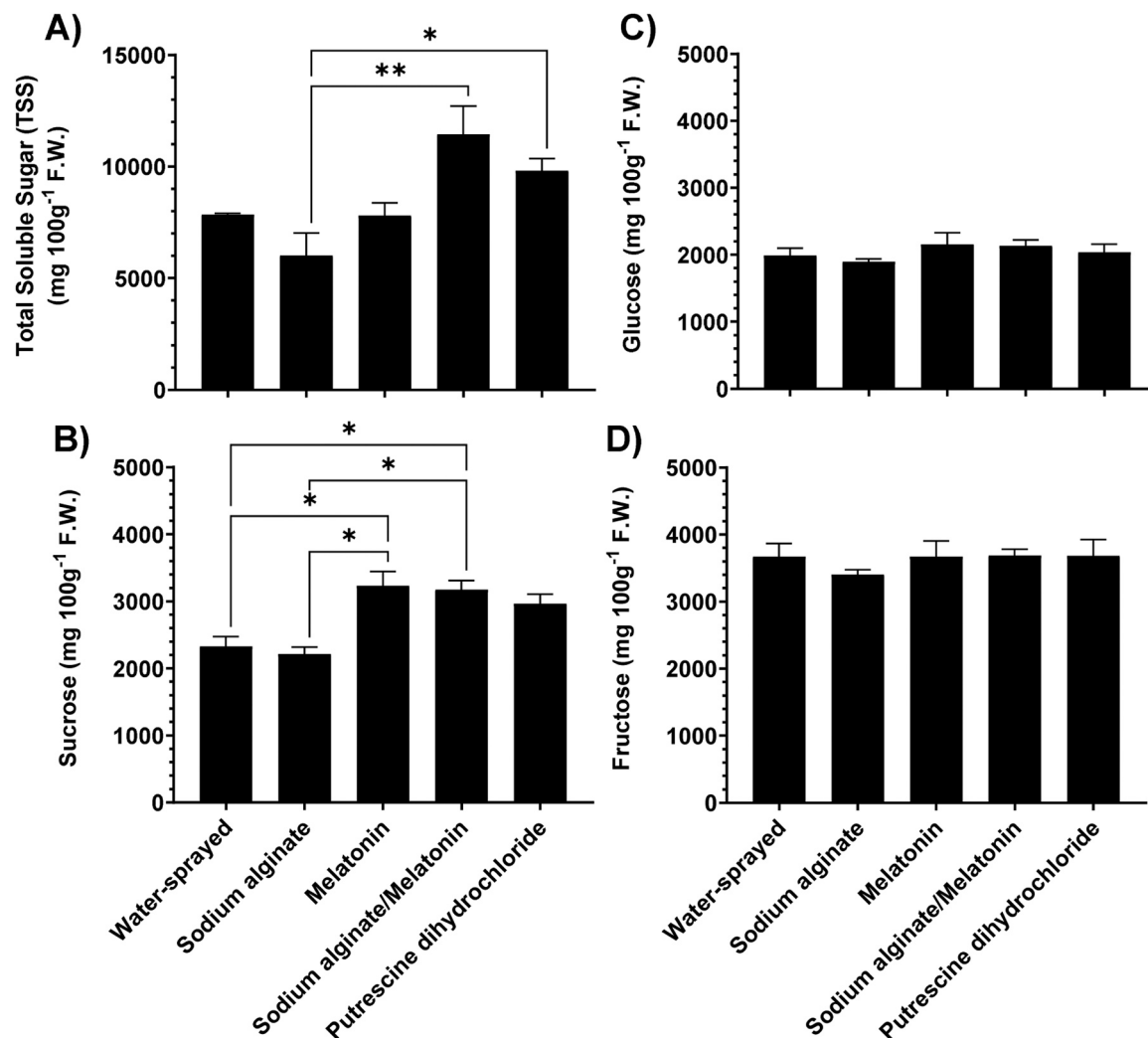


Fig. 2. The effect of pre-harvest application of Mel, NaA and Put on total soluble sugars (TSS) (A), sucrose (B), glucose (C), fructose (D) at fully ripe strawberry fruits (cv. 'Felicity Q3').

and with similar biological implications.

3.6. The effect of priming agents on VOC profile

A total of 118 VOCs were identified and 'quantified' in terms of their relative peak area to account for SPME fiber variations (Supplementary Table 2). The PCA score plot revealed a variation between replicate fruit as large as the variation between the different treatments, showing no clear separation between the treatments (Supplementary Figure 2). The PLS-DA analyses using the VOCs as X-variables and priming treatment as the Y-response was not able to establish robust prediction models for any of the treatments. These results suggest that the VOC profile was not systematically affected by the imposed priming treatments.

3.7. The effect of priming agents on allergenic protein and Mel biosynthesis gene expression

The expression levels of selected genes involved in the biosynthesis of known strawberry allergenic proteins and melatonin (Allergenic proteins: *Fra a2*, *Fra a3*, *Fra a1-A*, *Fra LPT46*, *Fra A4* Melatonin: *Fra TDC*, *Fra TSH*, *Fra SNAT*, and *Fra ASMT*) were evaluated following treatment with different priming agents (Fig. 6; Supplementary Figure 3; Supplementary Tables 4,5). Of the treatments, NaA generally caused moderate changes in gene expression, leading to the up-regulation of *Fra a3* and

Fra TDC, with a significant fold increase for *Fra a3* (4.891 fold-increase; $p = 0.0010$) (Supplementary Table 4). On the other hand, other genes, including *Fra a2*, *Fra a1-A*, *Fra A4*, and *Fra ASMT*, were predominantly down-regulated ($p < 0.05$), whereas the expression changes for other genes (e.g., *Fra LPT46*, *Fra SNAT*) were not statistically significant in the case of NaA (Fig. 6). In the case of Mel treatments, *Fra a3* was up-regulated (25.670-fold, $p = 0.0375$), while a significant down-regulation effect of melatonin on *Fra TDC* (-16.145-fold) and *Fra ASMT* (-16.011-fold, $p = 0.0010$) was further noted (Supplementary Table 4).

In the case of conjugation of Mel-NaA, significant up-regulation of *Fra a3* (14.774-fold, $p = 0.0010$) was achieved. However, the combination strongly down-regulated genes such as *Fra TDC* (-8.821-fold, $p = 0.0010$) and *Fra ASMT* (-9.553-fold, $p = 0.0010$) (Supplementary Table 4). The application of Put resulted in a pronounced elevation in the expression of *Fra a3* (24.710-fold, $p = 0.0345$) and a moderate increase in the expression of *Fra SNAT* (1.574-fold, not significant). Additionally, it significantly down-regulated *Fra TDC* (-4.976-fold, $p = 0.0010$) and *Fra ASMT* (-3.169-fold, $p = 0.0010$), while the changes for other genes were relatively mild (Supplementary Table 4).

Overall, the statistical analysis confirmed the significance of expression changes, particularly for *Fra a3*, *Fra TDC*, and *Fra ASMT*, in response to specific treatments. Notably, Mel and Put treatments resulted in significant upregulation for *Fra a3* ($p < 0.05$; Supplementary

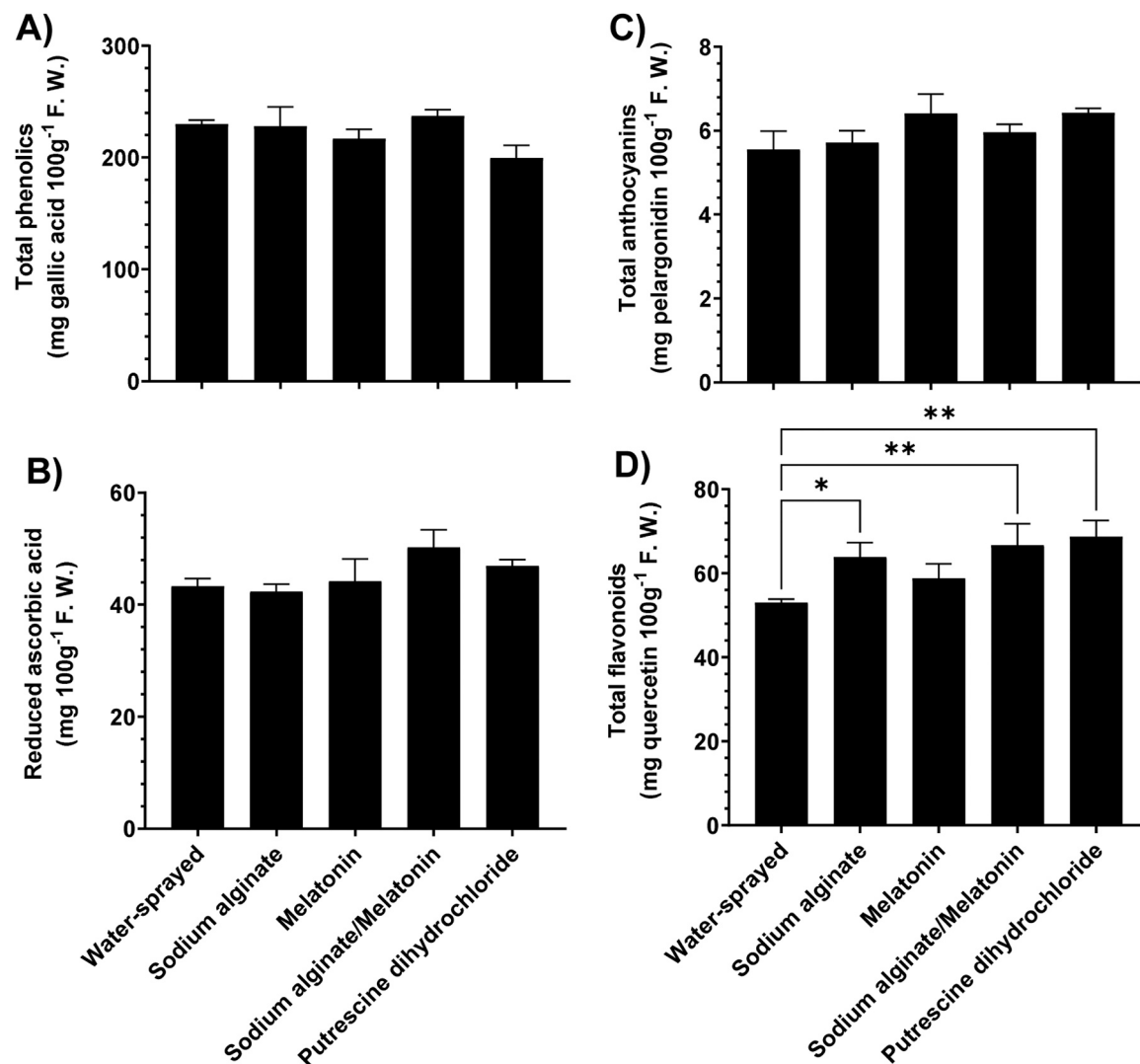


Fig. 3. The effect of pre-harvest application of Mel, NaA, Mel-NaA, and Put on total phenolic (A), reduced ascorbic acid (B), total anthocyanin (C) and total flavonoid (D) contents of strawberry fruit (cv. 'Felicity Q3').

Table 4), while Mel and Mel-NaA resulted in significant downregulation for *Fra TDC* and *Fra ASMT* ($p < 0.001$; Supplementary Table 4). As noted above as well, Mel and Put showed significant upregulation of *Fra a3*, with Mel exhibiting the highest expression increase for *Fra a3* (25.670-fold). In contrast, NaA and Mel-NaA treatments demonstrated moderate expression changes across most genes (Fig. 6).

4. Discussion

Enhancing fruit quality attributes by employing sustainable practices is one of the highest priorities for horticultural products. Exogenous application of plant growth regulators has been widely investigated in sustainable agricultural production and among them, natural compounds (e.g., Mel, Put) delivered by nanocarriers (e.g., alginate) are a promising approach to be used as priming agents in strawberry cultivation to enhance fruit quality and polyphenol profile [45]. Such approaches have been mainly studied at the post-harvest level. Revelant studies [36,35] demonstrated that functionalizing chitosan nanoparticles with glycine betaine and arginine chitosan can serve as an effective carrier for delivering such compounds as a fruit coating treatment at postharvest level that led to enhanced plum fruit quality attributes but also to extended shelf-life during cold storage conditions. The aim of this study was to investigate the efficacy of such nanocarriers and

evaluate their potential as a sustainable approach to improve strawberry fruit quality at pre-harvest level.

Visual quality is one of the most important parameters consumers assess when buying strawberry fruits; therefore, color value was the first quality attribute to be taken into account. NaA- treated strawberries showed higher L^* values which indicate brighter fruit and hence more visually attractive [55]. Strawberries treated with Mel-NaA were characterised by lower b^* , C and h and higher a^* / b^* values. Lower hue angle (h) and chroma (C) values are linked with more intense red color and lower color saturation, respectively [67]. Such sensory changes may increase the attractiveness to consumers. Even though chromaticity parameters linked to red color have been correlated with the anthocyanin content [67]; it is not the case in results reported herein. The co-occurrence of organic acids, pH and the occurrence of co-pigments can affect the final strawberry color perceived although the anthocyanin content can be similar.

Another important attribute for strawberries is flesh firmness as it affects the resistance to handling, storage life and the overall marketability of the strawberry and is influenced by both the cultivar and other factors (ripening stage, fruit size, temperature, etc.). Increased fruit firmness is connected to reduced post-harvest losses and extended shelf-life. In our study, fruit treated with Put were firmer than those subjected to other priming treatments. This comes in accordance with the findings

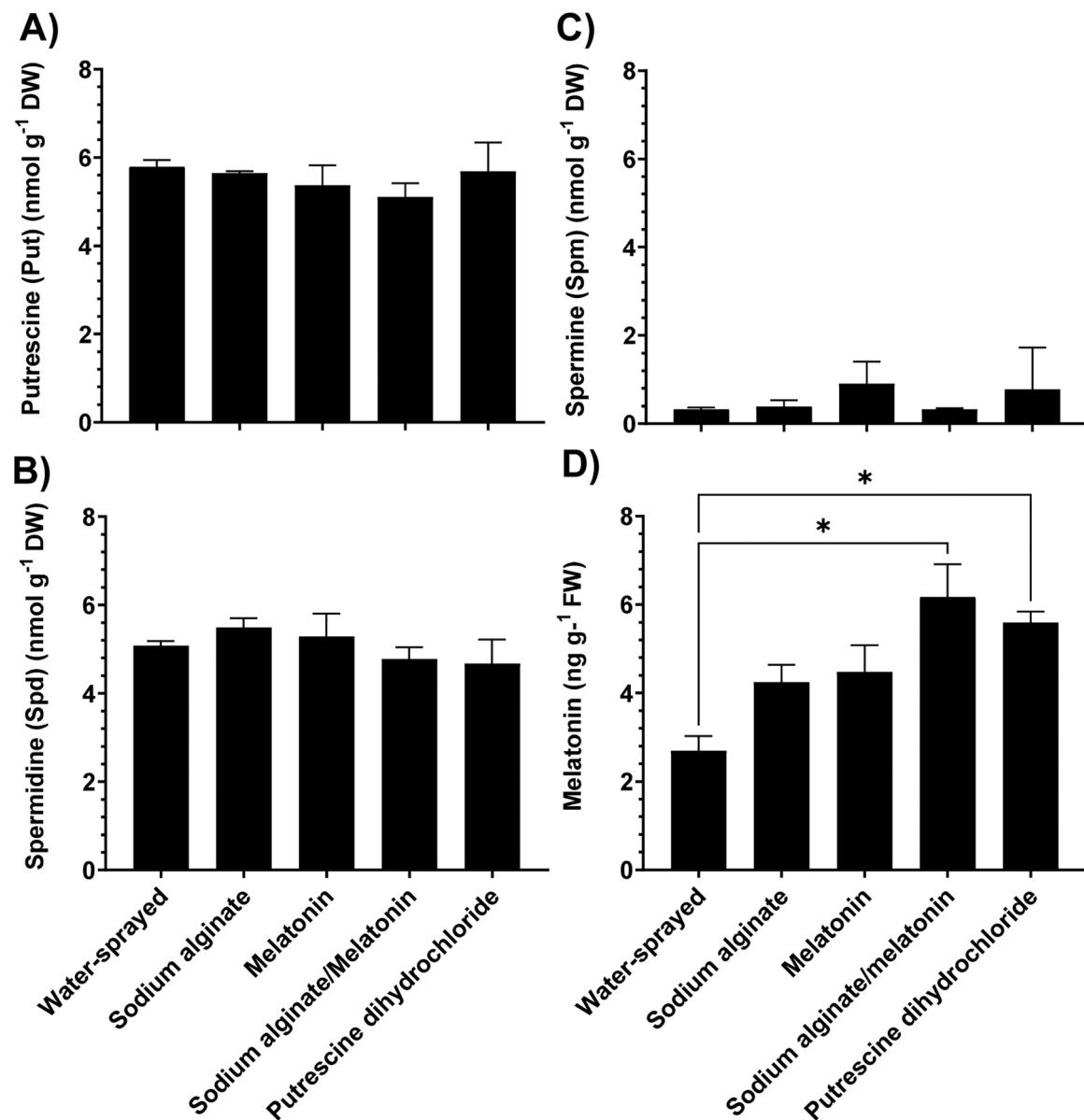


Fig. 4. The effect of pre-harvest application of Mel, NaA, Mel-NaA and Put on endogenous content of putrescine (A), spermidine (B), spermine (C) and melatonin (D) of strawberry fruits at fully ripe stage (cv. 'Felicity Q3').

of studies in other fruit crops, where pre-harvest Put application maintained the fruit firmness of pear fruit [60]. The exogenous application of Put on strawberry fruit has been reported to delay fruit ripening, which corresponded with changes in multiple physiological parameters such as firmness, anthocyanin, sugar and polyamine contents, among others [21]. To conclude that putrescine treatment has a beneficial effect on strawberry firmness retention, further studies are needed that will have a considerable bigger pool of fruits examined and will additionally consider the fruit size and weight effect as smaller fruit tend to be denser and firmer.

Sweeter fruits are usually more desirable to consumers and taste is highly depended on sugar content. Pre-harvest treatments Mel-NaA on strawberries had an overall significant effect on the total soluble sugar (TSS) and sucrose. Melatonin could regulate carbohydrate metabolism and support the plant's osmoregulatory response, as critical function during stressful conditions [5]. Several reports have shown that melatonin application can influence sugar metabolism by altering the expression of sugar transporter genes and enzymes involved in carbohydrate metabolism and TSS accumulation in different horticultural

crops like apple [73], pear [32], and peach [77].

Ascorbic acid, the well-known Vitamin C, is a water-soluble micro-nutrient that is not synthesised by the human body and is obtained through the diet, primarily from fruits and vegetables. Amongst the fruits, strawberries are considered an excellent source of *L*-ascorbic acid [47]. Previous studies have reported that the exogenous application of melatonin has been shown to increase ascorbic acid content in apple leaves [69], while Okatan et al. [48] reported higher levels of ascorbic acid in four strawberry cultivars after pre-harvest exogenous melatonin application. In addition, Li et al. [31] showed that the use of edible coatings, including alginate, maintained the increased ascorbic acid content of strawberry fruit under storage conditions. However, although Mel-NaA treatment resulted in an increased content of ascorbic acid, it was not statistically significant compared with the other treatments.

Secondary metabolites, particularly polyphenols are responsible for increased quality of vegetables and fruits, including color, flavor, firmness, and bitterness, contributing at the same time to the antioxidant capacity and plant defense mechanisms [61]. Anthocyanins are pigments found in many vegetables, fruits and flowers, with a health

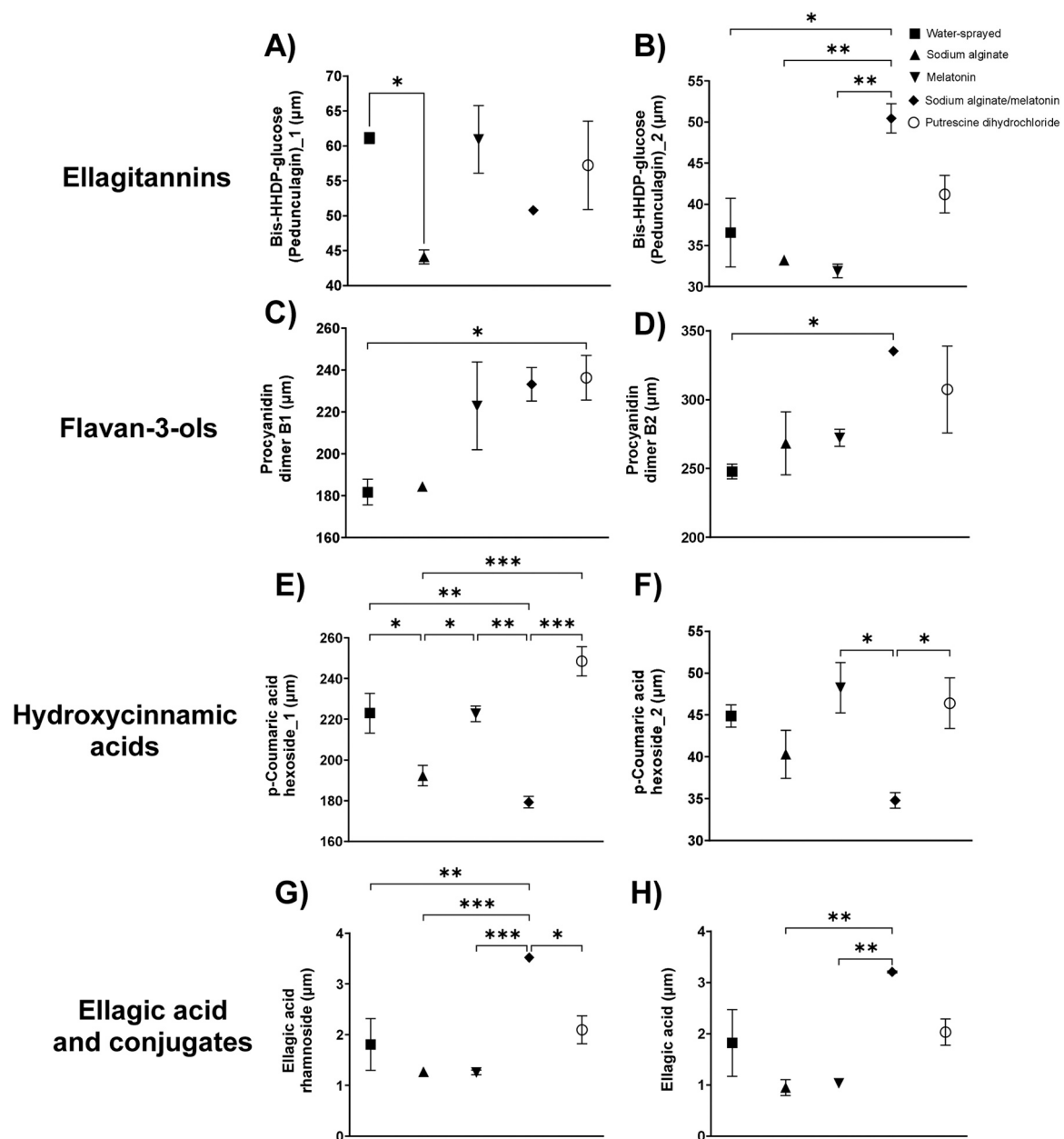


Fig. 5. The effect of pre-harvest application of Mel, NaA and Put on polyphenolic compounds of classes Ellagitannins (A, B), Flavan-3-ols (C, D), Hydroxycinnamic acids (E, F) and Ellagic acid and conjugates (G, H) of an early-harvested strawberry cultivar (*Fragaria x ananassa* cv. 'Felicity Q3').

beneficial role to plant and human health. Strawberries are a rich source of anthocyanins and numerous reports have focused on the quantification of their content [37]. In the current study, fruit treated with Put did not reveal a meaningful increase of total anthocyanin content compared with the other priming strategies (NaA alone or in combination with Mel). To our knowledge, no studies have been found to directly connect the increased anthocyanin content to pre-harvest application of Put, while its postharvest application has been reported to maintain the total anthocyanins in high levels under storage conditions [6].

Melatonin, spermidine, and putrescine can upregulate transcript levels of antioxidant enzymes in plants [68,76] and improve the quality of fruit, elevating at the same time the number of beneficial substances, like sucrose, polyphenols, and antioxidant compounds [70,72]. In the present study, melatonin alone or conjugated with sodium alginate were found to regulate polyphenol metabolism, as they increased the concentration of an important number of polyphenols, such as ellagitannins, flavan-3-ols, hydroxycinnamic acid, ellagic acid and their conjugates.

Particularly, Mel-NaA-treated fruit significantly impacted Bis-HHDP-glucose (Pedunculagin)_isomer 2 content compared to other treatments. Pedunculagin has two isomers (isomer 1 and 2) with same mass and same structure but different stereochemistry in the hydroxyl at C-1 of glucose which were both present in treated fruits of these study. These type of ellagitannins have been associated with the defense of strawberries against plant pathogens [20]. Xu et al. [71] reported results that are in accordance with current findings, as they indicated an increase in the content of total phenols, flavonoids, and proanthocyanidins of grape berries, after the exogenous application of melatonin. In specific, they found that melatonin treatment significantly increases the contents of non-flavonoid compounds such as coumaric acid [71]. Ellagitannins and ellagic acid derivatives were significantly enhanced with some of the priming treatments applied (Mel-NaA). These could impact the strawberry taste, as these polyphenols can impart astringent or mouth feeling sensations, and some of these are part of the characteristic strawberry fruit flavor [56]. In addition, these phenolics can

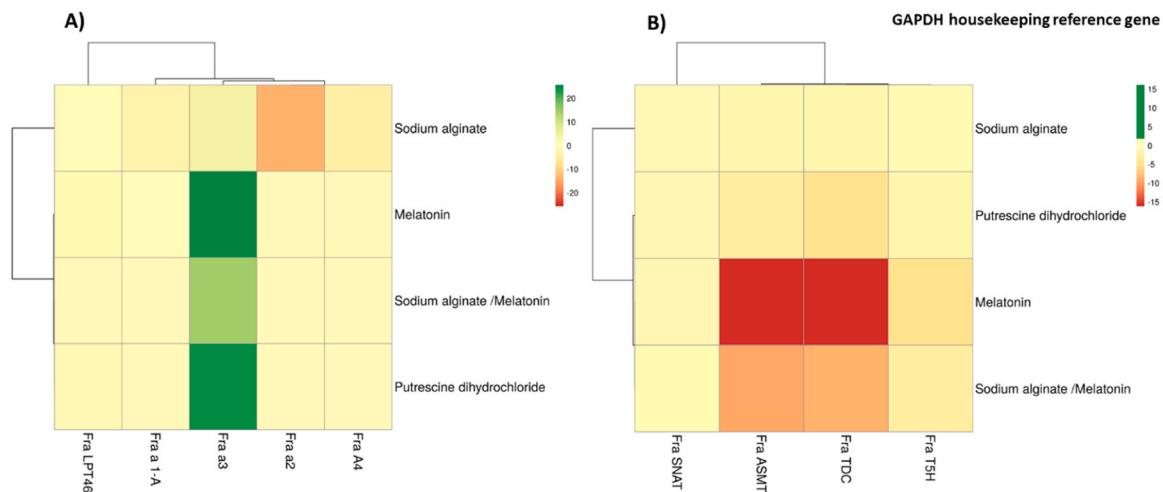


Fig. 6. Heat map of the relative expression levels of A) allergen-related genes (*Fra a2*, *Fra a3*, *Fra a 1-A*, *Fra LPT46* and *Fra A4*) and B) melatonin biosynthetic genes (*Fra TDC*, *Fra T5H*, *Fra SNAT* and *Fra ASMT*) on pre-harvest application of Mel, NaA, Mel-NaA and Put of strawberry fruits at fully ripe stage (cv. 'Felicity Q3') (n = 3). Relative mRNA abundance was evaluated by real-time RT-qPCR using three biological repeats. Up-regulation is indicated in green; down-regulation is indicated in red. A scale of color intensity is presented as a legend. Control (water-sprayed) samples and *GAPDH* housekeeping reference gene were used for calibrating gene expression values. Actual relative expression levels are shown in [Supplementary Table 4](#).

have protective effects against the development of plant pathogens (*Pseudomonas*); therefore, the treatments can have beneficial effects during strawberry development [64].

Interestingly, priming treatments did not result in the differential regulation of VOC compounds constituting aroma profile in comparison with water-sprayed control plants, suggesting that primed fruit retain their aroma quality with no aroma profile 'penalty', as seen in other cases such as in grapevine plants sprayed with swelling agent CPPU that resulted in the decreased content of several VOCs such as hexanal, phenyl ethanol, damascenone and linalool [53].

Current findings revealed that strawberry fruit treated with Mel-NaA exhibited the highest levels of endogenous melatonin, suggesting that alginate may be an effective carrier to enhance melatonin's efficacy with lower dose needs. Carriers like alginate enable the controlled, sustained release of priming agents such as melatonin, which can improve the efficiency of these active compounds. These biopolymer-based carriers like alginate and chitosan represent a promising, sustainable approach in agricultural technology, providing an eco-friendly method to deliver priming agents and effectively enhance not only plant stress resilience against different abiotic stress, but also fruit quality and yield [19]. For instance, Gohari et al. [18] reported that using chitosan as a bio-polymer-based nanocarrier for delivery of melatonin significantly boosted melatonin's efficacy in spearmint plants under salinity stress.

An additional point of concern following exogenous application of priming agents by direct spraying on fruit was the potential induction of allergenic proteins. Of the five known allergenic proteins in strawberry that were examined by RT-qPCR, four did not show any increase in transcript levels, in line with a biosafe approach. The exception was *Fra a3* which encodes an allergen of fruits and belong to the *PR10* family [46]. This gene family has been well-correlated and regulated in the case of pathogen infection, exhibiting antimicrobial activities against bacteria, fungi and viruses [41]. By modifying the biosynthesis of flavonoid and anthocyanin pathways, *Fra a3* has also been shown to be involved in stress response and fruit ripening [46]. *Fra a3* was the most strongly up-regulated gene across all treatments, particularly with Mel and Put. In a similar study by Petriccione et al. [51], application of chitosan also increased the transcript level of *Fra a3* at all ripening stages. Such an increase in *Fra a3* transcript levels could indeed suggest a potential increase in allergenic risk to sensitive consumers, raising concerns. However, theoretical population-level health risk remains low unless the protein accumulates in high concentrations in the consumable part of

the fruit [26], therefore rendering future protein content analyses as essential. In any case, it is important to note that *Fra a3* expression levels were lower in NaA-Mel treated samples, in comparison with Mel-treated ones, suggesting that delivery of Mel with NaA constitutes a more bio-safe approach in terms of potential allergenicity effects than direct agent application.

Tryptophan decarboxylase (TDC) is an enzyme responsible for the catalysis of conversion of L-tryptophan (Trp) to tryptamine, which is a precursor of serotonin and melatonin in plants [63]. A number of reports have clearly reported the positive effects of melatonin in biosynthesis of metabolites and delayed senescence in the case of strawberries [14,33, 38]. In this context, the enhanced transcript levels of *Fra TDC* is paramount with respect to melatonin biosynthesis. According to present findings, Mel and Mel-NaA treatments significantly down-regulated *Fra TDC* (-16.145-fold and -8.821-fold, respectively). Similarly, Put down-regulated *Fra TDC*, albeit to a lesser extent (-4.976-fold). *Fra ASMT* (Acetylserotonin O-Methyltransferase) is a critical enzyme in the biosynthesis of melatonin [10]. As in the case of *Fra TDC*, transcript levels of *Fra ASMT* were down-regulated by Mel, and Mel-NaA and Put, as well. Results suggest that these treatments could potentially modulate the melatonin biosynthetic pathway, redirecting metabolic fluxes towards alternative stress-response mechanisms.

5. Conclusions

Current results suggest that alginate, as a biopolymer-based carrier, may function as an effective and robust delivery system for melatonin in strawberry plants with special reference to secondary metabolism linked to fruits' phytochemical content. Ellagitannins and ellagic acid derivatives in particular were enhanced following application of the Mel-NaA conjugate. VOC profiling revealed no significant effect in aroma components following any of the priming treatments in comparison with water-sprayed fruit, suggesting no aroma 'penalty'. Overall, this approach could offer substantial benefits that can be additionally explored at post-harvest level towards fruit preservation and quality enhancement, positioning alginate-based delivery systems as a promising strategy in the development of advanced fruit coating technologies. Global transcriptomic analyses are scheduled in future experiments in order to further evaluate the potentially positive effect of these priming agents on the quality of strawberries and to improve the understanding of their mode of action. The extent to which these promising priming

agents can enhance yield efficiency beyond their beneficial effect on secondary metabolism, needs to be clarified in large scale experiments or trials under semi-commercial conditions.

Author contributions

V.F. designed the experiment. E.C.G., C.J.G., A.M.T., S.G., N.V., A.V., G.G., M.B. and R.A. performed the experiments. V.F. and G.A.M. wrote the paper. M.L.A.T.M.H and F.A.T. edited the manuscript. E.C.G. and V. F. revised the manuscript. All authors discussed and approved the final manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: George Manganaris reports financial support was provided by Horizon Europe. Vasileios Fotopoulos has patent #WO/2023/099627A1 pending to Cyprus University of Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.cpb.2025.100515](https://doi.org/10.1016/j.cpb.2025.100515).

Data availability

Data will be made available on request.

References

- C.A. Acevedo, D.A. Lopez, M.J. Tapia, J. Enrione, O. Skurtys, F. Pedreschi, et al., Using RGB image processing for designating an alginate edible film, *Food Bioprocess Technol.* 5 (2012) 1511–1520.
- G. Alharaty, H.S. Ramaswamy, The Effect of sodium alginate-calcium chloride coating on the quality parameters and shelf life of strawberry cut fruits, *J. Comp. Sci.* 4 (2020) 123.
- M.M. Ali, R. Anwar, A.U. Malik, A.S. Khan, S. Ahmad, Z. Hussain, et al., Plant growth and fruit quality response of strawberry is improved after exogenous application of 24-epibrassinolide, *J. Plant Growth Reg.* 41 (2022) 1786–1799.
- M.B. Arnao, J. Hernandez-Ruiz, The physiological function of melatonin in plants, *Plant Signal. Behav.* 1 (2006) 89–95.
- M.B. Arnao, J. Hernández-Ruiz, A. Cano, R.J. Reiter, Melatonin and carbohydrate metabolism in plant cells, *Plants* 15 (2021) 1917.
- E. Bal, B.A. Ürün, Effects of chitosan coating with putrescine on bioactive compounds and quality of strawberry cv. San Andreas during cold storage, *ErwerbsObstbau* 63 (2021) 7–14.
- S.K. Bose, P. Howlader, J. Xiaochen, W. Wenxia, Y. Heng, Alginate oligosaccharide postharvest treatment preserve fruit quality and increase storage life via abscisic acid signaling in strawberry, *Food Chem.* 283 (2019) 665–674.
- T. Bourtoom, Edible films and coatings: characteristics and properties, *Int. Food Res. J.* 15 (2008) 237–248.
- B. Buendía, M.I. Gil, J.A. Tudela, A.L. Gady, J.J. Medina, C. Soria, et al., HPLC-MS analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars, *J. Agr. Food Chem.* 58 (2010) 3916–3926.
- Y. Byeon, H.J. Lee, H.Y. Lee, K. Back, Cloning and functional characterization of the Arabidopsis N-acetylserotonin O-methyltransferase responsible for melatonin synthesis, *J. Pineal Res.* 60 (2016) 65–73.
- M. Cardarelli, A. El Chami, Y. Rouphael, M. Ciriello, P. Bonini, G. Erice, V. Cirino, B. Basile, G. Corrado, S. Choi, H.-J. Kim, G. Colla, Plant biostimulants as natural alternatives to synthetic auxins in strawberry production: physiological and metabolic insights, *Front. Plant Sci.* 14 (2024) 1337926.
- C.C. Chang, M.H. Yang, H.M. Wen, J.C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, *J. Food Drug Anal.* 10 (3) (2002).
- T.I. Edewor-Kuponiya, Determination of fructose content in *Anacardium occidentale*, *Int. J. Agric. Food Res.* 2 (2013) 13–19.
- M.M. El-Mogy, R.A. Ludlow, C. Roberts, C.T. Müller, H.J. Rogers, Postharvest exogenous melatonin treatment of strawberry reduces postharvest spoilage but affects components of the aroma profile, *J. Berry Res.* 9 (2019) 297–307.
- P. Filippou, C. Antoniou, V. Fotopoulos, Effect of drought and rewatering on the cellular status and antioxidant response of *Medicago truncatula* plants, *Plant Signal. Behav.* 6 (2011) 270–277.
- F. Gao, X. Mei, Y. Li, J. Guo, Y. Shen, Update on the roles of polyamines in fleshy fruit ripening, senescence, and quality, *Front. Plant Sci.* 12 (2021) 610313.
- E.C. Georgiadou, V. Goulas, I. Majak, A. Ioannou, J. Leszczynska, V. Fotopoulos, Antioxidant potential and phytochemical content of selected fruits and vegetables consumed in Cyprus, *Biotechnol. Food Sci.* 82 (2018) 3–14.
- G. Gohari, H. Farhadi, S. Panahirad, E. Zareei, P. Labib, H. Jafari, G. Mahdavinia, et al., Mitigation of salinity impact in spearmint plants through the application of engineered chitosan-melatonin nanoparticles, *Int. J. Biol. Macromol.* 224 (2023) 893–907.
- G. Gohari, M. Jiang, G.A. Manganaris, J. Zhou, V. Fotopoulos, Next generation chemical priming: with a little help from our nanocarrier friends, *Trends Plant Sci.* 29 (2024) 150–166.
- C.F. Grellet-Bournonville, PdlÁ. Di Peto, A.M. Cerviño Dowling, A.P. Castagnaro, G. Schmeda-Hirschmann, J.C. Díaz Ricci, et al., Seasonal variation of plant defense inductor ellagitannins in strawberry leaves under field conditions for phytosanitary technological applications, *J. Agric. Food Chem.* 69 (2021) 12424–12432.
- J. Guo, S. Wang, X. Yu, R. Dong, Y. Li, X. Mei, Y. Shen, Polyamines regulate strawberry fruit ripening by ABA, IAA, and ethylene, *Plant Physiol.* 177 (2018) 339–351.
- F. Habibzadeh, S. Hazrati, M. Gholamhoseini, D. Khodaei, D. Habashi, Evaluation of quantitative and qualitative characteristics of strawberry in response to bio- and chemical fertilizers, *Gesund Pflanz.* 71 (2019) 4.
- M. Hadjipieri, E.C. Georgiadou, F. Costa, V. Fotopoulos, G.A. Manganaris, Dissection of the incidence and severity of purple spot physiological disorder in loquat fruit through a physiological and molecular approach, *Plant Physiol. Biochem* 155 (2020) 980–986.
- M. Hadjipieri, E.C. Georgiadou, P. Drogoudi, V. Fotopoulos, G.A. Manganaris, The efficacy of acetylsalicylic acid, spermidine and calcium preharvest foliar spray applications on yield efficiency, incidence of physiological disorders and shelf-life performance of loquat fruit, *Sci. Hortic.* 289 (2021) 110439.
- M.L. Hernández, M. Mancha, J.M. Martínez-Rivas, Molecular cloning and characterization of genes encoding two microsomal oleate desaturases (FAD2) from olive, *Phytochemistry* 66 (2005) 1417–1426.
- K. Hoffmann-Sommergruber, Plant allergens and pathogenesis-related proteins: What do they have in common? *Int. Arch. Allergy Immunol.* 122 (2000) 155–166.
- A. Jimenez, G. Creissen, B. Kular, J. Firmin, S. Robinson, M. Verhoeven, P. Mullineaux, Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening, *Planta* 214 (2002) 751–758.
- Z.M. Jin, C.H. Wang, Z.P. Liu, W.J. Gong, Physiological and ecological characters studies on *Aloe vera* under soil salinity and seawater irrigation, *Process Biochem* 42 (2007) 710–714.
- A.S. Khan, Z. Singh, N.A. Abbasi, E.E. Swinny, Pre- or post-harvest applications of putrescine and low temperature storage affect fruit ripening and quality of 'Angeline' plum, *J. Sci. Food Agric.* 88 (2008) 1686–1695.
- M.R.Z. Khoshroshahi, M. Esna-Ashari, A. Ershadi, Effect of exogenous putrescine on post-harvest life of strawberry (*Fragaria ananassa* Duch.) fruit, cultivar Selva, *Sci. Hortic.* 114 (2007) 27–32.
- L. Li, J. Sun, H. Gao, Y. Shen, C. Li, P. Yi, et al., Effects of polysaccharide-based edible coatings on quality and antioxidant enzyme system of strawberry during cold storage, *Int. J. Polym. Sci.* 9746174 (2017) 8.
- L. Liu, A. Huang, B. Wang, H. Zhang, Y. Zheng, L. Wang, Melatonin mobilizes the metabolism of sugars, ascorbic acid and amino acids to cope with chilling injury in postharvest pear fruit, *Sci. Hortic.* 323 (2024) 112548.
- C.H. Liu, H.H. Zheng, K.L. Sheng, W. Liu, L. Zheng, Effects of melatonin treatment on the post-harvest quality of strawberry fruit, *Postharvest Biol. Technol.* 139 (2018) 47–55.
- F. Loreto, V. Velikova, Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes, *Plant Physiol.* 127 (2001) 1781–1787.
- R. Mahmoudi, F. Razavi, V. Rabiei, L. Palou, G. Gohari, Postharvest chitosan-arginine nanoparticles application ameliorates chilling injury in plum fruit during cold storage by enhancing ROS scavenging system activity, *BMC Plant Biol.* 22 (2022) 555.
- R. Mahmoudi, F. Razavi, V. Rabiei, G. Gohari, L. Palou, Application of glycine betaine coated chitosan nanoparticles alleviate chilling injury and maintain quality of plum (*Prunus domestica* L.) fruit, *Int. J. Biol. Macromol.* 207 (2022) 965–977.
- G.A. Manganaris, V. Goulas, A.R. Vicente, L.A. Terry, Berry antioxidants: small fruits providing large benefits, *J. Sci. Food Agric.* 94 (2014) 825–833.
- S. Mansouri, M. Koushesh Saba, H. Sarikhani, Exogenous melatonin delays strawberry fruit ripening by suppressing endogenous ABA signaling, *Sci. Rep.* 13 (2023) 14209.
- S. Mansouri, H. Sarikhani, M. Sayyari, M.S. Aghdam, Melatonin accelerates strawberry fruit ripening by triggering GAMYB gene expression and promoting ABA accumulation, *Sci. Hortic.* 281 (2021) 109919.
- M. Marcé, D.S. Brown, T. Capell, X. Figueras, A.F. Tiburcio, Rapid high-performance liquid chromatographic method for the quantitation of polyamines as their dansyl derivatives: application to plant and animal tissues, *J. Chromatogr. B Biomed. Appl.* 666 (1995) 329–335.

- [41] Y. Meller Harel, Y. Elad, D. Rav-David, M. Borenstein, R. Shulchani, B. Lew, E. R. Graber, Biochar mediates systemic response of strawberry to foliar fungal pathogens, *Plant Soil* 357 (2012) 245–257.
- [42] T. Metsalu, J. Vilo, ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap, *Nucleic Acids Res* 43 (2015) 566–570.
- [43] K.J. Meyers, C.B. Watkins, M.P. Pritts, R.H. Liu, Antioxidant and antiproliferative activities of strawberries, *J. Agric. Food Chem.* 51 (2003) 6887–6892.
- [44] B. Mezzetti, F. Giampieri, Y. Zhang, C. Zhong, Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world, *J. Berry Res.* 8 (2018) 205–221.
- [45] M.H. Milani, G. Gohari, G.A. Manganaris, V. Fotopoulos, Impacts of nanocomposites on the postharvest physiology and shelf life of agricultural crops. In *Nanocomposites for Environmental, Energy, and Agricultural Applications*, Woodhead Publishing, 2024, pp. 315–347.
- [46] C. Munoz, T. Hoffmann, N.M. Escobar, F. Ludemann, M.A. Botella, V. Valpuesta, W. Schwab, The strawberry fruit Fra a allergen functions in flavonoid biosynthesis, *Mol. Plant* 3 (2010) 113–124.
- [47] P. Muñoz, C. Castillejo, J.A. Gómez, L. Miranda, S. Lesemann, et al., 2023, QTL analysis for ascorbic acid content in strawberry fruit reveals a complex genetic architecture and association with GDP-L-galactose phosphorylase. *Horticulture Res.* 10, uhad006.
- [48] V. Okatan, M.A. Aşkın, M. Polat, I. Bulduk, A.M. Çolak, S.F. Güçlü, İ. Kahramanoğlu, et al., Effects of melatonin dose on fruit yield, quality, and antioxidants of strawberry cultivars grown in different crop systems, *Agriculture* 13 (2022) 71.
- [49] S. Pareek, S. Sharma, N. Sagar, G.A. González-Aguilar, Polyamines treatments, in: S. Pareek (Ed.), *Novel postharvest treatments of fresh produce*, CRC Press, 2018, pp. 79–101.
- [50] M. Perez-Llorca, P. Munoz, M. Müller, S. Munne-Bosch, Biosynthesis, metabolism and function of auxin, salicylic acid and melatonin in climacteric and non-climacteric fruits, *Front. Plant Sci.* 10 (2019) 136.
- [51] M. Petriccione, F. Mastrobuoni, L. Zampella, E. Nobis, G. Capriolo, M. Scortichini, Effect of chitosan treatment on strawberry allergen-related gene expression during ripening stages, *J. Food Sci. Technol.* 54 (2017) 1340–1345.
- [52] M.W. Pfaffl, G.W. Horgan, L. Dimpfle, Relative expression software tool (REST(C)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR, *Nucleic Acids Res* 30 (2002) e36.
- [53] J. Qiao, G. Su, L. Yuan, L. Wu, X. Weng, S. Liu, Y. Feng, D. Jiang, Y. Chen, Y. Ma, Effect of swelling agent treatment on grape fruit quality and the application of electronic nose identification detection, *Front. Plant Sci.* 14 (2024) 1292335.
- [54] G. Salazar-Orbea, R. García-Villalba, L.M. Sánchez-Siles, F.A. Tomás-Barberán, C. J. García, Untargeted metabolomics reveals new markers of food processing for strawberry and apple purees, *Molecules* 27 (2022) 7275.
- [55] F. Sampedro, X. Fan, D. Rodrigo, High hydrostatic pressure processing of fruit juices and smoothies: research and commercial application, *Case Stud. Nov. Food Process. Technol.* (2010) 34–72.
- [56] L. Sánchez-Rodríguez, N.S. Ali, M. Cano-Lamadrid, L. Noguera-Artiaga, L. Lipan, Á. A. Carbonell-Barrachina, E. Sendra, Flavors and aromas. In *Postharvest physiology and biochemistry of fruits and vegetables*, Woodhead Publishing, 2019, pp. 385–404.
- [57] A. Savvides, S. Ali, M. Tester, V. Fotopoulos, Chemical priming of plants against multiple abiotic stresses: Mission possible? *Trends Plant Sci.* 21 (2016) 329–340.
- [58] M. Sequera-Mutiozabal, C. Antoniou, A.F. Tiburcio, R. Alcázar, V. Fotopoulos, Polyamines: emerging hubs promoting drought and salt stress tolerance in plants, *Curr. Mol. Biol. Rep.* 3 (2017) 28–36.
- [59] S.A. Shehata, E.A. Abdeldaym, M.R. Ali, R.M. Mohamed, R.I. Bob, K. F. Abdelgawad, Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage, *Agronomy* 10 (2020) 1466.
- [60] V. Singh, S.K. Jawandha, P.P.S. Gill, M.S. Gill, Suppression of fruit softening and extension of shelf life of pear by putrescine application, *Sci. Hortic.* 256 (2019) 108623.
- [61] S. Sreekumar, H. Sithul, P. Muraleedharan, J.M. Azeez, S. Sreeharshan, Pomegranate fruit as a rich source of biologically active compounds, *BioMed. Res. Int.* (2014) e686921.
- [62] G.M. Symons, Y.J. Chua, J.J. Ross, L.J. Quittenden, N.W. Davies, J.B. Reid, Hormonal changes during non-climacteric ripening in strawberry, *J. Exp. Bot.* 63 (2012) 4741–4750.
- [63] J. Taboada, S. González-Gordo, J.M. Palma, F.J. Corpas, Tryptophan decarboxylase (TDC) in sweet pepper (*Capsicum annuum* L.): gene expression analysis during fruit ripening and after nitric oxide exposure, *Melatonin Res* 6 (2023) 277–295.
- [64] A. Tariq, A. Ambreen, "Plant phenolics production: a strategy for biotic stress management. *Plant Phenolics in Biotic Stress Management*, Springer Nature Singapore, Singapore, 2024, pp. 441–454.
- [65] UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). 2023. "Strawberry production in 2021, Crops/Regions/World list/Production Quantity (pick lists)".
- [66] T. Vandendriessche, B.M. Nicolai, M.L.A.T.M. Hertog, Optimization of HS SPME fast GC-MS for high-throughput analysis of strawberry aroma, *Food Anal. Meth* 6 (2013) 512–520.
- [67] S. Voća, J.S. Zlabur, N. Dobričević, L. Jakobek, M. Seruga, A. Galić, S. Pliestić, Variation in the bioactive compound content at three ripening stages of strawberry fruit, *Molecules* 19 (2014) 10370–10385.
- [68] T. Wang, M. Hu, D. Yuan, Z. Yun, Z. Gao, Z. Su, Z. Zhang, Melatonin alleviates pericarp browning in litchi fruit by regulating membrane lipid and energy metabolisms, *Postharvest Biol. Technol.* 160 (2020) 111066.
- [69] P. Wang, L. Yin, D. Liang, C. Li, F. Ma, Z. Yue, Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle, *J. Pineal Res.* 53 (2011) 11–20.
- [70] H. Xia, Y. Shen, T. Shen, X. Wang, X. Zhang, P. Hu, D. Liang, L. Lin, et al., Melatonin accumulation in sweet cherry and its influence on fruit quality and antioxidant properties, *Molecules* 25 (3) (2020).
- [71] L. Xu, Q. Yue, F. Bian, H. Sun, H. Zhai, Y. Yao, Melatonin enhances phenolics accumulation partially via ethylene signaling and resulted in high antioxidant capacity in grape berries, *Front. Plant Sci.* 8 (2017) 01426.
- [72] L. Xu, Q. Yue, G. Xiang, F. Bian, Y. Yao, Melatonin promotes ripening of grape berry via increasing the levels of ABA, H₂O₂, and particularly ethylene, *Hortic. Res.* 5 (2018) 41.
- [73] J. Yang, C. Zhang, Z. Wang, S. Sun, R. Zhan, Y. Zhao, B. Ma, F. Ma, M. Li, Melatonin-mediated sugar accumulation and growth inhibition in apple plants involves down-regulation of fructokinase 2 expression and activity, *Front. Plant Sci.* 10 (2019) 150.
- [74] S. Yoo, J.M. Krochta, Whey protein–polysaccharide blended edible film formation and barrier, tensile, thermal and transparency properties, *J. Sci. Food Agric.* 91 (2011) 2628–2636.
- [75] N. Zhang, et al., Roles of melatonin in abiotic stress resistance in plants (Available at:), *J. Exp. Bot.* 66 (3) (2015) 647–656, <https://doi.org/10.1093/jxb/eru336>.
- [76] W. Zhang, J. Cao, X. Fan, W. Jiang, Applications of nitric oxide and melatonin in improving postharvest fruit quality and the separate and crosstalk biochemical mechanisms, *Trends Food Sci. Technol.* 99 (2020) 531–541.
- [77] K. Zhou, Q. Cheng, J. Dai, Y. Liu, Q. Liu, R. Li, J. Wang, R. Hu, L. Lin, Effects of exogenous melatonin on sugar and organic acid metabolism in early-ripening peach fruits, *Plos One* 18 (2023) e0292959.



A comprehensive assessment of life cycle environmental impact and economic feasibility of different red raspberry (*Rubus idaeus* L) cultivation systems

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ABSTRACT

Red raspberry is considered a knowledge- and capital-intensive crop that targets a niche market globally; its quality attributes and enhanced health-promoting properties are highly appreciated by the consumers. In the context of the exponential growth in demand for this specialty crop that suffers from limited shelf life, it is imperative to expand raspberry cultivation by employing sustainably-sourced production models. In the current study, we used Cyprus as a case study that is characterised by increased production costs and lack of year-round production despite the fact that the latter is feasible under different production systems and cultivation methods in different altitude-related meso-climates. Towards that goal, the current study assessed the life cycle environmental impact and life cycle costs of two different cultivation methods - open-field production that took place from May to November 2022 and protected cultivation in high-tunnels, from August 2023 to April 2024, using in both cases the same cultivar (Kwanza[®]) and plant type. The results indicated that protected cultivation has better environmental performance (3.7 mPt - milli eco-points - per kg of raspberry produced compared to 7.4 mPt for open-field production). Noteworthy, production cost is excessive and substantially higher compared to other countries; open-field production has a life cycle cost of 22.5 €/kg, while protected cultivation achieved a lower life cycle cost, equal to 14.0 €/kg yet still high. From an output perspective, a key observation is the increased yield of raspberries in protected cultivation as well as the enhanced water use efficiency of the crop, due to a reduction of the water footprint by 76 %. It is also important to highlight the increased harvest efficiency of the crop under high tunnel, with 500 g per plant compared to 350 g on open field cultivation. Hence, it is safe to conclude that despite the increased start-up costs and knowledge-intensive practices, the productivity of the crop is increased during the off-season months, that can be sold for a premium. The results highlight the environmental and economic impact of the two cultivation methods and will be useful for producers and crop advisors seeking to expand the raspberry cultivation in climates that resembles south-eastern Europe and are characterised as vulnerable to adverse climate change scenarios.

1. Introduction

Raspberry (*Rubus idaeus* L.) is an economically significant crop with a

growing popularity and exponential growth in demand and production volumes (IRO, 2024). This is partially due to the fact that raspberry fruit represents an excellent natural source of biologically active components

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that provide significant health-promoting benefits (reviewed in [Manganaris et al., 2014](#) and is also getting increasingly recognized for its efficacy in preventing chronic diseases, including cancer and cardiovascular disorders ([Burton-Freeman et al., 2016](#)).

In the 21st century, several commercially important red raspberry cultivars have been developed through hybridisation or selective breeding from diverse genetic sources, extending their productivity and seasonality in tropical, sub-tropical, and Mediterranean regions ([Kempler et al., 2012](#)). These cultivars are characterised by enhanced traits, such as larger fruit size, better flavour, disease resistance and tolerance, enhanced harvest efficiency, and adaptability to different climates. Such improvements rendered raspberry cultivation commercially viable and contributed to its popularity as a widely cultivated fruit ([Hall et al., 2011](#)). Multiple public and private breeding programs around the world contribute to the development and release of new cultivars with a growing number of releases having royalties that increase farmers' overall costs of production.

The consumption of locally grown fruits minimises carbon footprint; however raspberries cannot be grown in open fields year-round in many regions ([Reisch et al., 2013](#)). On top of that, farmers must implement cost-effective cultivation practices to minimize financial risks and maintain profitability. Overall, the year-round availability of a specialty crop like raspberry requires a high level of technical expertise and is achieved through a combination of adopting appropriate plant material (different cultivars), using superior production protocols and cultivating across different climates.

Raspberry is considered a perennial fruit crop, yet it develops biennial canes that vary temporally and spatially with regards to fruit production, depending on raspberry type. The first type is floricanes-fruiting (i.e., non-remontant or summer-bearing). This type produces vegetative primocanes in the first year. These canes go through acclimation in autumn and overwinter, accumulating chilling units that induce floricanes into bearing flowers and fruits on lateral branches the following year. In contrast, primocane-fruiting (i.e., remontant, ever-bearing, or fall-bearing) types produce flowers and fruits at the top of first-year primocanes as well as on second-year floricanes after accumulating sufficient chilling units in winter ([Manganaris et al., 2024](#)).

Raspberry cultivation encompasses several labour-intensive techniques that are tailored to raspberry type, market requirements, and environmental conditions. Traditional raspberry cultivation involves open-field production, where plants are grown directly into the soil or sometimes containers, and the canopy is supported by trellises or stakes ([Dale et al., 1994](#)). Protected cultivation is an alternative production method that uses, plastic high tunnels, greenhouses, or other covers that extend the raspberry production season and enable harvest through modifications in the microclimate, while protecting against unpredictable weather patterns ([Dale 2012](#); [Palonen et al., 2017](#)). Protected cultivation is generally more dependent on the use of containers with soilless media, such as coir and peat, which enables hydroponic techniques to be used whereby nutrients are dissolved and provided frequently in irrigation water ([Ragaveena et al., 2021](#)). Overall, protected cultivation conceptually provides many benefits to raspberry production, leading to optimised growing conditions, but it is highly intensive and requires a high financial investment from growers ([Dale, 2012](#)). A careful selection of the production system must be made to ensure maximum production and profitability leading to economic viability.

Understanding the environmental footprint of agricultural practices can reveal information on whether the water, energy, and land are being used inefficiently or excessively ([Gruda et al., 2019](#); [Janick 2003](#)). As a commodity with a very short shelf life that requires cooling to maximize product longevity and quality, the raspberry supply chain has a significantly high environmental impact ([Blanc et al., 2018](#)). Several studies have been conducted to improve raspberry cultivation techniques, focusing on optimising plant growth, increasing yield, and improving fruit quality ([Girgenti et al., 2013](#); [Foster et al., 2015](#); [Kailasam et al.,](#)

[2020](#); [Sangiorgio et al., 2021](#); [Ponder and Hallmann., 2019](#); [Valiante et al., 2019](#)). However, all these studies focus on regions located in mainland Europe, where the climate is temperate.

In the current study, Cyprus case study is characterized by extreme temperatures and heatwaves compared to the rest of Europe. Additionally, its nature as an island poses significant challenges in terms of access to resources and higher production costs. More specifically, Cyprus is a Mediterranean island located in the easternmost part of the basin. It is characterised by different mesoclimates: a temperate climate in the mountainous areas with hot and dry summers, while the mainland and coastal areas are categorised as hot and arid, according to the Köppen–Geiger system ([Peel et al., 2007](#); [Zittis et al., 2017](#)). The mean annual precipitation is approximately 470 mm, and most of the water resources originate in the Troodos mountains, covering nearly 30 % of the island ([Christofi et al., 2020](#)). The average precipitation in mountainous Cyprus is estimated to be 600 mm ([Katsanos et al., 2016](#); [Michaelides et al., 2009](#)). Over the last decades, the observed rainfall trends in this part of the Mediterranean have been mostly declining ([Papadaskalopoulou et al., 2020](#)). Cyprus is among the EU Member States with the least available water per capita and remains vulnerable to climate change due to droughts and water scarcity ([RoC, 2021](#)), therefore the selection of sustainable cultivation practises that minimizes the use of water is crucial for the overall sustainability of Cypriot agriculture ([EC, 2023](#)). The rise of average air temperatures and more frequent heatwaves during the harvest season have caused problems to farmers who are still using traditional cultivation practices. Considering these challenges, there is an emerging need to explore alternative strategies that safeguard farmers' livelihoods ([del Pozo et al., 2019](#)).

Despite climate change concerns, the production of small fruits has expanded in the past two decades in the mountainous regions of Cyprus. In these areas the milder summer months and the relatively colder winter conditions permit open-field cultivation in the soil, which is considered an easier, low-cost production method. Growers use primocane-fruiting cultivars, and the production seasonality is limited to few months, mainly spanning from May until September ([Lazoglou et al., 2024](#)). Extreme heatwaves (over 30 °C) and lack of precise fertigation management techniques accelerate the ripening process, having a detrimental effect on fruit quantity and quality. Additionally, the induction of new flowers for next year's production is usually disrupted by high fall and winter temperatures, resulting in relatively small yields from the floricanes crop.

As a direct response to these challenges, the establishment of low-cost high tunnels made of plastic ("poly") has emerged as a promising solution, particularly in the flatlands of Cyprus. Notably, these regions experience significantly higher temperatures, reaching up to 40 °C during summer and ca. 17 °C during winter periods. These passively modified environmental conditions extend the cultivation period of plants and permit off-season production from fall through spring when conditions are generally unfavourable for traditional, open-field cultivation. Due to lower solar radiation and wind speeds, high-tunnel-grown plants are likely to require a reduced amount of water owing to their relatively lower vapour pressure deficit.

Another challenge for Cyprus is that due to its remote nature, as an island, all resource and energy prices are significantly higher than the average European ones. This is also reflected in the competitive prices of imported raspberries. The objective of this study was to compare Kwanza® primocane raspberry production in two distinct environments with different cultivation techniques and associated production periods in Cyprus: (i) open-field cultivation from May until December 2022 and (ii) protected cultivation in high tunnels from August 2023 until April 2024. The overarching aim was to compare the two cultivation techniques and generate recommendations whether farmers can consider replacing or complementing traditional open-field cultivation with protected cultivation without jeopardising yield and profitability, while minimizing their environmental impact. To our knowledge, this is the first reported attempt to evaluate the cultivation of raspberries in a

decentralised location with temperature extremes and water scarcity such as the conditions found in Cyprus. Another contribution of our study is the use of real-world data collected from the vegetative stage of a young Kwanza® planting until the end of primocane harvest in two distinct environments, with unique climatic conditions and cultivation methods in order to determine which is more profitable for Cyprus's red raspberry cultivation.

2. Methodology

A Life Cycle Assessment (LCA) was employed to evaluate the environmental performance of the two different raspberry cultivation methods. The current study adopted a methodological approach aligned with the LCA framework outlined in ISO14040/44:2006 (ISO, 2006), which includes four key stages:

1. Goal and Scope Definition, by establishing the study's objective and delineating the spatial and temporal boundaries of the chosen system.
2. Life Cycle Inventory (LCI), by compiling an inclusive inventory of all incoming and outgoing exchanges between the system and the environment.
3. Life Cycle Impact Assessment (LCIA), by calculating the environmental impact indicators, drawing from the inventory flows, and corresponding characterisation factors.
4. Interpretation of Results to discern the broader significance of the LCA assessment findings.

Table 1
Selected set of midpoint impact indicators.

Impact category	Impact acronym	Unit
Climate change	GWP	kg CO _{2,eq}
Ozone depletion	ODP	kg CFC-11 _{eq}
Ionising radiation	IRP	kBq U-235 _{eq}
Photochemical ozone formation	PCOP	kg NMVOC _{eq}
Particulate matter	PM	disease incidence
Human toxicity, non-cancer	HTPNC	CTUh
Human toxicity, cancer	HTPC	CTUh
Acidification	AP	mol H _{eq} ⁺
Eutrophication, freshwater	FEP	kg P _{eq}
Eutrophication, marine	MEP	kg N _{eq}
Eutrophication, terrestrial	TEP	mol N _{eq}
Ecotoxicity, freshwater	ETP	CTUe
Land use	LU	Pt
Water use	WDP	m ³
Resource use, fossils	ADPF	MJ
Resource use, minerals	ADPM	kg Sb _{eq}

The Life Cycle Assessment was performed using the SimaPro 9.2 Academic License, with the ecoinvent v3.7 database. The Environmental Footprint 3.0 was chosen as the assessment method. Environmental Footprint 3.0 is affiliated with the Environmental Footprint initiative and is the preferred method of LCA practitioners, since it provides both midpoint and endpoint indicators, and it is widely used by recent studies on the same sector (Valiante et al., 2019). The midpoint impact indicators employed to evaluate the environmental impact of raspberry production in this study, together with the abbreviations used and the units of measurement, are illustrated in Table 1. The endpoint single score indicator of this impact assessment method is the Environmental Footprint, and it is expressed in ecopoints (Pt). One milli ecopoint (mPt) corresponds to the annual environmental impact of an average European citizen.

2.1. Goal and scope definition

As previously mentioned, the scope of the study was to compare the environmental impact of raspberry production using two distinct cultivation methods: (a) soil-less open-field cultivation, with lower establishment costs but possibly higher water usage and lower yield, and (b) soil-less protected cultivation with a high-tunnel, entailing higher initial investment but with higher yield potential while conserving water. The research seeks to guide producers toward more cost-effective and eco-efficient production options, particularly in Mediterranean decentralized microclimates, as exemplified by the case study in Cyprus. Hence, the geographical scope refers to Southeastern Europe.

A cradle-to-gate approach was adopted for comparing both cultivation systems, focusing specifically on pre-harvest activities from nursery supply to primocane harvest. Data for the soil-less open-field cultivation method was sourced from a field located in Chandria village (altitude ca. 1200 m) in the upper highlands of Troodos, Cyprus (Fig. 1a). This cultivation cycle spanned from May to December 2022. Data for the protected cultivation system was sourced from an operation, located in Peristerona (altitude ca. 600 m), situated northwest of Nicosia (Fig. 1b). The protected cultivation cycle spanned from August 2023 to April 2024.

The system boundaries include all in-field processes (irrigation, fertiliser application and plant protection), while post-harvest processes fall beyond the scope of the study and are assumed to be the same regardless of the cultivation method (Fig. 2). During the cultivation period, a mix of different fertilizers and pesticides was applied to maintain plant growth while electricity was consumed by irrigation pumps and smart farm management equipment.

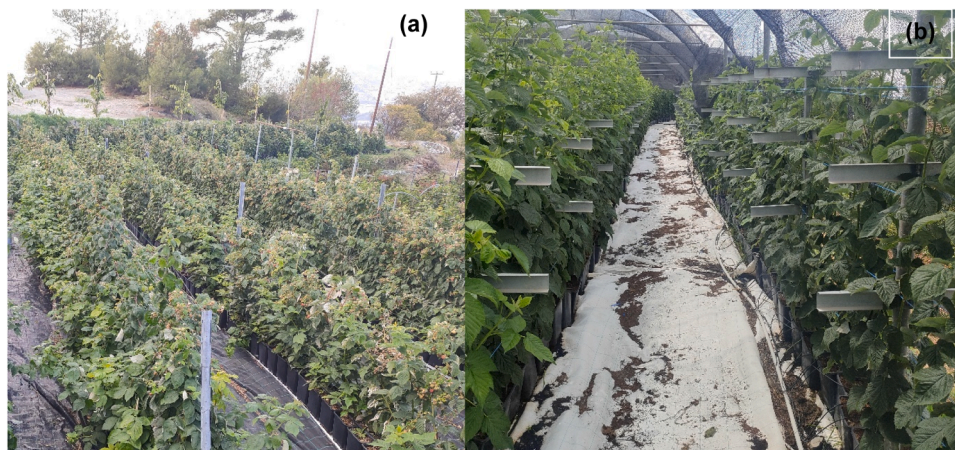


Fig. 1. (a) Open-field cultivation at harvesting stage and (b) Protected cultivation at flowering stage.

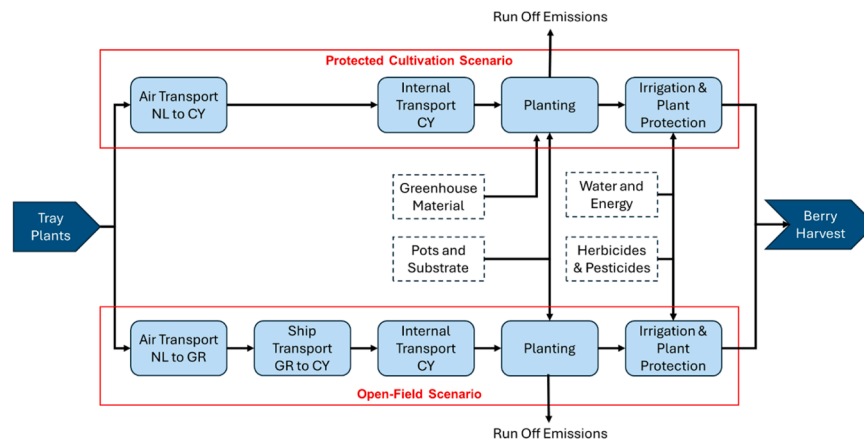


Fig. 2. System boundaries for the two examined scenarios. Market values were used as the characterisation factors of all resources used (fertilisers, pesticides, soil, pots, irrigation and construction material, cover, pots). Such values consider both the impact of the production process of each resource and a typical transportation impact. However, a separate consideration was made for plant delivery from the nursery located in The Netherlands (either via ship or plane) and transportation of plant material via truck to the field, since the itinerary is different for each type of cultivation.

The functional unit (FU) is defined as the physical quantity that is used to provide the reference to which all data in the system are normalized and enables the comparison of the findings. In this study, the FU was chosen to be 1 kg of fresh fruit produced (mass-based FU), which is the most used unit for agricultural products (Pergola et al., 2017; Vinyes et al., 2015).

2.2. Life cycle inventory (LCI)

The LCI was constructed using data from the two cultivation models being compared and it was assumed they were representative of small fruit cultivation in the greater area. To collect these data, a questionnaire was distributed to a local agronomist, and the responses were processed and utilized accordingly.

The Life Cycle Inventory is illustrated in Table 2, using normalized data per hectare (ha) of cultivated area. As far as the use of land is concerned, the total number of hectares per farm was 0.2 hectares, typical of small-scale farm holdings. However, the data provided in Table 2 were normalised for 1 hectare of cultivated land. The data presented are referring to the most recent agricultural year that consisted of two individual cycles, from May 2022 to December 2022 and from August 2023 to April 2024. The assumptions used for the formulation of the inventory are presented in the following sections. It needs to be noted that the quantities presented in the material section of Table 2 correspond to the annual equivalent value. For example, since the drip emitters are replaced every five years, then the annual equivalent value is one fifth of the total number of drip emitters used.

2.2.1. Plant sourcing and potting

Juvenile plants were initially propagated and grown in The Netherlands. Each plant was provided as a fresh tray plant with healthy leaves and roots (average root length 5 cm). Propagation and nursery production are beyond the scope of this analysis, as these are not directly related to the operation in Cyprus and are the same regardless of the production system. The same cultivar (Kwanza®) and plant type were planted in both locations but at different time points for each location. Plantlets were transplanted into pots as soon as possible to avoid plant stress. Specifically, for the open field, the tray plants were transported by truck from The Netherlands to Greece and then from Greece to Cyprus by ship, reaching the plot (Chandria, 1200 m altitude) by truck. The tray plants were transplanted into 10 L pots in May 2022 and placed in the open field (Fig. 3). This trip from The Netherlands to the field takes about a week to complete. However, when planting takes place during the summer, air freight is imperative due to weather conditions

so that the plants arrive within a day without being affected by the high temperatures. Thus, for the protected cultivation scenario, the tray plants were transported by airplane from The Netherlands to Cyprus directly and then by truck to the plot (Peristerona) and were transplanted in 10 L pots in September 2023 under protected cultivation conditions in a high-tunnel. In both cases, pot dimensions were $24.7 \times 24.7 \times 25.6$ cm and were made of polypropylene.

2.2.2. Fertilizer and plant protection

Before planting, a space of 1.8 m between lines of plants was created, while 4 pots were put in one linear meter. There was no ploughing, tillage, harrowing, or hoeing because propylene ground cover of 50–100 μ m was used under the pots to avoid weed emergence. Primocane yield was determined at different periods of the year for each plot. In the protected cultivation system (Peristerona), harvest was conducted weekly from December 2023 until April 2024, while in the open field system (Chandria) harvest was conducted every two days from October to December 2022. Fertigation was carried out using a standard drainage calculation that factored in retention of approximately 25 % during the vegetative stage from pots after the irrigation interval of the day. However, to avoid accumulation of salts from flowering to the end of harvest, 35 % of drainage was applied (Ben Hadj Daoud et al. 2024).

Common practices including pest management through the utilisation of a backpack mist blower and necessary weed and canopy management were carried out manually. Irrigation and fertilisation were conducted multiple times every day using a drip irrigation system. Fertilisation was set using targeted Electrical Conductivity (EC) and pH value was adjusted at 6. EC values fluctuated around $2 \mu\text{S}/\text{cm}$ during the vegetative state, $1.6 \mu\text{S}/\text{cm}$ during flowering, and $1.4 \mu\text{S}/\text{cm}$ during harvest. Crop management involved several treatments against pests (five per year) and diseases (five per year) that were carried out by operators equipped with a portable sprayer. Two alternative pesticides were used, with active ingredients sulphur (70 %), natural pyrethrins (5 %), and flupyradifurone for the first one and copper for the second one. Monopotassium phosphate (0–52–34), potassium sulphate (0–0–50), potassium nitrate (13–0–36), calcium nitrate (15.5–0–26.5Ca) and trace elements were used as solid fertilisers dissolved in 1000 L tanks and injected at 1 % rate in each irrigation (Malghani et al., 2010). The substrate used was a combination of coco peat (40 %), peat-moss (40 %) and perlite (20 %). Fertilizer run-off was assumed to be 5 % for N (nitrates) and 2 % for P and K, (Song et al., 2023). No specific fertiliser run-off data were collected in this study given the system used, drip fertigation and there were no measurements from the field.

Table 2

Life Cycle Inventory for the two raspberry cultivation methods in Cyprus, open-field and protected cultivation. Data were collected from May to December 2022 and from August 2023 to April 2024, respectively.

Resource	Unit	Open-field	Protected-cultivation
Land	ha	1	1
Plants	Unit	18,200	18,200
Plants	kg	3640	3640
Perlite	kg	36,400	36,400
Coco peat	kg	72,800	72,800
Peat moss	kg	72,800	72,800
Transportation			
Air transport of tray plants (NL to CY)	tkm	N/A	10,920
Road transport of tray plants (NL to GR)	tkm	10,192	N/A
Ship transport of tray plants (GR to CY)	tkm	3640	N/A
Road transport of tray plants (Internal CY)	tkm	146	146
Material			
Containers (Polypropylene)	Unit	3640	3640
Containers (Polypropylene)	kg	3640	3640
Irrigation Pipes (Polyvinyl chloride)	m	910	910
Irrigation Pipes (Polyvinyl chloride)	kg	364	364
Drip Emitters (Polyvinyl chloride)	Unit	3280	3280
Drip Emitters (Polyvinyl chloride)	kg	1640	1640
Ground Cover (Polypropylene)	kg	250	250
High-tunnel Arches (Iron)	kg	N/A	8000
High-tunnel Material (Polyethylene)	kg	N/A	80
Fertilizers & Pesticides			
P	kg	108	96
K	kg	324	288
N	kg	216	192
Pesticides	kg	198	158
Water and Energy			
Electricity for irrigation	kWh	19,980	3960
Diesel for irrigation	L	250	200
Water for irrigation	m ³	52,998	17,666
Run-off Emissions			
P	kg	10.8	9.6
K	kg	6.48	5.76
N	kg	2.16	1.92
Products			
Raspberries	kg	6370	9100

2.2.3. Utilities and resources

For pumping purposes, electricity was used to operate a submersible pump (Franklin Electric, Model n. 236 616 9061 at 50 Hz). Levelling/terracing took place two times per year, each one before the beginning of each cycle. Regarding water consumption, the values differ significantly

for the two cultivation methods (Table 2). For pesticide application, it was calculated that 250 L/ha of diesel were used in the open-field while 200 L/ha were used in protected cultivation. Regarding irrigation, PVC pipelines provide water through the drippers with the boost of a submersible pump. It is estimated that 1640 emitters were required for 0.1 ha for both cultivation types. A gutter made of polypropylene, with 0.8 mm thickness and 150–600 mm wide was used in both production scenarios.

The high tunnel consists of 7 arches in a row, with netting on the side, while the front and back were closable with plastic and were sealed during periods with winter frost risks. The arches are made of galvanised iron, and consists of 6 m of DN50 pipes, 6 m of DN32 pipes and 6 m of DN20 pipes. The total weight of the pipes is 40 tonnes. Each arch was 5.4 m wide, and the tunnel length was 42 m. This means that the total area covered by the high tunnel was $7 \times 5.4 \times 42$ m or approximately 1600 m². The peak of the arch was 3.5 m in height, so the plastic (polyethylene) cover required is approximately 2300 m² or 400 kg, assuming 170 gsm for the high tunnel cover. The plastic cover and the pipes require replacement every 5 years.

2.2.4. Background systems

Background systems in the ecoinvent database refer to the pre-existing data and processes used in LCA to model the broader environmental impacts of a product or service. These systems refer to the entire supply chain, including the production of raw materials, energy, transportation, and other inputs, providing comprehensive coverage of upstream and downstream activities. They rely on global (GLO) or regional average data, ensuring consistency and standardization across LCA studies. By connecting with the foreground system, i.e. the processes directly under study, background systems offer a holistic view of environmental impacts, essential for transparency, comparability, and informed decision-making in LCA. Table 3 summarises all the background systems retrieved from the ecoinvent database for the modelling. The Allocation at the Point of Substitution (APOS) factors were selected, since these were the only ones available with the academic license. However, since the system studied does not involve multifunctional processes, then the choice between Allocation at the Point of Substitution (APOS) and Consequential (CONS) allocation does not have a significant impact to the results.

2.3. Life cycle costing data

The Life Cycle Costing (LCC) methodology international standards published in 2008 (ISO15686–5) define the general approach of an LCC



Fig. 3. (a) Rooted tray plant in 10 L pot with drip irrigation 15 days after planting (b) Raspberry canes 2 months after planting.

Table 3
Background systems – emission factors (Source: ecoinvent database v3.7).

Resource	Emission Factor Reference
Containers	Polypropylene, granulate {GLO} market for APOS, U
Ground cover	Polypropylene, granulate {GLO} market for APOS, U
High-tunnel material	Polyethylene, high density, granulate {GLO} market for APOS, U
High-tunnel arches	Pig iron {GLO} market for APOS, U
Irrigation emitters	Polyvinylchloride, bulk polymerised {GLO} market for APOS, U
Fertiliser	Nitrogen fertiliser, as N {GLO} market for APOS, U Phosphate fertiliser, as P2O5 {GLO} market for APOS, U Potassium fertiliser, as K2O {GLO} market for APOS, U
Pesticide	Pesticide, unspecified {GLO} market for APOS, U
Perlite	Perlite {GLO} market for APOS, U
Peat moss	Peat moss {GLO} market for APOS, U
Fertiliser application	Application of plant protection product, by field sprayer {RoW} processing APOS, U
Electricity	Electricity, medium voltage {CY} market for APOS, U
Diesel	Diesel {Europe without Switzerland} market for APOS, U
Water	Tap water {Europe without Switzerland} market for APOS, U
International transportation (road)	Transport, freight, lorry 16-32 metric ton, euro5 {RER} market for transport, freight, lorry 16-32 metric ton, EURO5 APOS, U
National transportation (road)	Transport, freight, lorry 3.5-7.5 metric ton, euro5 {RER} market for transport, freight, lorry 3.5-7.5 metric ton, EURO5 APOS, U
International transportation (air)	Transport, freight, aircraft, unspecified {GLO} market for transport, freight, aircraft, unspecified APOS, U
International transportation (sea)	Transport, freight, sea, container ship {GLO} market for transport, freight, sea, container ship APOS, U

analysis. However, since there are no shared methodologies to perform the analysis (Mohamad et al., 2014), for this study we have adopted an ad hoc method, including the costs of the whole life cycle of raspberry production “from cradle to gate”, following the “whole life costing” method expressed by Gluch and Baumann (2004). The LCC analysis uses the same functional unit and has the same system limitations that have been considered in the LCA. Table 4 reports the purchase costs per unit of raw materials, utilities, and chemicals that were used during the two cultivation periods for each method. These have been collected by a questionnaire that was distributed to local farms, and the average values were utilised. For protected cultivation, there is an extra labour cost of 5 workers for two working weeks at 5 €/h.

3. Results and discussion

3.1. Environmental impact assessment

Fig. 4 presents the environmental impact of the two raspberry cultivation methods in Cyprus. Protected cultivation led to an improved environmental performance, reduced by 49.6 % compared to open-field

Table 4
Purchase cost of resources for open-field and protected cultivation using high tunnels of raspberries in Cyprus.

Resource	Price	Unit
Raspberry plants	2	€/unit
Containers	2.5	€/unit
Substrate	0.09	€/kg
High-tunnel cover	0.8	€/m ²
Nozzles and tubes	2.5	€/m ²
Pesticides	300	€/kg
Fertilisers	0.5	€/kg
Water	0.45	€/m ³
Electricity	0.2	€/kWh
Diesel	1.7	€/L

cultivation. More specifically, the environmental footprint of protected cultivation was at 3.7 mPt per kg of raspberry produced, whereas the environmental footprint of open-field cultivation was 7.4 mPt per kg of raspberry produced.

Furthermore, protected cultivation was superior in all 17 indicators. Among all 17 indicators, land use and water use were the most dominant components, followed by resource usage (fossil fuels) and climate change.

Table 5 presents the absolute values of these four indicators for the two scenarios, expressed in physical units per kg of raspberry produced. Water usage is the indicator with the highest relative improvement between the two types of cultivation (dropping from 0.361 m³ to 0.085 m³ per kg of raspberry, thus improving by 76 %), which was anticipated since the protected cultivation has significantly lower water demand. The other three indicators are improved by 30 % (global warming potential), 37 % (land use) and 33 % (fossil fuel depletion) and are the main responsible factors for the superior performance of protected cultivation.

Fig. 5 illustrates the same results with a different point of view. The overall environmental footprint (expressed in ecopoints per cultivated hectare) is broken down into the different resource types contributing to it. This allows for the identification of environmental hotspots among the two cultivation methods. While the material and the transportation impacts are increased (the latter almost doubled) for the protected cultivation scenario, due to high tunnel material and the air transportation of the plants respectively, their relative importance is very small compared to the impact of water, fertilisers and soil used (in that order). Although the substrate impact is the same for both scenarios, the water and the plant protection contributions are significantly reduced, leading to an overall improved environmental performance for the protected cultivation method. These results agree with the findings by Foster et al. (2015) and Valiente et al. (2019), who identified the water footprint and the use of fertilisers as the main factors that contribute to the overall environmental impact of raspberry cultivation. Similarly, Vázquez-Ibarra et al. (2021) underscored the crucial role of irrigation systems, cultivation support, and coverings to the overall environmental impact.

3.1.1. Impact of rainfall

Since water is the most critical resource affecting the environmental performance, one environmental factor that could potentially improve the performance of open field cultivation is the rainfall. The previous scenarios have assumed that the open-field water requirements are fully covered by irrigation. This assumption is based on the fact that during the cultivation period of the open field system there is no significant rainfall and supplemental irrigation is necessary. Rainfall for the open field cultivation in the mountains occurs only between November and May, so the only months that can be affected are November and December. Even if we consider the extreme, where the entire water requirements for 2 months are satisfied by rainfall, the overall environmental footprint of open-field cultivation will drop to 6.4 mPt per kg of raspberry produced, which is still significantly higher compared to the environmental footprint of protected cultivation (3.5 mPt per kg of raspberry produced).

3.2. Economic analysis

Based on the price of the resources and utilities (Table 4) and the life cycle inventory (Table 2), the cost breakdown of the compared raspberry cultivation methods is presented in Table 6. The fact that certain resources need to be replaced after a certain time period is also considered. The results reveal that the life cycle cost of protected cultivation is 37.8 % lower compared to the open-field production (14.0 €/kg for the high-tunnel compared to 22.5 €/kg for the open-field). Even though the total annual expenses are similar for the type of cultivation (127,262 € for protected cultivation and 143,341 € for open-field), the

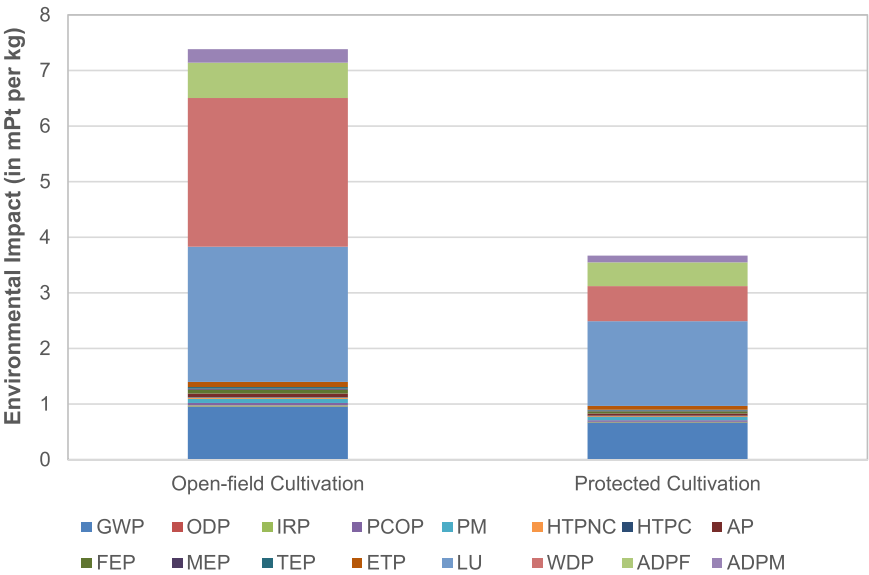


Fig. 4. Environmental impact assessment for the two cultivation methods, open-field and protected cultivation using high tunnels in Cyprus (expressed in mPt per kg of raspberry produced).

Table 5
Selected midpoint impact indicators for the two cultivation methods, open-field and protected cultivation using high tunnels in Cyprus (expressed in unit per kg of raspberry produced).

Indicator	Open-field cultivation	Protected cultivation	Unit
GWP	0.037	0.026	kg CO ₂ eq
LU	25.118	15.724	Pt
WDP	0.361	0.085	m ³ depriv.
ADPF	0.498	0.336	MJ

critical factor here is the improved yield and water use efficiency for protected cultivation.

4. Conclusions and further perspectives

Currently in Cyprus, there is a relatively small number of raspberry

and other small fruit growers, mostly located in the mountainous and more temperate parts of the island, even though the different meso-climates can be considered appropriate for year-round production. This is mainly due to the lack of knowledge and risk-adverse nature of farmers, as they are concerned about the costs of introducing new cultivars and cultivation methods. Additionally, concerns over economic losses due to environmental factors, including heat stress during the summer period or heavy rains during winter, are important factors influencing farmers choice to use the mountainous areas for production. Moreover, small-scale enterprises in the agricultural sector in Cyprus and worldwide lack specialised and scientific guidance to make evidence-based decisions that would enable them to flourish (Bechtsis et al., 2022; Kasimati et al., 2024).

The selection of plant material, planting period, and cultivation method between protected cultivation in poly tunnels and open-field depends on various factors like the local climate, the availability of human resources, and market demand. While open-field cultivation is

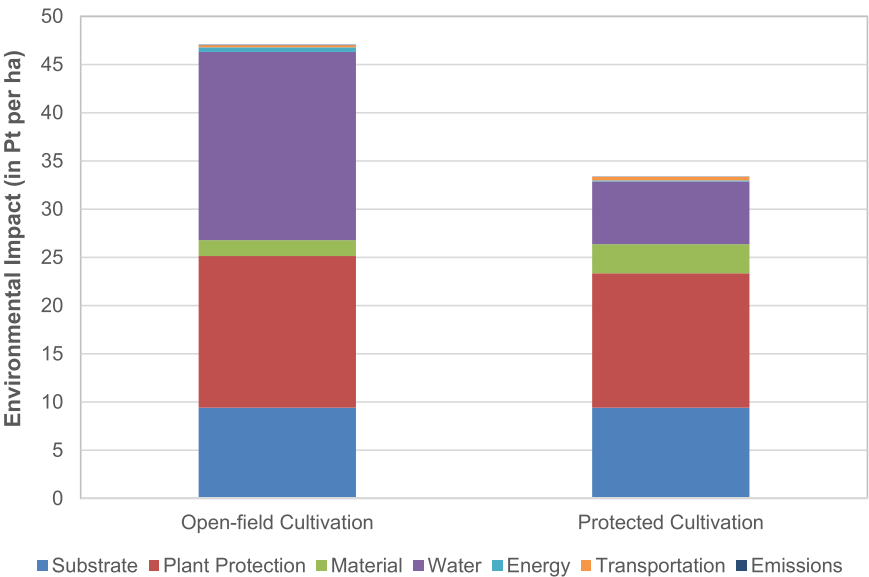


Fig. 5. Environmental hotspots for the two cultivation methods, open-field and protected cultivation using high tunnels in Cyprus (expressed in mPt per ha of cultivated area) for open-field and protected cultivation in high-tunnels in Cyprus.

Table 6

Overall cost analysis for open-field and protected raspberry cultivation methods using high tunnels in Cyprus.

Resource	Price		Change every	Open-field	Protected cultivation
Ground cover	8000	€/ha	5 year	1600	1600
High-tunnel cover	13,600	€/ha	5 year	0	2720
Nozzle/tubes	25,000	€/ha	5 year	5000	5000
Plants	2	€/unit	1 year	36,400	36,400
Pots	2.5	€/unit	5 year	9100	9100
Substrate	0.09	€/L	1 year	16,380	16,380
Electricity	0.2	€/kWh	1 year	3996	792
Water	0.15	€/m ³	1 year	7950	2650
Diesel	1.7	€/L	1 year	425	340
Pesticides	300	€/L	1 year	59,250	47,400
Fertilisers	0.50	€/kg	1 year	3240	2880
Labour	5	€/h	1 year	0	2000
Total Cost (€)				143,341	127,262
Yield (kg)				6370	9100
Cost per kg (€/kg)				22.5	14.0

believed to be suitable for a more concentrated, cost-efficient harvest, protected cultivation with high tunnels has the potential for higher yields, with improved water use efficiency, while extending the raspberry harvest season throughout the year which in turn may allow growers to sell their fruits to the market during the “off season” for a premium. The careful selection of cultivation methods holds the potential to mitigate the adverse impacts of climate change for small fruit cultivation in Cyprus while still maintaining on-farm profitability. By embracing innovative solutions, farmers may not only adapt to the changing environment but also secure their economic prosperity in the years to come.

Our analysis has revealed that complementing or replacing traditional open-field cultivation in the mountains with protected cultivation using plastic high tunnels on the plains will have a lower environmental impact while being more financially profitable for the farmer. More specifically, protected cultivation will have a 49.6 % lower total environmental footprint. Additionally, the yield in protected cultivation is 43 % higher than open-field cultivation resulting to greater profit margin for growers. More importantly, the amount of water used is 4-times less in protected cultivation, subsequently limiting the electricity costs of the production. This is a very critical point for Cyprus, since water usage is crucial for sustainable production, especially in areas with water scarcity issues (Dale, 2012; Jain and Janakiram, 2016).

In addition to higher yield efficiency, water use is more effective due to lower evapotranspiration losses. The raspberry cultivar Kwanza is cultivated globally with an observed yield of 1.5 to 2.5 kg per plant in a climate-controlled Dutch Glasshouse (Herckens et al., 2019), while a yield of 1.8 to 2.4 kg per plants has been observed under Californian Mediterranean Climate (Daugovish et al., 2021). The variations are due to the planting density, planting date and the local mesoclimate. The literature agrees with our findings that Kwanza is more suited to a protected environment, rather than open field, since it allows higher precision in climate and irrigation management, higher yield and less water usage.

The life cycle production cost for 1 kg of raspberries is 37.8 % lower for the protected cultivation compared to open field. Higher profitability is achieved from off-season as well as limited operational costs, despite the increased start-up and worker costs which can be depreciated and managed more efficiently. It must be noted though that the production cost per kg is still relatively high compared to other European countries where fresh raspberries are sold around 16 € per kg. The remote nature of the island of Cyprus, with no road interconnections to mainland, results to significantly higher resource and energy prices (on average 30 % higher than in Europe), as it can be observed in Table 5. This also means that imported raspberries, although they may arrive in Cyprus in bad shape, are still sold at higher prices compared to the rest of the Europe

(at approximately 21–22 € per kg). Additionally, the yield of each plant for the conventional cultivation techniques is relatively low, resulting in high operational labour cost to harvest. For example, harvesting efficiency in Europe is 4–6 € per hour per person while in Cyprus it is no more than 2 € per hour per person. Thus, the initial focus of the local farmers is to compete with the imported berries.

The local production of raspberries in protected cultivation using high tunnels in Cyprus (not only in the plains, but potentially in the mountains) can also have a lower environmental footprint, compared to imported fruits from other countries. Also, more heat-tolerant and short-cycle crops can be used as an alternative when raspberries are not being produced and buffer against plastic environmental footprint. Recycling of used tunnel plastic at the end of its useable life will also lessen the environmental impact of protected cultivation.

In terms of the limitations of our study, the analysis is performed on a cradle-to-gate basis (from the acquisition of nursery plants until the raspberries leave the gate of the field at the end of their productive lifespan), and the Life Cycle Inventory is populated using real data for the years 2022, 2023 and 2024, collected from two farms located on the island of Cyprus. One main limiting factor is that these values encompass data from only one primocane harvest cycle (8-month duration), which may not reflect conditions over a longer term due to year-to-year variations. A wider sample, either for multiple harvesting cycles, from multiple farms in Cyprus and abroad would be an appropriate next step to validate the results. Moreover, the use of data from questionnaires (sourced in their majority from utilities/suppliers' bills and smart meters on the field) may offer a very realistic and representative view of the problem but does not allow a complete understanding of the relationship between different factors and their potential further improvement of the situation. Thus, a model of the cultivation systems could be developed, relating irrigation, fertilisation, choice of substrate mixture in pots, and other pre-harvest factors on field activities with the expected raspberry yield, to enable us to analyse alternative scenarios.

CRedit authorship contribution statement

Angeliki Xyderou Malefaki: Writing – original draft, Visualization, Software, Resources, Investigation, Data curation. **Nicolas Valanides:** Writing – original draft, Investigation, Data curation, Conceptualization. **George A Manganaris:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Lisa Wasko DeVetter:** Writing – review & editing, Validation, Supervision. **Sofia Papadaki:** Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Magdalini Krokida:** Writing – review & editing, Supervision, Funding acquisition. **Antonia Vyrkou:** Writing – original draft, Visualization, Software, Resources, Methodology, Data curation. **Athanasios Angelis-Dimakis:** Writing – review & editing, Supervision, Software, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

- Bechtsis, D., Tsolakis, N., Iakovou, E., Vlachos, D., 2022. Data-driven secure, resilient and sustainable supply chains: gaps, opportunities, and a new generalised data sharing and data monetisation framework. *Int. J. Prod. Res.* 60 (14), 4397–4417. <https://doi.org/10.1080/00207543.2021.1957506>.
- Ben Hadj Daoud, H., Rosario Butera, M., Pedro Rosa Duarte, J., 2024. Perspective chapter: growing berries in substrate. *Intech. Open*. <https://doi.org/10.5772/intechopen.1008343>.
- Blanc, S., Accastello, C., Girgenti, V., Brun, F., Mosso, A., 2018. Innovative strategies for the raspberry supply chain: an environmental and economic assessment. *Calitatea* 19 (165), 139–142.
- Burton-Freeman, B.M., Sandhu, A.K., Edirisinghe, I., 2016. Red raspberries and their bioactive polyphenols: cardiometabolic and neuronal health links. *Adv. Nutr.* 7 (1), 44–65. <https://doi.org/10.3945/an.115.009639>.
- Christofi, C., Bruggeman, A., Kuells, C., Constantinou, C., 2020. Hydrochemical evolution of groundwater in the gabbro of the Troodos Fractured Aquifer. A comprehensive approach. *Appl. Geochem.* 114, 104524. <https://doi.org/10.1016/j.apgeochem.2020.104524>.
- Dale, A., 2012. Protected cultivation of raspberries. *Acta Hort.* 946, 349–354. <https://doi.org/10.17660/ActaHortic.2012.946.57>.
- Dale, A., Hanson, E.J., Yarborough, D.E., McNicol, R.J., Stang, E.J., Brennan, R., Morris, J.R., Hergert, G.B., 1994. Mechanical harvesting of berry crops. In: Janick, J. (Ed.), *Mechanical harvesting of berry crops*. *Hortic. Rev. (Am Soc Hortic Sci)* 255–282. <https://doi.org/10.1002/9780470650561.ch8>.
- Daugovish, O., Gaskell, M., Ahumada, M., Howell, A.D., 2021. Blackberry and raspberry cultivar evaluations in Coastal California. *Hort. Technol.* 31 (4), 552–558. <https://doi.org/10.21273/HORTTECH04843-21>.
- del Pozo, A., Brunel-Saldias, N., Engler, A., Ortega-Farías, S., Acevedo-Opazo, C., Lobos, G.A., Jara-Rojas, R., Molina-Montenegro, M.A., 2019. Climate change impacts and adaptation strategies of agriculture in Mediterranean-Climatic regions (MCRs). *Sustainability*, 11 (10), 2769. <https://doi.org/10.3390/su11102769> (2023). At a glance: Cyprus' Cap Strategic Plan, Available online at: <https://agriculture.ec.europa.eu/cap-my-country/cap-strategic-plans/cyprus-en>.
- Foster, T., Brozović, N., Butler, A.P., 2015. Analysis of the impacts of well yield and groundwater depth on irrigated agriculture. *J. Hydrol.* 523, 86–96. <https://doi.org/10.1016/j.jhydrol.2015.01.032>.
- Gluch, P., Baumann, H., 2004. The life cycle costing (LCC) approach: a conceptual discussion of its usefulness for environmental decision-making. *Build. Environ.* 39 (5), 571–580. <https://doi.org/10.1016/j.buildenv.2003.10.008>.
- Gruda, N., Bisbis, M., Tanny, J., 2019. Impacts of protected vegetable cultivation on climate change and adaptation strategies for cleaner production—a review. *J. Clean. Prod.* 225, 324–339. <https://doi.org/10.1016/j.jclepro.2019.03.295>.
- Hall, H.K., Hummer, K.E., Jamieson, A., Jennings, S., Weber, C., 2011. Raspberry breeding and genetics. Janick J. (Ed) *Plant Breeding Reviews*. <https://doi.org/10.1002/9780470593806.ch2>.
- Herckens, K., Boonen, M., Bylemans, D., 2019. Year-round production of the primocane raspberry 'Kwanza'. *Acta Hort.* 1265, 145–152. <https://doi.org/10.17660/ActaHortic.2019.1265.20>.
- IRO (International Raspberry Organization). 2024. International raspberry organization homepage. <https://www.internationalraspberry.net/>.
- ISO, 2006. 14040 International standard. Environmental management - life cycle assessment - principles and framework. International Organization for Standardization: Geneva, Switzerland.
- Janick, J., 2003. *Horticultural Reviews, Volume 29: Wild apple and fruit trees of central Asia*. John Wiley & Sons.
- Jain, R., Janakiram, T., 2016. Vertical gardening: a new concept of modern era. In: Patel, N.L., Chawla, S.L., Ahlawat, T.R. (Eds.), *Vertical gardening: a new concept of modern era*. *Commercial Horticulture* 527–536.
- Kailasam, S., Achanta, S.D.M., Rama Koteswara Rao, P., Vatambeti, R., Kayam, S., 2022. An IoT-based agriculture maintenance using pervasive computing with machine learning technique. *International Journal of Intelligent Computing and Cybernetics* 15 (2), 184–197. <https://doi.org/10.1108/IJICC-06-2021-0101>.
- Katsanos, D., Retalis, A., Michaelides, S., 2016. Validation of a high-resolution precipitation database (CHIRPS) over Cyprus for 30 years. *Atmos. Res.* 169, 459–464. <https://doi.org/10.1016/j.atmosres.2015.05.015>.
- Kasimati, A., Papadopoulos, G., Manstretta, V., Giannakopoulou, M., Adamides, G., Neocleous, D., Vassiliou, V., Savvides, S., Stylianou, A., 2024. Case studies on sustainability-oriented innovations and smart farming technologies in the wine industry: a comparative analysis of pilots in Cyprus and Italy. *Agronomy* 14 (4), 736. <https://doi.org/10.3390/agronomy14040736>.
- Kempler, C., Hall, H., Finn, C.E., 2012. Raspberry. In: Badenes, M.L., Byrne, D.H. (Eds.), *Fruit Breeding*. Springer, London, pp. 263–304.
- Lazoglou, G., Hadjinicolaou, P., Sofokleous, I., Bruggeman, A., Zittis, G., 2024. Climate change and extremes in the Mediterranean island of Cyprus: from historical trends to future projections. *Environ. Res. Comm.* 6 (9), 095020. <https://doi.org/10.1088/2515-7620/ad7927>.
- Manganaris, G.A., Goulas, V., Vicente, A.R., Terry, L.A., 2014. Berry antioxidants: small fruits providing large benefits. *J. Sci. Food Agric.* 94, 825–833. <https://doi.org/10.1002/jsfa.6432>.
- Manganaris, G.A., Valanides, N., Gohari, R., Milivojevic, J., DeVetter, L.W., Fotopoulos, V., 2024. Exploring the potential of priming agents towards enhanced performance of *Rubus* species. *Acta Horticulturae*. <https://doi.org/10.17660/ActaHortic.2024.1388.2>.
- Malghani, A.L., Malik, A.U., Sattar, A., Hussain, F., Abbas, G., Hussain, J., 2010. Response of growth and yield of wheat to NPK fertilizer. *Sci. Int* 24 (2), 185–189.
- Michaelides, S.C., Tymvios, F.S., Michaelidou, T., 2009. Spatial and temporal characteristics of the annual rainfall frequency distribution in Cyprus. *Atmos. Res.* 94 (4), 606–615. <https://doi.org/10.1016/j.atmosres.2009.04.008>.
- Mohamad, R.S., Verrastro, V., Cardone, G., Bteich, M.R., Favia, M., Moretti, M., Roma, R., 2014. Optimisation of organic and conventional olive agricultural practices from a life cycle assessment and life cycle costing perspectives. *J. Clean Prod.* 70, 78–89. <https://doi.org/10.1016/j.jclepro.2014.02.033>.
- Palonen, P., Pinomaa, A., Tommila, T., 2017. The influence of high tunnel on yield and berry quality in three florican raspberry cultivars. *Sci. Hort.* 214, 180–186. <https://doi.org/10.1016/j.scienta.2016.11.049>.
- Peel, M.C., Finlayson, B.L., McMahon, T.A., 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* 11 (5), 1633–1644. <https://doi.org/10.5194/hess-11-1633-2007>.
- Pergola, M., Persiani, A., Pastore, V., Palese, A.M., Arous, A., Celano, G., 2017. A comprehensive Life cycle assessment (LCA) of three apricot orchard systems located in Metapontino area (Southern Italy). *J. Clean. Prod.* 142, 4059–4071. <https://doi.org/10.1016/j.jclepro.2016.10.030>.
- Ponder, A., Hallmann, E., 2019. The effects of organic and conventional farm management and harvest time on the polyphenol content in different raspberry cultivars. *Food Chem.* 301, 125295. <https://doi.org/10.1016/j.foodchem.2019.125295>.
- Ragaveena, S., Shirley Edward, A., Surendran, U., 2021. Smart controlled environment agriculture methods: a holistic review. *Reviews in Environmental Science and Bio/Technology* 20 (4), 887–913. <https://doi.org/10.1007/s11157-021-09591-Z>.
- Reisch, L., Eberle, U., Lorek, S., 2013. Sustainable food consumption: an overview of contemporary issues and policies. *Sustain.: Sci. Practice Policy* 9 (2), 7–25. <https://doi.org/10.1080/15487733.2013.11908111>.
- RoC (Republic of Cyprus), 2021, "Republic of Cyprus second voluntary national report - sustainable development goals", ISBN 978-9963-50-541-8 Available online at: https://publications.gov.cy/en/assets/user/publications/2021/2021_116/2021_116.pdf.
- Sangiorgio, D., Cellini, A., Spinelli, F., Farneti, B., Khomenko, I., Muzzi, E., Savioli, S., Pastore, C., Rodriguez-Estrada, M.T., Donati, I., 2021. Does organic farming increase raspberry quality, aroma and beneficial bacterial biodiversity? *Microorganisms* 9 (8), p.1617. <https://doi.org/10.3390/microorganisms9081617>.
- Song, K., Qin, Q., Yang, Y., Sun, L., Sun, Y., Zheng, X., Lu, W., Xue, Y., 2023. Drip fertigation and plant hedgerows significantly reduce nitrogen and phosphorus losses and maintain high fruit yields in intensive orchards. *J. Integr. Agric.* 22 (2), 598–610. <https://doi.org/10.1016/j.jia.2022.08.008>.
- Vásquez-Ibarra, L., Iriarte, A., Rebolledo-Leiva, R., Vásquez, M., Angulo-Meza, L., González-Araya, M.C., 2021. Considering the influence of the variability in management practices on the environmental impacts of fruit production: a case study on raspberry production in Chile. *J. Clean. Prod.* 313, 127609. <https://doi.org/10.1016/j.jclepro.2021.127609>.
- Valiante, D., Sirtori, I., Cossa, S., Corengia, L., Pedretti, M., Cavallaro, L., Vignoli, L., Galvagni, A., Gomarasca, S., Pesce, G.R., Boccadelli, A., 2019. Environmental impact of strawberry production in Italy and Switzerland with different cultivation practices. *Sci. Total Environ.* 664, 249–261. <https://doi.org/10.1016/j.scitotenv.2019.02.046>.
- Vinyes, E., Gasol, C.M., Asin, L., Alegre, S., Muñoz, P., 2015. Life Cycle assessment of multiyear peach production. *J. Clean. Prod.* 104, 68–79. <https://doi.org/10.1016/j.jclepro.2015.05.041>.
- Zittis, G., Bruggeman, A., Camera, C., 2020. 21st Century projections of extreme precipitation indicators for Cyprus. *Atmosphere (Basel)* 11 (4), p.343. <https://doi.org/10.3390/atmos11040343>.

The postharvest application of biodegradable polymers and a priming agent as a potential tool to enhance phytochemical content, aroma profile and market life of strawberry fruit

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Cold storage

ABSTRACT

Strawberry (*Fragaria x ananassa* Duch.) is a highly perishable crop with limited market life. The aim of our work was to dissect the efficacy of an array of molecules with potential priming effect on postharvest performance, antioxidant potential and aroma profile of strawberry fruit. Freshly harvested fruit (cv. 'Savana') of uniform size and ripening stage (commercial ripeness >80 % of the surface red color) were subjected to the following postharvest dip treatments: control (untreated), hydro-primed, NOSH-A, chitosan (CTS) and sodium alginate (NaA). NOSH-A is a proprietary priming agent that acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently. CTS is a biobased, biologically safe and biodegradable polymer that has been exploited as a nanocarrier to efficiently deliver an array of compounds, while NaA is another biodegradable polymer applied in smart nano-delivery systems. Treated fruit were subjected to 4, 8 and 12 d of cold storage (CS, 4 °C, 90 % RH) and additional maintenance at room temperature for 1 d to simulate short, medium and extended refrigerated storage, respectively. Quality attributes and fungal incidence and severity were determined, without any striking differences among the treatments applied. Polyphenolic compounds analysis by HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical compounds such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, and catechin, particularly after 8 days CS compared to freshly harvested fruit. Such changes were more evident when the priming agent NOSH-A was applied, being more pronounced in the case of pedunculagin 2 isomer that registered a significant increment. HS-SPME-GC analysis identified 140 unique volatile organic compounds (VOCs) with chitosan-treated strawberries showing the most distinct VOC profile after extended cold storage with higher contents of methyl hexanoate. Results reported herein shed light in the efficacy of an array of agents on parameters linked to secondary metabolism of strawberry fruit at postharvest level.

1. Introduction

Strawberry (*Fragaria x ananassa* Duch.) is a delicate fruit crop with a considerable growth in terms of production volumes, mainly attributed

to its appealing appearance and high nutritional profile and phytochemical content (El-Mogy et al., 2019; Manganaris et al., 2014). However, strawberry fruit suffers from limited storage potential leading to spoilage and reduced marketability (Sun et al., 2022). Noteworthy,

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postharvest losses can reach up to 50 %, exacerbated by fungal diseases and sensitivity to mechanical damage, and thus significantly impacting the economic returns for producers (Moghadas et al., 2025). Towards preservation of strawberry quality and nutritional value after harvest, an array of postharvest strategies including compounds with hormonal activity (i.e. salicylic acid, abscisic acid, methyl jasmonate) has been also tested (Darwish et al., 2021).

Coatings have been reported as cost-effective and environmentally friendly solutions towards preservation of fruit quality (Adiletta et al., 2021; Guimarães et al., 2018); they create a continuous, thin layer of edible substances, such as polysaccharides, lipids and proteins used alone or in blends, on the fruit surface by spraying or dipping them and obtaining single or double layers (Suhag et al., 2020). Such coatings are usually composed of biodegradable and biocompatible materials that are "generally recognized as safe" (GRAS) or recognized as food additives by the Food and Drug Administration (FDA) or the European Union (Ncama et al., 2018). Coatings form a physical protective barrier between the fruit and the environment (Jongsri et al., 2016) and alter respiration and transpiration, thus slowing down the ripening and/or softening process (Maringgal et al., 2020). At the same time, coatings may contribute to the protection against mechanical damages, microbiological infestations, tissue softening and enzymatic browning, with special reference to fresh-cut commodities (Oms-Oliu et al., 2010).

The most commonly used polysaccharides are cellulose derivatives, alginates, starches, pectin, pullulan, carrageenan and chitosan. Among them, chitosan (CTS)-based coatings are considered to be the best ECs, with non-toxic, biodegradability, and antimicrobial actions and with a wide use in several horticultural commodities (Petriccione et al., 2014). CTS is a deacetylated derivative of chitin, the second most abundant renewable biopolymer in nature; it is a linear polysaccharide composed of β -(1,4)-linked glucosamine units (2-amino-2-deoxy- β -D-glucopyranose) together with some quantities of N-acetylglucosamine units (2-acetamino-2-deoxy- β -D-glucopyranose) and is present in green algae, the exoskeletons of arthropods and the cell walls of fungi and yeasts (De Queiroz Antonino et al., 2017).

Alginate (Alg) is also a linear copolymer, commonly produced by seaweeds (brown algae) and is structured by (1 $\rightarrow$ 4)-linked- α -L-guluronate and (1 $\rightarrow$ 4)-linked- β -D-mannuronate moieties (Nair et al., 2020), widely used as an edible coating for the preservation of fruits and vegetables (Gohari et al., 2024). It is known as a hydrophilic biopolymer capable of forming transparent, uniform, water soluble and high-quality films (Mahcene et al., 2020). Sodium alginate (NaA) is the most common salt of alginate, with excellent colloidal properties, characterised by high degree of reactivity with many metal cations that leads to the formation of gels or insoluble polymers (Jiang et al., 2013).

Biodegradable polymers are extensively being tested as a sustainable, alternative to traditional non-biodegradable materials, postharvest treatment on strawberry fruits, showing promising results in maintaining the freshness extending the shelf-life (Bahmani et al., 2024). The nanoencapsulation-based coatings showed promising results in extending the shelf life of fresh produce (Neethirajan & Jayas, 2011). A nano-coating can contain bioactive compounds in the form of nanoparticles, that due to their improved mechanical properties, have greater chemical reactivity and more bioactivity than conventional particles (Neethirajan & Jayas, 2011). In fact, positive effects on shelf-life extension and/or quality maintenance, after the application of CTS nano-coatings enriched with nanomaterials, essential oils, or antimicrobial agents, have been reported in a considerable number of studies dealing with strawberry (Huang et al., 2022; Lin et al., 2020; Nguyen et al., 2020; Robledo et al., 2018). The use of alginate nano-coatings have also shown a positive effect on strawberry (Dhital et al., 2018; Emamifar & Bavaisi, 2020; Liu et al., 2021). However, each polymer type has unique mechanisms and benefits, making them suitable for various applications in the food industry. The selection of polymer depends on the cultivar properties and scope in terms of storage potential (i.e. destined for exportation).

The priming agents have received considerable attention over the recent years in order to ameliorate plant response under adverse conditions with however limited information available when they are directly applied in the fruit. The exploitation of non-toxic synthetic and natural priming agents towards sustainably-sourced and environmentally sound products has recently received considerable attention. In the current study we aimed to dissect the efficacy of a novel priming agent (NOSH-A) at postharvest level. NOSH-A acts as a donor that releases nitric oxide (NO), hydrogen sulfide (H₂S), and aspirin (acetylsalicylic acid) concurrently; it was initially formulated as an anticancer drug but it also displays protective effects against abiotic stress conditions in plants (Antoniou et al. 2020; Gohari et al. 2024). The simultaneous donation of multiple signal/hormonal molecules renders it an attractive candidate of multifunctional priming, whereby multiple benefits can be achieved through the synergistic activity of different agents. However, no data on its use to combat postharvest abiotic conditions as cold storage have been reported. To this end, our study aimed to evaluate the effect of postharvest dip treatments with either NOSH-A along with two biodegradable polymers (chitosan and sodium alginate) on quality attributes, volatilome fingerprinting and phytochemical properties of strawberry fruit.

2. Materials and methods

2.1. Fruit material and treatment application

Strawberry fruit of cultivar 'Savana' was used for the needs of the current study. 'Savana' is a rustic and highly productive strawberry variety that allows early production that can be marketed for a premium, covering 'low supply' periods. Fruits of uniform size and ripening stage (commercial ripeness >80 % of the surface red color and without any softness symptoms), were hand-harvested and immediately transferred to the laboratory. After removal of defective fruit, they were separated into 15 lots of 60 fruit each. Each three lots were subjected to immersion with the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A (50 μ mol L⁻¹), (4) chitosan (CTS, 0.1 g 100 mL⁻¹), (5) sodium alginate, (NaA, 0.1 g 100 mL⁻¹). In all cases, solutions were freshly prepared and 0.1 mL 100 mL⁻¹ Tween20 was added as surfactant. Fruit were immersed in the treatment solutions for 10 min, then kept for drying for 30 min at room temperature and subsequently transferred to cold storage (4 °C, 90 % RH) with each lot being kept in a covered punnet. Fruit were maintained at cold storage for 4, 8 and 12 d, respectively and were analyzed after additional maintenance at room temperature (20 °C) for an additional day (4 + 1, 8 + 1, 12 + 1). For biological/enzymatic analyses, the fruit were immediately flash frozen in liquid nitrogen, and stored at -80 °C until needed. For the determination of polyphenolic compound analysis, samples were freeze-dried (Freeze Dryer-Christ Alpha 1-4 LD plus).

2.2. Quality attributes

Fruit weight was measured using 20 fruit per treatment and storage condition applied and accordingly weight loss was determined. Soluble solid content (SSC) and titratable acidity (TA) were measured in fruit juice isolated using a professional juicer. SSC was quantified with a refractometer (Atago, PR-32 α , Japan) and results expressed as °Brix. TA was determined with the use of an automatic multiple positions titrator (862 Compact Titrator, Metrohm AG, Switzerland). Briefly for each measurement, 5 mL of diluted juice in 45 mL distilled H₂O was used for titrating 0.1 mol L⁻¹ NaOH to a pH end point of 8.1. Results were expressed as g citric acid 100 mL⁻¹. Ripening index (RI) was calculated as the SSC/TA ratio. Strawberry samples (0.3 g) were extracted with 10 mL of 80 % v/v ethanol and sugars [total soluble sugars (TSS), sucrose, glucose, and fructose] were determined spectrophotometrically as described elsewhere (Hadjipieri et al., 2020).

2.3. Aroma profile

The volatilome fingerprinting of juiced strawberry samples using HS-SPME-GC-MS was adapted from Vandendriessche et al. (2013). In short, fruit were cut and blended with 1.0 M NaCl (0.5 mL: 1 g), snap-frozen in liquid N₂ and stored at -80 °C until needed. After overnight thawing at 4 °C, 5 g of the juice mixture was transferred into a 20 mL headspace (HS) vial (Filter Service, Belgium). Prior to solid phase micro extraction (SPME), the samples were incubated for 35 min at 40 °C on a heated tray to populate the headspace with VOCs. The volatiles were extracted by exposing an SPME fiber (DVB/CAR/PDMS, 50/30 mm film thickness; Supelco Inc., USA) to the headspace for 30 min at 40 °C. After extraction, aroma compounds were thermally desorbed into the injector set to 250 °C and equipped with an SPME liner (0.75 i.d., Supelco Inc., USA). Separation was conducted on a 30 m × 250 µm × 0.250 µm HP-5MS column (Agilent Technologies), using helium as carrier gas. The data were evaluated using MassHunter Workstation (Unknowns Analysis v10.1, Agilent Technologies) and the volatile compounds listed in Supplementary Table 1 were identified with the NIST 2020 database (NIST20, USA). Analyses were performed on three biological replicates per treatment.

2.4. Phytochemical analysis

Phenolic compounds were extracted following the procedure of Shehata et al. (2020) and spectrophotometrically determined at 765 nm. Analysis were conducted in triplicate and the results were expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ of fresh weight (FW).

Total anthocyanin content was extracted from the samples following the procedure of Bal and Ürün (2020) and its concentration was calculated as pelargonidin equivalents and expressed on a fresh weight base as mg 100 g⁻¹. Total flavonoid content was estimated from the samples following the procedure of Meyers et al. (2003) and results were expressed on a fresh weight base as mg 100 g⁻¹ quercetin equivalents. Ascorbic acid (AsA) assay was performed as described by Georgiadou et al. (2018) and results expressed as mg 100 g⁻¹ FW.

The polyphenolic compound analysis by HPLC-DAD-ESI-MS/MS was performed according to Salazar-Orbea et al. (2022). One hundred mg of lyophilized samples were extracted with 1 mL of methanol/water/acetic acid (70:29:1, v/v/v). The samples were homogenized in a vortex for 1 min and then sonicated for 30 min at room temperature. Samples were then centrifuged for 15 min at 20000 g at 10 °C (Thermo Scientific™ Sorvall™ ST 16, Germany). The supernatant was filtered through a 0.22 µm PVDF filter and analyzed by triplicate. Phenolics identification and quantification were carried out on an Agilent 1100 HPLC system equipped with a photodiode array detector (G1315D) and coupled in series to an HCT Ultra Bruker Daltonics ion trap mass spectrometer through an electrospray ionization (ESI) interface HPLC-DAD-ESI-MS/MS. The chromatographic separation was performed using a Poroshell 120 EC column (3 × 100 mm, 2.7 µm) from Agilent Technologies (Waldbronn, Germany). Phenolic compounds were identified by their UV spectra, retention time, molecular weight, and MS/MS fragmentation pattern. Phenolic compounds quantification was performed using the authentic standards of castalagin (280 nm), catechin (280 nm) *p*-coumaric acid (320 nm), pelargonidin (520 nm), ellagic acid (360 nm) and quercetin (360 nm) to quantify ellagitannins, flavan-3-ols, hydroxycinnamic acids, anthocyanins, ellagic acid conjugates and flavonols respectively.

2.5. Polyamine content

The levels of free putrescine (Put), spermidine (Spd) and spermine (Spm) were determined by high-performance liquid chromatography (HPLC) separation of dansyl derivatives, as analytically described in Marcé et al. (1995). Analyses were performed on three biological replicates per treatment.

2.6. Polyphenol oxidase (PPO) activity

PPO extraction was based on the methodology described in Alegria et al. (2016) with slight modifications. In brief, PPO was extracted from strawberry tissues (0.4 g) adding 1.5 mL of cold phosphate buffer (0.1 mol L⁻¹, pH 6.5) and 0.04 g of polyvinylpyrrolidone. Next, samples were vortexed for 1 min and centrifuged at 20.000×g for 30 min at 4 °C. During the entire process, samples were kept in an ice-bath to prevent protein denaturation. PPO activity was assayed spectrophotometrically measuring the catechol oxidation rate at 420 nm for 1 min (TECAN, Infinite 200® PRO). The reaction mixture was adapted to 96-well microplate with 10 µL of enzymatic extract and 290 µL of catechol (0.05 M). Results were expressed as U mg⁻¹ fresh weight (FW).

2.7. Cellular damage indicators

Malondialdehyde (MDA) content resulting from the thiobarbituric acid (TBA) reaction was estimated to determine lipid peroxidation (Filippou et al., 2011). The MDA content was measured at 532 and 600 nm and was estimated using the Lambert-Beer law, with extinction coefficient of 155 mmol⁻¹ L cm⁻¹ and expressed as nmol g⁻¹ fresh weight (FW).

Hydrogen peroxide (H₂O₂) content was calculated spectrophotometrically based on the oxidation of iodide (I⁻) to iodine (I), after its reaction with potassium iodide (KI), using the procedure described by Loreto and Velikova (2001). The content of H₂O₂ was measured at 390 nm and was estimated based on a standard curve of known concentrations of H₂O₂ (µmol g⁻¹ FW).

2.8. Severity index and fungal incidence of strawberry fruit

Fungal incidence due to *Bortyris cinerea* infection was determined by the percentage of strawberries exhibiting visible signs of fungal contamination relative to the total number (n = 24) of fruit in each treatment after 12 d of cold storage and additional maintenance at room temperature for 1 d and 4 d, respectively. The infection severity was determined by a diagrammatic scale (Fig. 1) that was developed in accordance with a relevant study by Filippi et al. (2021) with scores from 0 (no visible infection) to 7 (rotten). The scores from all fruit in each treatment were summed to generate the cumulative severity index. Higher cumulative scores reflected to more severe fungal contamination across the sample set.

2.9. Statistical analysis

Statistical analysis was carried out for quality attributes using the software package SPSS v25.0 (SPSS Inc., Chicago, IL, United States). The comparison of averages of each treatment was carried out using One-way Anova analysis followed by Duncan's multiple range test at significance level 5 % ($p \leq 0.05$). For biochemical and phytochemical analysis, both statistical analysis (one-way ANOVA analysis was conducted including Tukey's-HSD post hoc test ($p \leq 0.05$) and figures were conducted using GraphPad version 10.4.1 (GraphPad Software, San Diego, CA, USA).

For the VOC-data, multivariate statistical analysis, PCA, PLS and variable selection using Jack-knifing, was conducted using the Unscrambler X (CAMO, Norway). Specific VOCs one-way ANOVA analysis was conducted including Tukey's-HSD post hoc test ($p \leq 0.05$) using JMP-Pro (v17, SAS Institute Inc., Cary, NC).

With reference to severity index of fungal diseases, a non-parametric test (Kruskal-Wallis; $p \leq 0.05$) was performed to assess differences in infection severity among the treatment groups followed by pairwise Mann-Whitney *U* test ($p \leq 0.05$) comparing each treatment against the untreated group.

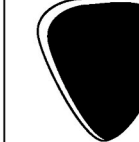
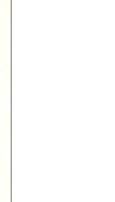
0 - 0.5	1	2	3	4	5	6	7
							
							
no infection / infection only in the stem (0%)	5%	10%	20%	40%	60%	80%	Rotten (100%)

Fig. 1. Diagrammatic scale for the severity of infection (0–7) of fungal spoilage in strawberry fruits and the corresponding percentage (%) of coverage. The scale was developed based on a relevant study by [Filippi et al. \(2021\)](#).

3. Results and discussion

3.1. Quality attributes

A gradual increase of weight loss in all treatments applied, being higher than the threshold of 5 % after 6–8 d of cold storage, was monitored. The treatment of strawberries with water (hydroprimed) prior to storage showed a negative effect compared with control, while none of the agents applied showed any striking difference in terms of controlling weight losses ([Supplementary Figure 1](#)). Weight loss is a key parameter that defines both quantitative and qualitative (i.e. shrivelling) losses of strawberry fruit during cold storage. As a non-climacteric fruit, strawberries must be harvested at the fully mature stage to obtain the best visual and nutritional quality, resulting in high fruit losses during refrigerated storage. Chitosan-based coatings have been reported to prolong the shelf-life of several fruits and fresh-cut commodities ([Adiletta et al., 2021](#)), including strawberries ([Robledo et al., 2018](#)) by slowing down the weight loss and at the same time safeguarding vitamin

content (i.e. ascorbic acid) and antioxidant capacity during cold storage ([Pagliarulo et al., 2016](#)). Results reported herein showed that weight losses were not reduced when the agents tested were applied. Among the formulations examined, a reduction in weight loss in CTS-treated strawberries as compared with NOSH-A- and NaA-treated fruits was registered. Higher losses in hydroprimed versus untreated strawberries was also monitored, indicating a negative effect due to immersion.

Harvested strawberries had an average SSC content of 8.3 % that is considered well above the threshold that defines a strawberry as of high quality in terms of taste ([Table 1](#)). The SSC contents after removal from cold storage and additional maintenance at room temperature for 1 day was in the range 7.6–8.6 % for all storage treatments and durations applied. Cold storage generally leads to a decrease in the soluble solids content of strawberries, although the extent of this decrease can vary based on factors such as cultivar, storage conditions, and specific treatments applied. In the current study, lower values after 12 d CS were not statistically significant. On the other hand, titratable acidity showed some alterations among different storage treatments, yet no specific

Table 1

Effect of storage duration^a and postharvest treatment^b applied on the soluble solids content (SSC), titratable acidity (TA), and ripening index (RI=SSC/TA) of strawberry fruits.

Storage	SSC (°Brix) (%)				
	Untreated	Hydroprimed	NOSH-A	CTS	NaA
Harvest	8.33 ± 0.11				
4 d CL + 1 d SL	8.10 ± 0.23 a	8.43 ± 0.35 a	8.57 ± 0.42 a	8.25 ± 0.33 a	8.37 ± 0.20 a
8 d CL + 1 d SL	8.50 ± 0.06 a	8.22 ± 0.12 ab	7.98 ± 0.21 b	8.08 ± 0.16 ab	8.43 ± 0.15 ab
12 d CL + 1 d SL	8.37 ± 0.29 a	7.85 ± 0.20 a	7.98 ± 0.19 a	7.58 ± 0.32 a	7.60 ± 0.26 a
Storage	TA (g citric acid 100 mL ⁻¹)				
	Untreated	Hydroprimed	NOSH-A	CTS	NaA
Harvest	0.97 ± 0.03				
4 d CL + 1 d SL	0.98 ± 0.07 ab	1.12 ± 0.07 a	0.80 ± 0.07 b	1.05 ± 0.07 a	0.99 ± 0.06 ab
8 d CL + 1 d SL	0.94 ± 0.04 a	0.93 ± 0.09 a	0.98 ± 0.03 a	0.90 ± 0.02 a	0.99 ± 0.05 a
12 d CL + 1 d SL	0.82 ± 0.02 a	1.05 ± 0.13 a	0.92 ± 0.10 a	0.99 ± 0.04 a	0.96 ± 0.05 a
Storage	RI (SSC/TA) (%)				
	Untreated	Hydroprimed	NOSH-A	CTS	NaA
Harvest	8.60 ± 0.21				
4 d CL + 1 d SL	8.30 ± 0.58 b	7.55 ± 0.51 b	10.84 ± 0.50 a	7.88 ± 0.51 b	8.51 ± 0.66 b
8 d CL + 1 d SL	9.04 ± 0.38 a	9.00 ± 0.83 a	8.18 ± 0.37 a	8.97 ± 0.07 a	8.54 ± 0.37 a
12 d CL + 1 d SL	10.21 ± 0.55 a	7.74 ± 0.95 a	8.81 ± 0.79 a	7.75 ± 0.63 a	8.02 ± 0.73 a

^a Cold storage (4 °C, 90 % RH) duration was 4, 8 and 12 d followed by an additional day maintenance at room temperature for (20 °C, 90 % RH).

^b Fruits were subjected to the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A (50 µmol L⁻¹ µM), (4) chitosan (CTS, 0.1 g 100 mL⁻¹), (5) sodium alginate, (NaA, 0.1 g 100 mL⁻¹). Values within each row (day of shelf-life) followed by the same letter are not statistically significant according to Duncan's multiple range test at significance level $P \leq 0.05$.

pattern was recorded. Elevated acidity levels were monitored for the treatments after 12 d cold storage plus one day shelf-life resulting in higher ripening index for untreated fruit, potentially due to an advanced senescencing processes. In general, coatings have been reported to enhance quality attributes and sensorial properties (Maringgal et al., 2020), yet this cannot be validated by the results reported herein.

The contents of sucrose, glucose and fructose, as well as the total soluble sugars were additionally determined; in line with the SSC results, no statistically significant differences were observed among storage treatments and durations applied (Fig. 2). Glucose is the predominant sugar in strawberries, often found in higher concentrations than fructose and sucrose (Basson et al., 2010). Although fructose is a major sugar in strawberries, it is generally less prevalent than glucose

(Lee et al., 2018). However, in the current study, fructose registered the highest contents as also elsewhere indicated (Simkova et al., 2024). Therefore, sugar contents appear to be a cultivar specific characteristic, while our data confirm that sucrose is present in strawberries in lower concentrations.

3.2. Volatile organic compounds

Using HS-SPME-GC-MS, 140 unique volatile organic compounds (VOCs) were identified among samples from the various treatments and storage durations (Supplementary Table 1). The compounds listed are in line with what has been observed by other groups (reviewed in Ulrich et al., 2018). While all fruit were harvested at a fully red ripe stage, over

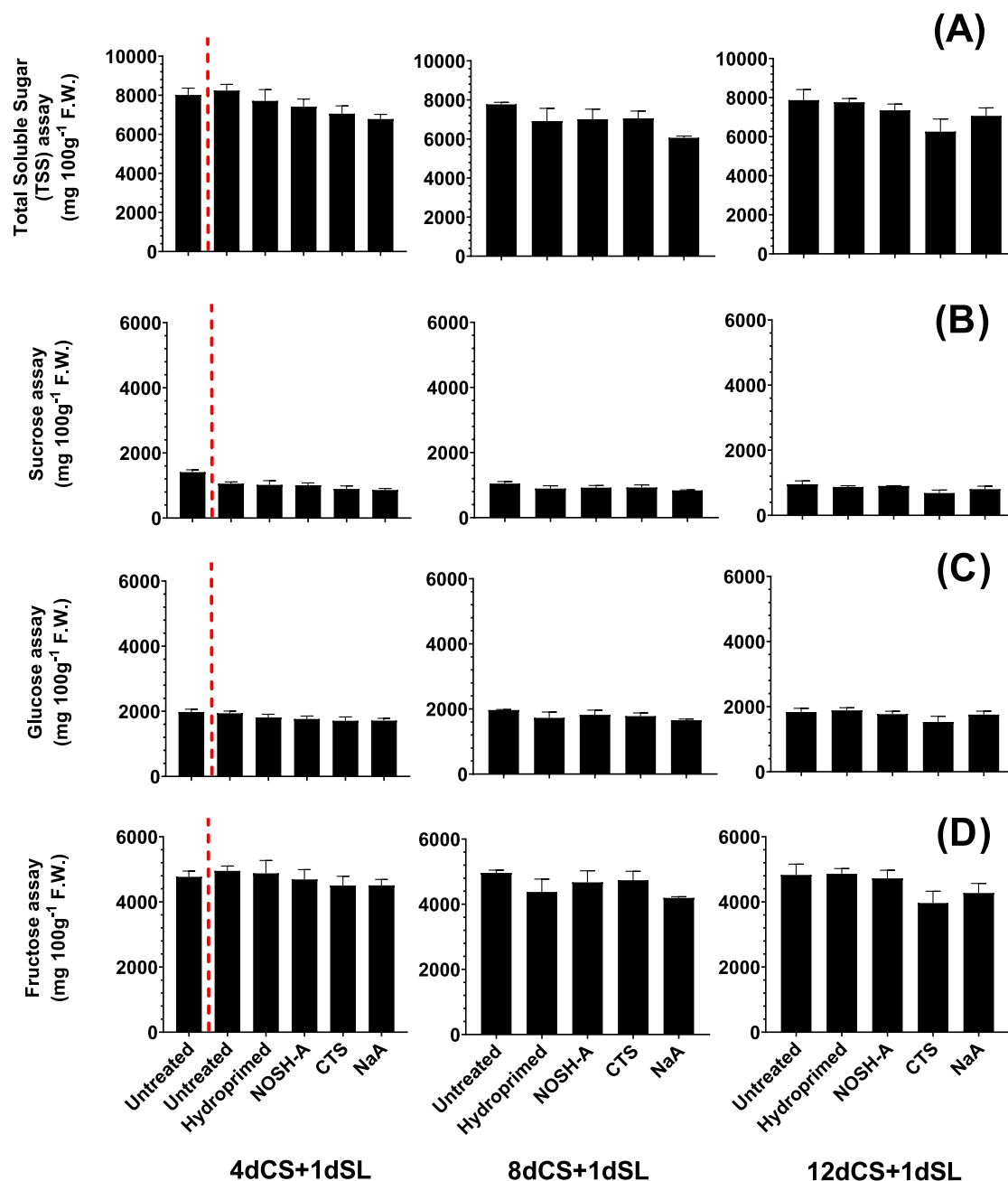


Fig. 2. Effect of storage duration and postharvest treatment applied on the contents of total soluble solids and individual sugars (sucrose, glucose, fructose) in strawberry fruits (ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.) Fruits were subjected to the following postharvest treatments: (1) control (untreated), (2) hydro-primed, (3) NOSH-A (50 50 $\mu\text{mol L}^{-1}$), (4) chitosan (CTS, 0.1 g 100 mL⁻¹), (5) sodium alginate, (NaA, 0.1 g 100 mL⁻¹). Storage duration was 4, 8 and 12 days at 4 °C, 90 % RH) and maintenance for an additional day at room temperature (20 °C).

time the VOC profile still changed as can be observed from the principal component analysis (PCA). Score plot of PCA visualising the shift in VOC composition of the fruit's aroma profiles within the first to principal components summarised 43 % of the VOC variation (Fig. 3A). The scoreplot showed that the untreated fruit presented the largest changes with time as represented by their arrow trajectories. Fruit treated with CTS remained closest to their starting position indicating their VOC profiles changed the least. The hydro-, NOSH-A and NaA-primed fruit took intermediate positions, showing large overlap between their replicate samples. In spite of these differences large similarities are still present. Using a PLS on time, a total of 47 volatiles were identified as being statistically significant contributors to explain time related

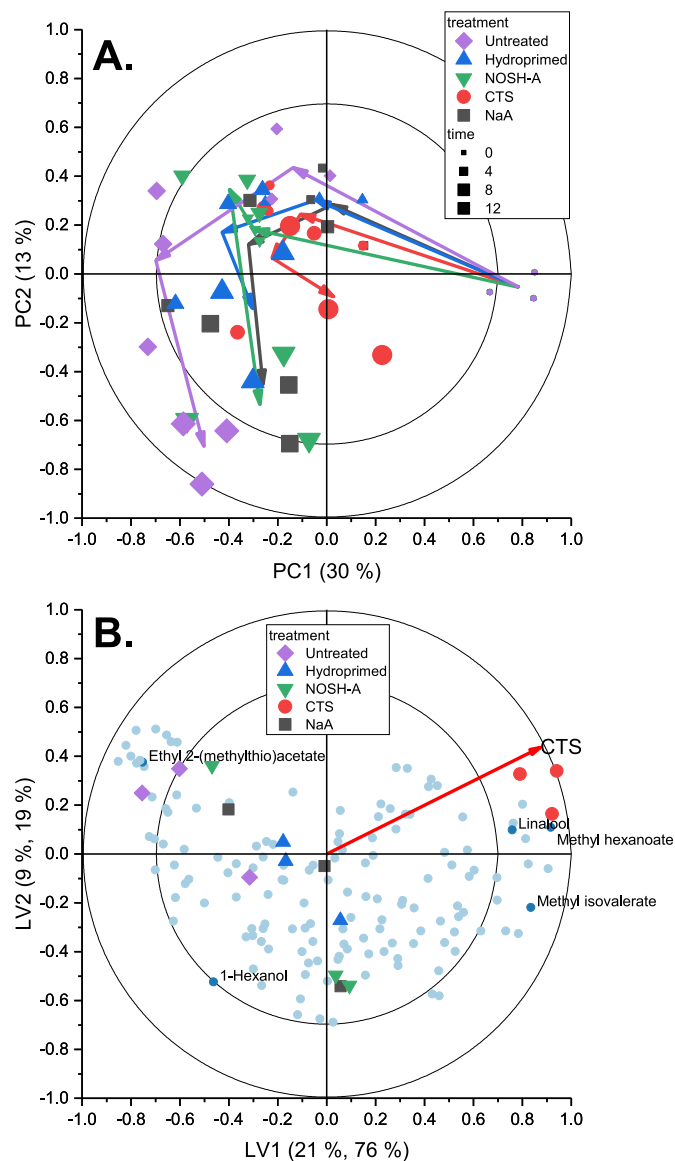


Fig. 3. Multivariate analysis of the VOC patterns of primed strawberry fruit. (A) Score plot of principal component analysis (PCA) visualising the shift in VOC composition of the fruit's aroma profiles. (B) Biplot of a partial least square discriminant analysis (PLS-DA) applied to the data after 12 days cold storage and additional maintenance at room temperature for 1 day to discriminate CTS-treated fruit from the other treatments. The large symbols represent the scores of the triplicate objects per treatment. The small symbols represent the X-loadings of the individual VOCs with the labelled VOCs being the five selected significant contributors to this PLS-DA model. The arrow represents the Y-loading indicating the direction of CTS treated fruit.

changes in common between all treatments (Supplementary Table 1) with a crossvalidation R^2 of 92 % (data not shown).

Based on the scoreplot from Fig. 3A, the largest differences in the VOC profile can be expected at the end of cold storage (12 d) plus 1 day SL. Therefore, a preliminary PLS-DA regression analysis on the 12 + 1 d data only, revealed that CTS-treated fruit was indeed most different from the other treatments which, amongst them, could not be well separated. Therefore, a final PLS-DA was performed contrasting, at 12 + 1 d, CTS treated fruit against all other treatments (Fig. 3B). This PLS-DA calibration model, based on the first two latent variables (LV), used 30 % of the variation in VOCs (21 % + 9 %) to discriminate with 95 % accuracy CTS treated fruit from the other treatments (76 % + 19 %) and resulted in the selection of five VOCs significantly contributing to this PLS-DA model. In particular, methyl hexanoate (CAS 106-70-7), methyl isovalerate (CAS 556-24-1) and linalool (CAS 78-70-6) were positively correlated to CTS-treated fruit, while ethyl 2-(methylthio)acetate (CAS 4455-13-4) and 1-hexanol (CAS 111-27-3) were negatively correlated. By using only these five VOC's, a stable PLS-DA model was obtained giving a crossvalidation R^2 of 66 % (data not shown). While a good separation could be obtained based on this limited selection of VOCs, only methyl hexanoate showed a statistically significant difference for CTS treated fruit (Supplementary Figure 2).

Esters are the predominant compounds contributing to the characteristic strawberry aroma, with significant contributions from terpenoids, furanones, and sulfur compounds (Abouelenen et al., 2023; Zheng et al., 2023). Methyl hexanoate is one of the most abundant and most frequently identified ester in strawberry (Ulrich et al., 2018) and has been shown, after an initial increase, to decrease during storage of ripe fruits (Li et al. 2021, Yan et al., 2024). Its significantly high value in CTS treated fruit at 12 + 1 d suggests CTS might be inhibiting post-harvest fruit senescence-related processes affecting their VOC profile. Interestingly, other relevant studies on strawberry showcased that the application of chitosan coatings can help maintain the aroma profile by enhancing the levels of desirable esters and delaying the buildup of off-flavors (Almenar et al., 2009; Perdones et al., 2016).

Overall, the observed similarities in VOC changing over time were larger than the differences between treatments. Cold storage is used to extend the shelf life of strawberries but can significantly alter their aroma profile. Noteworthy, VOC profile of 'Elsanta' strawberries differed between 4 or 8 °C of cold storage and additionally among fruit harvested in different years, indicating that aroma change at harvest and during storage is highly dependent on environmental factors during growth (Baldwin et al., 2023).

3.3. Phytochemical composition

Total phenols and ascorbic acid content remained unaffected by the agents and the storage duration applied (Fig. 4A and B). Marked differences were visible after 4 d and 8 d of cold storage, for total anthocyanin and total flavonoids contents, respectively. After 4 d CS, NOSH-A-treated strawberries were characterized by higher anthocyanin content, while NaA-treated fruits registered the highest flavonoid content (Fig. 4C and D). Polyphenolic compounds analysis by HPLC-DAD-ESI-MS/MS revealed that the strawberry samples contained a characteristic phenolic compound profile, including ellagitannins and ellagic acid conjugates [two pedunculagin (bis-hexahydroxydiphenyl glucose) isomers, ellagic acid and an ellagic acid pentoside and ellagic acid rhamnoside], anthocyanins (pelargonidin 3-glucoside and 3-rutinoside), flavan 3-ols (catechin) and proanthocyanidins (procyanidin B1), flavonols (quercetin 3-glucuronide and kaempferol 3-glucuronide, 3-glucoside and 3-malonylglucoside), and hydroxycinnamic acid derivatives [p-coumaroyl hexose (2 isomers) and feruloyl hexose] (Fig. 5). Such data are in agreement with previous studies on strawberry phenolic compounds (Buendía et al., 2010).

Noteworthy, polyphenolic compounds analysis by HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical

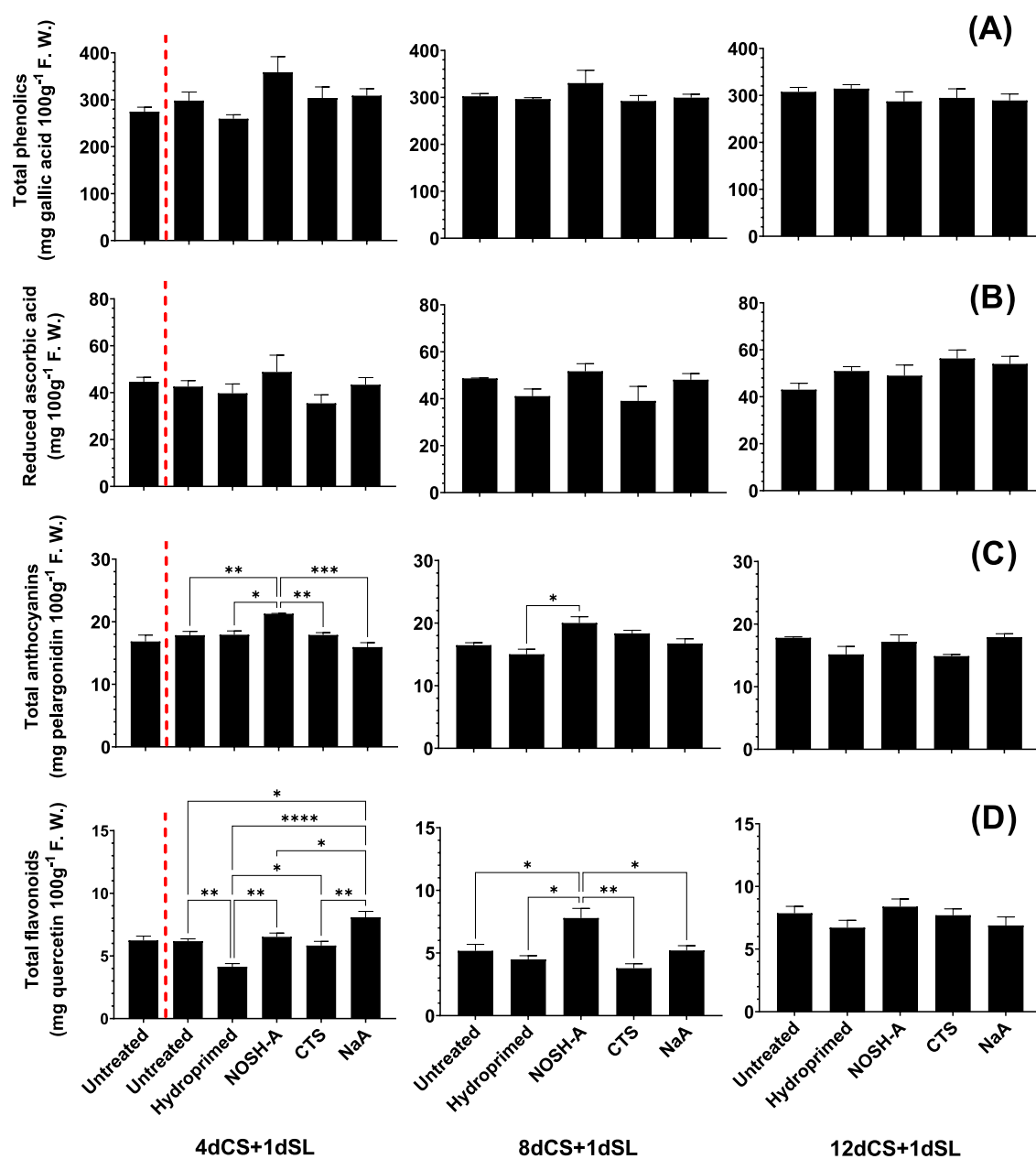


Fig. 4. Effect of storage duration and postharvest treatment applied on the contents of total phenolics, reduced ascorbic acid, anthocyanins and flavonols in strawberry fruits (ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.) Storage conditions and postharvest treatments are described in Fig. 2.

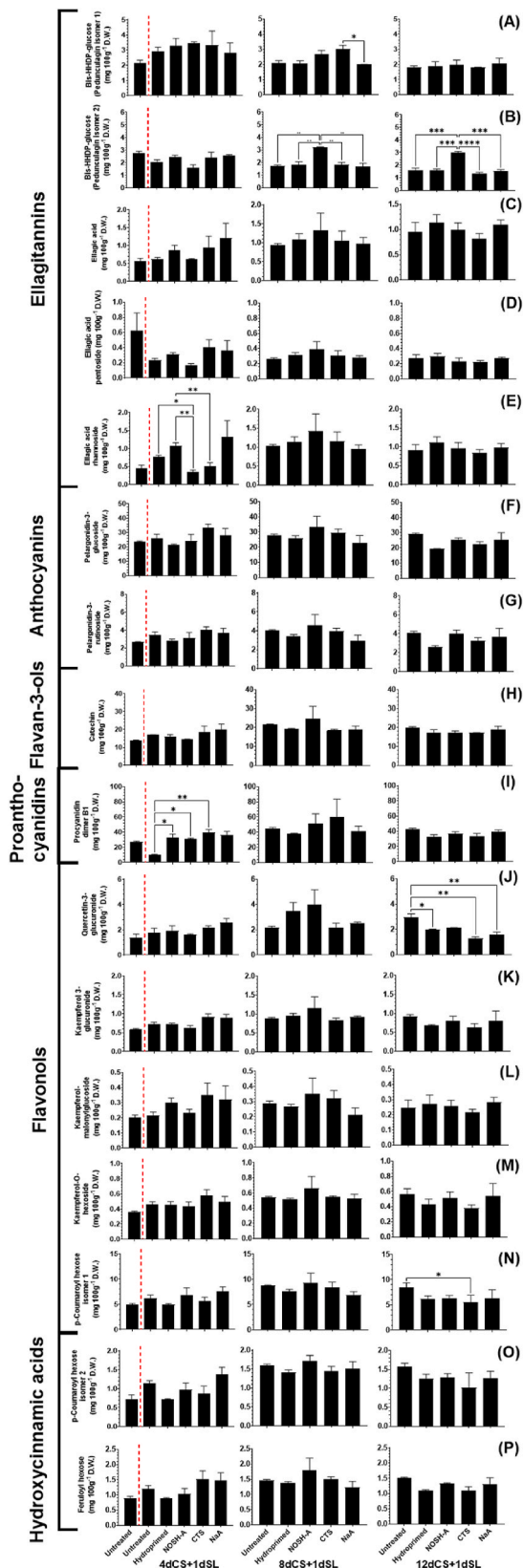


Fig. 5. Content of individual classes (ellagitannins, anthocyanins, flavan-3-ols, proanthocyanidins, flavonols and hydroxycinnamic acids) of phenolic compounds in strawberry fruits (ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$).

compounds such as ellagic acid, pelargonidin-3-glucoside, pelargonidin-3-rutinoside, and catechin after 8 days CS compared to freshly harvested fruit. Such changes were more evident when the priming agent NOSH-A was applied, being more pronounced in the case of ellagitannins and pedunculagin 2 isomer in particular that registered a significant increment. However, as a whole, the different agents applied did not show significant changes in an array of key phytochemicals found in strawberry fruit when tested for the same storage conditions with few exceptions. Among agents tested, NOSH-A potential as ‘phytochemical enhancer’ worths to be further tested in other postharvest experimental set ups.

3.4. PPO activity

PPO activity presented significant changes among the agents applied after 4 and 8 d CS and maintenance at room temperature for 1 d. At 4 + 1 d, PPO activity in untreated-, hydroprimed-, CTS- and NaA-treated strawberries was higher than NOSH-A-treated fruit presented the lowest PPO activity ($p \leq 0.001$) after 4 d CS. A lower PPO activity ($p \leq 0.05$) in NOSH-A-treated fruit compared to CTS- and NaA-treated fruits was also registered after 8 d CS (Fig. 6A). Phenols are secondary metabolites with different roles in plants, primarily displaying antioxidant function that helps plant to cope with oxidative stress induced by ROS (Panahirad et al., 2020). PPO oxidizes phenols and additionally causes browning in fruits converting polyphenolic substrates to dark pigments in the presence of oxygen (Panahirad et al., 2019). CTS-based coatings have been reported to enhance phenolic content in strawberry (Wang & Gao, 2013) and other crops, being used as nano carrier for delivery of phenylalanine (Gohari et al., 2021) and additionally cause reduced PPO activity in litchi (Jiang et al., 2005). Results reported herein provide insights that NOSH-A can be considered as a potential agent that can enhance polyphenolic content at postharvest level (Figs. 5 and 6A).

3.5. Cellular damage indicators

MDA and H₂O₂ contents were not significantly affected neither by the treatments nor the cold storage regimes applied (Fig. 6B and C). MDA and H₂O₂ serve as oxidative stress markers. During postharvest storage of fruits, oxidative stress can lead to an increase in H₂O₂ levels. This, in turn, promotes lipid peroxidation in cellular membranes, resulting in elevated MDA levels (Aghdam & Bodbodak, 2013). Any change in MDA and H₂O₂ over storage time might be a sign of extra stress, i.e. cold storage in our study. In this regard, Bahmani et al. (2024) reported that postharvest cold storage of strawberries at 4 °C for 12 d led to cellular damage, as evidenced by elevated levels of stress markers such as MDA and H₂O₂ with fruit coatings using chitosan-functionalized nanocomposites alleviating such symptoms and thus preserving fruit quality under cold storage conditions. The same study indicated that biodegradable polymers, namely chitosan and chitosan-putrescine nano-composites effectively preserve strawberry fruit by enhancing their antioxidant capacity and scavenging free radicals. However, such effects on CTS-treated strawberries of the current study experimental set up were not observed.

3.6. Polyamine content

Putrescine (Put) contents showed differences after removal from 4 d cold storage; untreated fruit had higher Put content than hydroprimed-, NaA- ($p \leq 0.05$) and CTS- ($p \leq 0.01$) ones; likewise, NOSH-A treatment resulted in higher Put content than CTS treatment in fruit ($p \leq 0.05$). Spm was significantly higher in untreated fruit than NaA ($p \leq 0.05$) after 8 d cold storage. Spd was not significantly affected for all storage durations and treatments applied (Fig. 7).

Polyamines (PAs) are aliphatic nitrogenous bases with low molecular weight, consisting of two or more amino groups and having potential biological activity. The main polyamines in plants, are Put, Spd and Spm

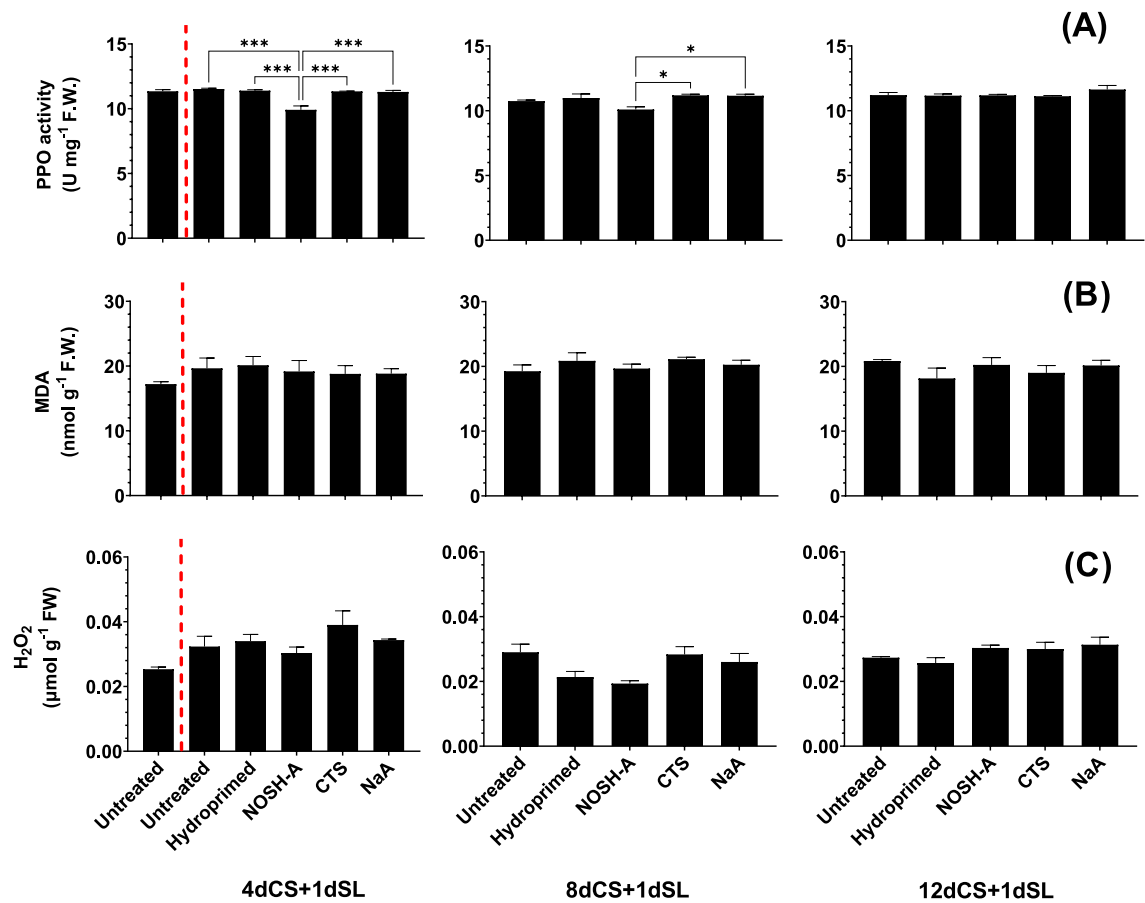


Fig. 6. Effect of storage duration and postharvest treatment applied on polyphenol oxidase (PPO) activity and malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents in strawberry fruits (ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$). Storage conditions and postharvest treatments are described in Fig. 2.

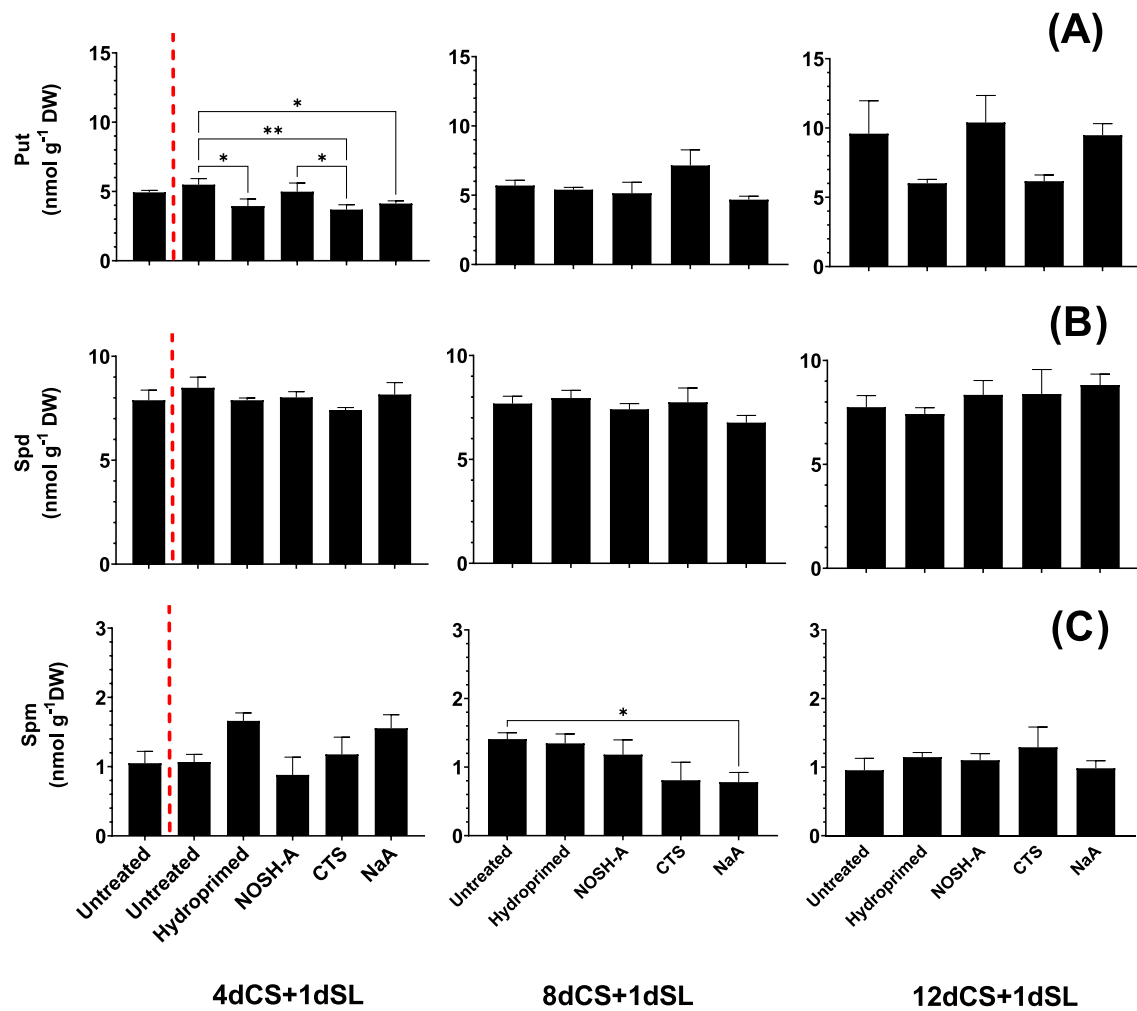


Fig. 7. Effect of storage duration and postharvest treatment applied on the contents of free spermidine, spermine and putrescine contents in strawberry fruits (ns = $p > 0.05$, * = $p \leq 0.05$, ** = $729 p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$). Storage conditions and postharvest treatments are described in Fig. 2.

which are involved in the regulation of diverse physiological processes (Sequera-Mutiozabal et al. 2017), while they are widely used in post-harvest applications to prolong the shelf-life of perishable horticultural crops (Pareek et al. 2018). Polyamines act as anti-senescence agents in fruits by inducing ROS detoxification, leading to reduced colour changes, increased fruit firmness, delayed ethylene and respiration rate emissions, induced mechanical resistance and reduced chilling symptoms (Handa et al., 2018). Several studies have indicated that the exogenous application of polyamines increases storage life and quality attributes of several fruit crops, including strawberry fruit either alone (Khosroshahi et al., 2007) or in combination with CTS (Bal & Ürün, 2020). Increasing polyamine contents enhances the stress tolerance via reinforcement of antioxidative properties (Seo et al., 2019). Yet, in our study, results showed that the agents applied had no significant effect on polyamine metabolism; exception for some slight changes in Put and Spd contents monitored after 4 and 8 d CS, respectively that can be additionally attributed to fruit response to the applied treatments and temperature changes.

3.7. Fungal incidence and severity index

The fungal incidence and severity were monitored on strawberry

fruit subjected to extended (12-d) CS. Results indicated that among the priming agents applied, CTS-treated fruit had the lowest incidence and severity (Fig. 8). These data can be linked with the fact that CTS-treated fruit after extended cold storage had a distinctive VOC profile that at certain extent can affect postharvest fruit senescence-related processes (Fig. 3). On the other hand, both NOSH-A and NaA, well known for their enhanced performance under abiotic conditions, did not display marked activity against fungal diseases. Interestingly, in another soft fruit crop (blueberry), NaA coatings incorporating cyclolipopeptides from *Bacillus subtilis* demonstrated potent antifungal properties, significantly reducing fungal counts (Xu et al., 2020).

After additional maintenance at room temperature for 4 d, fungal incidence increased across all treatments. The limited effectiveness of NOSH-A and NaA at postharvest level suggests the need to explore the association with other compounds or combinatory approaches with antifungal agents. For instance, while NaA alone may primarily serve as a barrier to moisture and gas exchange, its antifungal properties can be significantly enhanced when combined with natural antimicrobial agents (Janik et al., 2023).

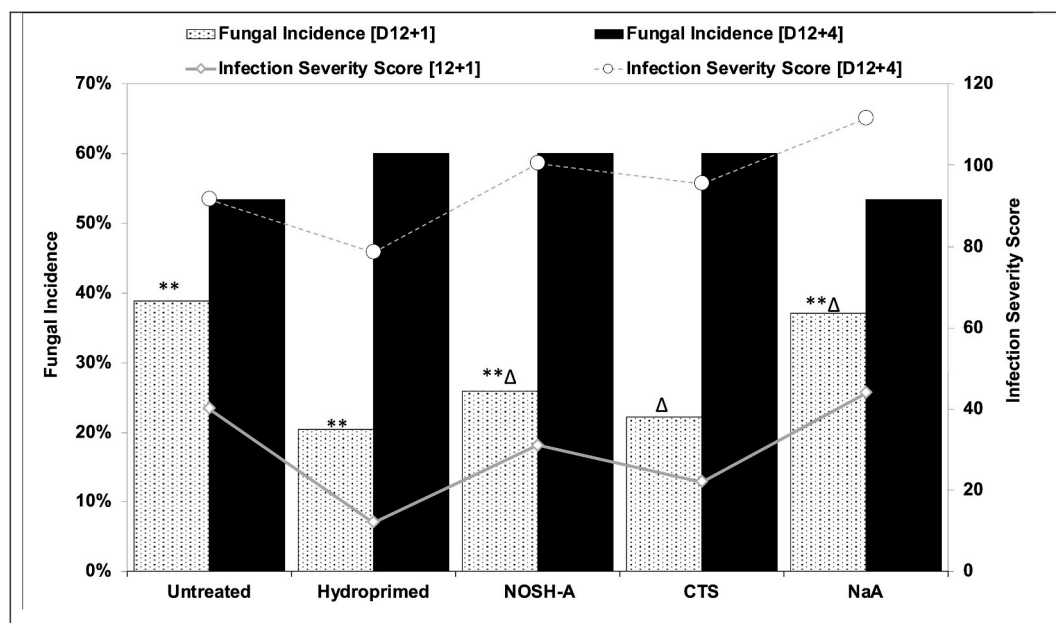


Fig. 8. Fungal incidence (%) and infection severity of strawberry fruits after 12 d of cold storage and additional maintenance at room temperature for 1 [D12 + 1] and 4 [D12 + 4] days, respectively. Same symbol (** or Δ) indicates significant difference for the infection severity between the treatments in the Mann-Whitney *U* test ($p \leq 0.05$) for the D12 + 1.

4. Conclusions

Our aim was to dissect to what extent the use of biocompatible polymers can be considered as an effective postharvest treatment to enhance postharvest quality attributes with special reference to aroma and phytochemical properties. Fruit treated with CTS remain closest to their starting position indicating their VOC profiles changed the least. Methyl hexanoate is one of the most abundant and most frequently identified ester in strawberry; its significantly higher value in CTS-treated fruit after extended cold storage (12 d) suggests that CTS might be inhibiting postharvest fruit senescence-related processes that can affect their VOC profile. HPLC-DAD-ESI-MS/MS showed an increment in an array of phytochemical compounds after cold storage compared to freshly harvested fruit. Noteworthy, the application of the proprietary priming agent NOSH-A led to enhanced contents for some phytochemical compounds and its potential use as ‘antioxidant potency enhancer’ at postharvest level can be further dissected in future post-harvest experiments. On the other side, no clear beneficial effects in terms of qualitative attributes were observed of the agents applies. Future attention could address whether other specific agents, with known preharvest priming activity to combat abiotic stress conditions such as drought and/or salinity, can be additionally exploited at a postharvest storage level for other stress types, i.e. biotic stress conditions linked to fungal resistance.

CRedit authorship contribution statement

Egli C. Georgiadou: Writing – review & editing, Methodology, Formal analysis, Data curation. **Carlos Javier Garcia Hernandez Gil:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Anna Maria Taliadorou:** Writing – review & editing, Methodology. **Eleni D. Myrtsi:** Methodology, Data curation. **Gholamreza Gohari:** Writing – review & editing, Conceptualization. **Alice Varaldo:** Methodology, Formal analysis. **Sofia Torrado:** Methodology, Formal analysis. **Alessandra Marcon Gasperini:** Writing – review & editing, Methodology, Formal analysis. **Francisco Tomás-Barberán:** Writing – review & editing, Supervision, Methodology, Formal analysis, Data curation. **Maarten L.A.T.M. Hertog:** Writing – review & editing,

Methodology, Formal analysis, Data curation. **Vasileios Fotopoulos:** Writing – review & editing, Validation, Methodology, Investigation. **George A. Manganaris:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.117877>.

Data availability

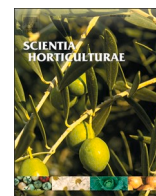
Data will be made available on request.

References

- Abouelenen, D., Acquaticci, L., Alessandrini, L., Borsetta, G., Caprioli, G., Mannozi, C., et al. (2023). Volatile profile of strawberry fruits and influence of different drying methods on their aroma and flavor: A review. *Molecules*, 28, 15. <https://doi.org/10.3390/molecules28155810>
- Adiletta, G., Di Matteo, M., & Petriccione, M. (2021). Multifunctional role of chitosan edible coatings on antioxidant systems in fruit crops: A review. *International Journal of Molecular Sciences*, 22, 2633. <https://doi.org/10.3390/ijms22052633>
- Aghdam, M. S., & Bodbodak, S. (2013). Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates

- and jasmonates treatments. *Scientia Horticulturae*, 156, 73–85. <https://doi.org/10.1016/j.scienta.2013.03.028>
- Alegria, C., Gonçalves, E. M., Moldão-Martins, M., Cisneros-Zevallos, L., & Abreu, M. (2016). Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. *Postharvest Biology and Technology*, 120, 232–239. <https://doi.org/10.1016/j.postharvbio.2016.07.004>
- Almenar, E., Hernández-Muñoz, P., & Gavarra, R. (2009). Evolution of selected volatiles in chitosan-coated strawberries (*Fragaria x ananassa*) during refrigerated storage. *Journal of Agricultural and Food Chemistry*, 57, 974–980. <https://doi.org/10.1021/jf802319v>
- Antoniou, C., Xenofontos, R., Chatzimichail, G., Christou, A., Kashi, K., & Fotopoulos, V. (2020). Exploring the potential of nitric oxide and hydrogen sulfide (NOSH)-releasing synthetic compounds as novel priming agents against drought stress in *Medicago sativa* plants. *Biomolecules*, 10, 120. <https://doi.org/10.3390/biom10010120>
- Bahmani, R., Razavi, F., Mortazavi, S. N., Juárez-Maldonado, A., & Gohari, G. (2024). Chitosan-putrescine nanoparticle coating attenuates postharvest decay and maintains ROS scavenging system activity of strawberry cv. 'Camarosa' during cold storage. *Folia Horticulturae*, 36, 149–160. <https://doi.org/10.2478/fhort-2024-0009>
- Bal, E., & Ürün, B. A. (2020). Effects of chitosan coating with putrescine on bioactive compounds and quality of strawberry cv. San Andreas during cold storage. *Erwerbs-obstbau*, 63, 7–14. <https://doi.org/10.1007/s10341-020-00531-9>
- Baldwin, A., Dhorajiwala, R., Roberts, C., Dimitrova, S., Tu, S., Jones, S., et al. (2023). Storage of halved strawberry fruits affects aroma, phytochemical content and gene expression, and is affected by pre-harvest factors. *Frontiers in Plant Science*, 14. <https://doi.org/10.3389/fpls.2023.1165056>
- Basson, C. E., Groenewald, J. H., Kossmann, J., Cronjé, C., & Bauer, R. (2010). Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: Invertase is the main sucrose hydrolysing enzyme. *Food Chemistry*, 121, 1156–1162. <https://doi.org/10.1016/j.foodchem.2010.01.064>
- Buendía, B., Gil, M. I., Tudela, J. A., Gady, A. L., Medina, J. J., Soria, C., López, J. M., & Tomás-Barberán, F. A. (2010). HPLC-MS Analysis of proanthocyanidin oligomers and other phenolics in 15 strawberry cultivars. *Journal of Agricultural and Food Chemistry*, 58, 3916–3926. <https://doi.org/10.1021/jf9030597>
- Darwish, O. S., Ali, M. R., Khojah, E., Samra, B. N., Ramadan, K. M. A., & El-Mogy, M. M. (2021). Pre-harvest application of salicylic acid, abscisic acid, and methyl jasmonate conserve bioactive compounds of strawberry fruits during refrigerated storage. *Horticulturae*, 7, 568. <https://doi.org/10.3390/horticulturae7120568>
- De Queiroz Antonino, R. S. C. M., Lia Fook, B. R. P., de Oliveira Lima, V. A., de Farias Rached, R. I., Lima, E. P. N., da Silva Lima, R. J., Peniche Covas, C. A., & Lia Fook, M. V. (2017). Preparation and characterization of chitosan obtained from shells of shrimp (*Litopenaeus vannamei* boone). *Marine Drugs*, 15, 141. <https://doi.org/10.1007/s13201-019-0967-z>
- Dhital, R., Mora, N. B., Watson, D. G., Kohli, P., & Choudhary, R. (2018). Efficacy of limonene nano coatings on post-harvest shelf life of strawberries. *LWT—Food Science and Technology*, 97, 124–134. <https://doi.org/10.1016/j.lwt.2018.06.038>
- El-Mogy, M. M., Ludlow, R. A., Roberts, C., Müller, C. T., & Rogers, H. J. (2019). Postharvest exogenous melatonin treatment of strawberry reduces postharvest spoilage but affects components of the aroma profile. *Journal of Berry Research*, 9, 297–307. <https://doi.org/10.3233/JBR-180361>
- Emamifar, A., & Bavaisi, S. (2020). Nanocomposite coating based on sodium alginate and nano-ZnO for extending the storage life of fresh strawberries (*Fragaria x ananassa* Duch.). *Journal of Food Measurement and Characterization*, 14, 1012–1024. <https://doi.org/10.1007/s11694-019-00350-x>
- Filippi, D., Nienow, A. A., Chiomento, J. L. T., et al. (2021). Development and validation of a set of standard area diagrams to assess severity of gray mold in strawberry fruit. *European Journal of Plant Pathology*, 160, 277–286. <https://doi.org/10.1007/s10658-021-02238-3>
- Filippou, P., Antoniou, C., & Fotopoulos, V. (2011). Effect of drought and rewetting on the cellular status and antioxidant response of *Medicago truncatula* plants. *Plant Signaling & Behavior*, 6, 270–277. <https://doi.org/10.4161/psb.6.2.14633>
- Georgiadou, E. C., Goulas, V., Majak, I., Ioannou, A., Leszczynska, J., & Fotopoulos, V. (2018). Antioxidant potential and phytochemical content of selected fruits and vegetables consumed in Cyprus. *Biotechnology and Food Science*, 82, 3–14. <https://doi.org/10.34658/bfs.2018.82.1.3-14>
- Gohari, G., Jiang, M., Manganaris, G. A., Zhou, J., & Fotopoulos, V. (2024). Next generation chemical priming: With a little help from our nanocarrier friends. *Trends in Plant Science*, 29, 150–166. <https://doi.org/10.1016/j.tplants.2023.11.024>
- Gohari, G., Zareei, E., Kulak, M., Labib, P., Mahmoudi, R., Panahirad, S., Jafari, H., Mahdavinia, G., Juárez-Maldonado, A., & Lorenzo, J. M. (2021). Improving the berry quality and antioxidant potential of flame seedless grapes by foliar application of chitosan-phenylalanine nanocomposites (CS-Phe NCs). *Nanomaterials*, 11(9), 2287. <https://doi.org/10.3390/nano11092287>
- Guimaraes, A., Abrunhosa, L., Pastrana, L. M., & Cerqueira, M. A. (2018). Edible films and coatings as carriers of living microorganisms: A new strategy towards biopreservation and healthier foods. *Comprehensive Reviews in Food Science and Food Safety*, 17, 594–614. <https://doi.org/10.1111/1541-4337.12345>
- Hadjipieri, M., Georgiadou, E. C., Costa, F., Fotopoulos, V., & Manganaris, G. A. (2020). Dissection of the incidence and severity of purple spot physiological disorder in loquat fruit through a physiological and molecular approach. *Plant Physiology and Biochemistry*, 155, 980–986. <https://doi.org/10.1016/j.plaphy.2020.06.043>
- Handa, A. K., Fatima, T., & Mattoo, A. K. (2018). Polyamines: Bio-Molecules with diverse functions in plant and human health and disease. *Frontiers in Chemistry*, 6, 10. <https://doi.org/10.3389/fchem.2018.00010>
- Huang, G., Huang, L., Geng, C., Lan, T., Huang, X., Xu, S., Shen, Y., & Bian, H. (2022). Green and multifunctional chitosan-based conformal coating as a controlled release platform for fruit preservation. *International Journal of Biological Macromolecules*, 219, 767–778. <https://doi.org/10.1016/j.ijbiomac.2022.08.038>
- Janik, W., Nowotarski, M., Ledniewska, K., et al. (2023). Modulation of physicochemical properties and antimicrobial activity of sodium alginate films through the use of chestnut extract and plasticizers. *Scientific Reports*, 13, Article 11530. <https://doi.org/10.1038/s41598-023-38794-3>
- Jiang, T., Feng, L., & Wang, Y. (2013). Effect of alginate/nano-Ag coating on microbial and physicochemical characteristics of shiitake mushroom (*Lentinus edodes*) during cold storage. *Food Chemistry*, 141, 954–960. <https://doi.org/10.1016/j.foodchem.2013.03.093>
- Jiang, Y., Lib, J., & Jiang, W. (2005). Effects of chitosan coating on shelf life of cold-stored litchi fruit at ambient temperature. *LWT—Food Science and Technology*, 38, 757–761. <https://doi.org/10.1016/j.lwt.2004.09.004>
- Jongsri, P., Wangsomboondee, T., Rojsithisak, P., & Seryapheap, K. (2016). Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit. *LWT—Food Science and Technology*, 73, 28–36. <https://doi.org/10.1016/j.lwt.2016.05.038>
- Khosroshahi, M. R. Z., Esna-Ashari, M., & Ershadi, A. (2007). Effect of exogenous putrescine on post-harvest life of strawberry (*Fragaria ananassa* Duch.) fruit, cultivar Selva. *Scientia Horticulturae*, 114, 27–32. <https://doi.org/10.1016/j.scienta.2007.05.006>
- Lee, J., Kim, H. B., Noh, Y. H., Min, S. R., Lee, H. S., Jung, J., et al. (2018). Sugar content and expression of sugar metabolism-related gene in strawberry fruits from various cultivars. *Journal of Plant Biotechnology*, 45, 90–101. <https://doi.org/10.5010/JPB.2018.45.2.090>
- Li, H., Brouwer, B., Oud, N., Verdonk, J. C., Tikunov, Y., Woltering, E., Schouten, R., & Pereira da Silva, F. (2021). Sensory, GC-MS and PTR-ToF-MS profiling of strawberries varying in maturity at harvest with subsequent cold storage. *Postharvest Biology and Technology*, 182, Article 111719. <https://doi.org/10.1016/j.postharvbio.2020.105871>
- Lin, M., Fang, S., Zhao, X., Liang, X., & Wu, D. (2020). Natamycin-loaded zein nanoparticles stabilized by carboxymethyl chitosan: Evaluation of colloidal/chemical performance and application in postharvest treatments. *Food Hydrocolloids*, 106, Article 105871. <https://doi.org/10.1016/j.foodhyd.2020.105871>
- Liu, C., Jin, T., Liu, W., Hao, W., Yan, L., & Zheng, L. (2021). Effects of hydroxyethyl cellulose and sodium alginate edible coating containing asparagus waste extract on postharvest quality of strawberry fruit. *LWT—Food Science and Technology*, 148, Article 111770. <https://doi.org/10.1016/j.lwt.2021.111770>
- Loreto, F., & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology*, 127, 1781–1787. <https://doi.org/10.1104/pp.010497>
- Mahcene, Z., Khellil, A., Hasni, S., Akman, P. K., Bozkurt, F., Birech, K., ... Tornuk, F. (2020). Development and characterization of sodium alginate based active edible films incorporated with essential oils of some medicinal plants. *International Journal of Biological Macromolecules*, 145, 124–132. <https://doi.org/10.1016/j.ijbiomac.2019.12.093>
- Manganaris, G. A., Goulas, V., Vicente, A. R., & Terry, L. A. (2014). Berry antioxidants: Small fruits providing large benefits. *Journal of the Science of Food and Agriculture*, 94, 825–833. <https://doi.org/10.1002/jsfa.6432>
- Marcé, M., Brown, D. S., Capell, T., Figueras, X., & Tiburcio, A. F. (1995). Rapid high-performance liquid chromatographic method for the quantitation of polyamines as their dansyl derivatives: Application to plant and animal tissues. *Journal of Chromatography B Biomedical Applications*, 666, 329–335. [https://doi.org/10.1016/0378-4347\(94\)00586-T](https://doi.org/10.1016/0378-4347(94)00586-T)
- Maringgal, B., Hashim, N., Mohamed Amin Tawakkal, I. S., & Muda Mohamed, M. T. (2020). Recent advance in edible coating and its effect on fresh/more fresh fruits quality. *Trends in Food Science & Technology*, 96, 253–267. <https://doi.org/10.1016/j.tifs.2019.12.024>
- Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, 51, 6887–6892. <https://doi.org/10.1021/jf034506n>
- Moghadas, H. C., Smith, J. S., & Tahergorabi, R. (2025). Recent advances in the application of edible coatings for shelf-life extension of strawberries: A review. *Food and Bioprocess Technology*, 18, 1079–1103. <https://doi.org/10.1007/s11947-024-03517-7>
- Nair, M. S., Tomar, M., Punia, S., Kukula-Koch, W., & Kumar, M. (2020). Enhancing the functionality of chitosan- and alginate-based active edible coatings/films for the preservation of fruits and vegetables: A review. *International Journal of Biological Macromolecules*, 164, 304–320. <https://doi.org/10.1016/j.ijbiomac.2020.07.083>
- Ncama, K., Magwaza, L. S., Mditshwa, A., & Tesfay, S. Z. (2018). Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review. *Food Packaging and Shelf Life*, 16, 157–167. <https://doi.org/10.1016/j.foodpack.2018.03.011>
- Neethirajan, S., & Jayas, D. S. (2011). Nanotechnology for the food and bioprocessing industries. *Food and Bioprocess Technology*, 4, 39–47. <https://doi.org/10.1007/s11947-010-0328-2>
- Nguyen, V. T. B., Nguyen, D. H. H., & Nguyen, H. V. H. (2020). Combination effects of calcium chloride and nano-chitosan on the postharvest quality of strawberry (*Fragaria x ananassa* Duch.). *Postharvest Biology and Technology*, 162, Article 111103. <https://doi.org/10.1016/j.postharvbio.2019.111103>
- Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., ... Martín-Belloso, O. (2010). Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, 57, 139–148. <https://doi.org/10.1016/j.postharvbio.2010.04.001>

- Pagliarulo, C., Sansone, F., Moccia, S., Russo, G. L., Aquino, R. P., Salvatore, P., ... Volpe, M. G. (2016). Preservation of strawberries with an antifungal edible coating using peony extracts in chitosan. *Food and Bioprocess Technology*, 9, 1951–1960. <https://doi.org/10.1007/s11947-016-1779-x>
- Panahirad, S., Naghshiband-Hassani, R., Ghanbarzadeh, B., Zaare-Nahandi, F., & Mahna, N. (2019). Shelf life quality of plum fruits (*Prunus domestica* L.) improves with carboxymethylcellulose-based edible coating. *HortScience*, 54, 505–510. <https://doi.org/10.21273/HORTSCI13751-18>
- Panahirad, S., Naghshiband-Hassani, R., & Mahna, N. (2020). Pectin-based edible coating preserves antioxidative capacity of plum fruit during shelf life. *Food Science and Technology International*, 26, 583–592. <https://doi.org/10.1177/1082013220916559>
- Pareek, S., Sharma, S., Sagar, N., & González-Aguilar, G. A. (2018). Polyamines treatments. In S. Pareek (Ed.), *Novel postharvest treatments of fresh produce* (pp. 79–101). CRC Press.
- Perdones, A., Escriche, I., Chiralt, A., & Vargas, M. (2016). Effect of chitosan-lemon essential oil coatings on volatile profile of strawberries during storage. *Food Chemistry*, 197, 979–986. <https://doi.org/10.1016/j.foodchem.2015.11.054>
- Petriccione, M., De Sanctis, F., Pasquariello, M. S., Mastrobuoni, F., Rega, P., Scortichini, M., & Mencarelli, F. (2014). The effect of chitosan coating on the quality and nutraceutical traits of sweet cherry during postharvest life. *Food and Bioprocess Technology*, 8, 394–408. <https://doi.org/10.1007/s11947-014-1411-x>
- Robledo, N., López, L., Bunker, A., Tapia, C., & Abugoch, L. (2018). Effects of antimicrobial edible coating of thymol nanoemulsion/quinoa protein/chitosan on the safety, sensorial properties, and quality of refrigerated strawberries (*Fragaria × ananassa*) under commercial storage environment. *Food and Bioprocess Technology*, 11, 1566–1574. <https://doi.org/10.1007/s11947-018-2124-3>
- Salazar-Orbea, G., García-Villalba, R., Sánchez-Siles, L. M., Tomás-Barberán, F. A., & García, C. J. (2022). Untargeted metabolomics reveals new markers of food processing for strawberry and apple purees. *Molecules*, 27, 7275. <https://doi.org/10.3390/molecules27217275>
- Seo, S. Y., Kim, Y. J., & Park, K. Y. (2019). Increasing polyamine contents enhances the stress tolerance via reinforcement of antioxidative properties. *Frontiers in Plant Science*, 10, 1331. <https://doi.org/10.3389/fpls.2019.01331>
- Sequera-Mutiozabal, M., Antoniou, C., Tiburcio, A. F., Alcázar, R., & Fotopoulos, V. (2017). Polyamines: Emerging hubs promoting drought and salt stress tolerance in plants. *Current Molecular Biology Reports*, 3, 28–36. <https://doi.org/10.1007/s40610-017-0052-z>
- Shehata, S. A., Abdeldaym, E. A., Ali, M. R., Mohamed, R. M., Bob, R. I., & Abdelgawad, K. F. (2020). Effect of some Citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage. *Agronomy*, 10, 1466. <https://doi.org/10.3390/agronomy10101466>
- Simkova, K., Veberic, R., Hudina, M., Grohar, M. C., Pelacci, M., Smrke, T., et al. (2024). Non-destructive and destructive physical measurements as indicators of sugar and organic acid contents in strawberry fruit during ripening. *Scientia Horticulturae*, 327, Article 112843. <https://doi.org/10.1016/j.scienta.2024.112843>
- Suhag, R., Kumar, N., Petkoska, A. T., & Upadhyay, A. (2020). Film formation and deposition methods of edible coating on food products: A review. *Food Research International*, 136, Article 109582. <https://doi.org/10.1016/j.foodres.2020.109582>, 2020.
- Sun, Y., Huang, Y., Wang, X. Y., Wu, Z. Y., & Weng, Y. X. (2022). Kinetic analysis of PGA/PBAT plastic films for strawberry fruit preservation quality and enzyme activity. *Journal of Food Composition and Analysis*, 108. <https://doi.org/10.1016/j.jfca.2022.104439>
- Ulrich, D., Kecke, S., & Olbricht, K. (2018). What do we know about the chemistry of strawberry aroma? *Journal of Agricultural and Food Chemistry*, 66, 3291–3301. <https://doi.org/10.1021/acs.jafc.8b01115>
- Vandendriessche, T., Nicolai, B. M., & Hertog, M. L. A. T. M. (2013). Optimization of HS SPME Fast GC-MS for high-throughput analysis of strawberry aroma. *Food Analytical Methods*, 6, 512–520. <https://doi.org/10.1007/s12161-012-9471-x>
- Wang, S. Y., & Gao, H. (2013). Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (*Fragaria × ananassa* Duch.). *LWT—Food Science and Technology*, 52, 71–79. <https://doi.org/10.1016/j.lwt.2012.05.003>
- Xu, L., Zhang, B., Qin, Y., Li, F., Yang, S., Lu, P., et al. (2020). Preparation and characterization of antifungal coating films composed of sodium alginate and cyclolipopeptides produced by *Bacillus subtilis*. *International Journal of Biological Macromolecules*, 143, 602–609. <https://doi.org/10.1016/j.ijbiomac.2019.12.051>
- Yan, B., Wang, Y., Bai, Y., Liu, Z., Liu, H., Chen, X., Shen, Y., & Duan, L. (2024). Insights into the senescent mechanisms of harvested strawberry fruit at the physiological, molecular and metabolic levels. *Fruit Research*, 4, Article e018. <https://doi.org/10.48130/frures-0024-0011>
- Zheng, S., Cai, J., Huang, P., Wang, Y., Yang, Z., & Yu, Y. (2023). Determination of volatile profiles of woodland strawberry (*Fragaria vesca*) during fruit maturation by HS-SPME GC-MS. *Journal of the Science of Food and Agriculture*, 103, 7455–7468. <https://doi.org/10.1002/jsfa.12827>



Research Paper

Application of priming agents in red raspberries prior to transplantation and at pre-flowering stages results in improved yield efficiency and enhanced secondary metabolism

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ABSTRACT

Red raspberry (*Rubus idaeus* L.) fruit has high nutritional value and there is an increasing demand in its global cultivation, highlighting the need for sustainable practices to improve both fruit and plant productivity. Chemical priming has recently gained attention as a sustainable horticultural crop management approach to enhance plant performance. In the current study, the effects of multiple chemical priming agents were investigated on their potential to improve yield efficiency, enhance antioxidant potential and fruit quality attributes, with special reference to aroma of 'Vica Abril' raspberry plants. Treatments included: (1) NOSH-aspirin (NOSH-A, 100 µM), (2) melatonin (Mel, 100 µM), (3) sodium alginate (NaA, 0.5 % w/v), (4) sodium alginate-melatonin conjugate (NaA/Melatonin, 100 µM/0.5 % w/v), and (5) glycine-betaine (GB, 10 mM). Additionally, control treatments included application of water (hydro-primed) and DMSO (0.1 % v/v) (solvent control for NOSH-A). Treatment application was initially performed pre-planting at the root zone and subsequently at 27, 46 and 74 days after planting (DAP). Melatonin treatment significantly enhanced fruit yield, particularly during the early harvests, while NOSH-A enhanced sucrose and ascorbic acid content and all priming agents increased total flavonoid content. Treatments with NaA alone or in conjugated form with Mel led to a considerable increment of kaempferol, several anthocyanins and ellagic acid derivatives, among the 13 polyphenolic compounds identified. The analysis of volatile organic compounds (VOCs) in raspberry fruits identified a total of 98 distinct compounds. Besides d-limonene content, no striking differences in aroma composition was monitored among treatments tested. The application of priming agents, most promptly melatonin, is a promising technological approach that needs to be further exploited towards increased crop productivity and/or enhanced raspberry fruit quality.

1. Introduction

High nutritional properties of red raspberry (*Rubus idaeus* L.) are widely recognised in the horticultural sector that has led to an exponential growth of its demand on global market. However, the cultivation of raspberry is capital- and knowledge-intensive, requiring optimum cultivation protocols (Manganaris et al., 2024). Advanced breeding programs have led to the development of heat- and low-chilling tolerant cultivars which can withstand an array of adverse climatic conditions.

Noteworthy, the development of new-cultivar types, namely "true primocanes", which do not have any chilling requirements to be productive, have a significant positive impact towards year-round production. Modern cultivars, are being tested in different meso-climates and can be highly productive when combined with advanced production systems, i. e. soilless production and plastic tunnels (Sønsteby et al., 2013; Demchak and Hanson, 2013). Despite recent advances, raspberry cultivation is particularly vulnerable at exposure to high temperatures ($\geq 35^\circ\text{C}$) that poses a significant threat to plant productivity (Sønsteby and Heide,

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2008). This is a particular challenge to Mediterranean climates, since heat stress during developmental stages can affect flowering and hence plant productivity and fruit quality. Heat-sensitive cultivars may prolong their vegetative stage until milder temperatures are present, while in heat-tolerant cultivars, flowering is induced prematurely at early developmental stages.

Plant priming has emerged as a promising approach to improve crop resilience and combat negative impacts on crop productivity induced by adverse environmental conditions. Priming refers to the physiological memory of plants when being exposed to an environmental stressor which triggers the induction of several metabolic and molecular pathways that allow them to respond more effectively upon exposure to future stressors (Savvides et al., 2016; Guzmán et al., 2022; Gohari et al., 2024). Priming can be achieved using various natural or synthetic compounds in small, non-toxic doses. Priming agents (PAs) are generally recognized as safe, since they do not leave any harmful residues on fruits and are naturally present. Furthermore, they have been shown to trigger defense mechanisms against biotic and abiotic stress factors in an array of agricultural commodities.

Chemical priming agents, like melatonin, are increasingly recognised not only as antioxidant molecules, but as plant growth regulators, that have the ability to up-regulate genes related to physiological plant responses to biotic and abiotic stress, thus acting as stress elicitors, while also often leading to improvements in nutritional value of treated crops (Agathokleous et al., 2021). Organic and inorganic nanoparticles and polymers are another distinct category of priming agents. Additionally, NOSH-A, a novel synthetic donor of nitric oxide (NO), hydrogen sulfide (H_2S) and acetylsalicylic acid, represents a promising tool for plant priming to develop stress-resilient crops and advance sustainable production systems, thanks to its functional role in stress tolerance mechanisms involving oxidative stress avoidance, stomata regulation, and gene expression regulation (Antonίου et al., 2020). Notably, the use of nanocarriers (including biopolymers such as sodium alginate) as smart delivery vectors for priming agents towards improved plant performance is recently attracting increasing attention (reviewed in Gohari et al., 2024). However, such compounds have been scarcely analysed.

Towards the need for sustainable solutions, the incorporation of PAs in early developmental stages of raspberry cultivation presents a promising technological approach. The current study aimed to offer insights into the practical application of chemical PAs in raspberry cultivation under Mediterranean conditions, including melatonin, glycine-betaine, and NOSH-A and sodium alginate (alone or as conjugate with melatonin), along with appropriate solvent controls. Our working hypothesis was to dissect whether early-stage priming with different chemical agents, either through direct application or through smart delivery with the use of biodegradable polymers, will improve physiological responses of raspberry plants in the field and can potentially lead to the improvement of yield and phytochemical fruit attributes associated with taste, aroma and antioxidant capacity.

2. Materials and methods

2.1. Chemicals and standards

Chemical priming agents were obtained from Sigma-Aldrich (glycine-betaine and sodium alginate) and from Chem Cruz (melatonin). NOSH-A was provided by Avicenna Pharmaceuticals Inc., NY, USA. The analytical grade solvents, used to extract the samples, methanol and acetone, were obtained from Sigma-Aldrich and Scharlau, respectively. The HPLC grade water and acetonitrile solvents were purchased from Merck. Folin-Ciocalteu reagent, sodium hydroxide, potassium chloride, 2,6-dichloroindophenol sodium salt hydrate, aluminum chloride, trichloroacetic acid, thiobarbituric acid, metaphosphoric acid, and potassium acetate were from Sigma-Aldrich. Sodium carbonate, sodium acetate and hydrogen peroxide were obtained from Fluka, Scharlau and Supelco, respectively. Absolute ethanol was

from Scharlau, hydrochloric acid and DMSO from Supelco.

The purity of all standards was higher than 95 %. Fructose, glucose and sucrose standards were obtained from Sigma-Aldrich, Himedia and Melford, respectively. Ascorbic acid, pelargonidin, and gallic acid were purchased from Sigma-Aldrich.

For the determination of volatile compounds, sodium chloride (NaCl) and SPME fiber assembly Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) was used and purchased from Sigma-Aldrich and Supelco, respectively.

2.2. Plant material, experimental design and treatment application

Experiment was conducted in Chandria village, Limassol District Cyprus located 1250 m above sea level. The climate is continental in terms of winter and chilling accumulation, with typical Mediterranean conditions during spring and autumn. Annual rainfall ranges from 800–1200 mm depending on the season. Over the last decade, the summer period has shifted from mild Mediterranean temperatures and occasional rains, to very hot and dry environment, similar to the coastal areas of the island.

Plant material (cv. 'Vica Abril', Viveros California, Spain) was received as tray plants in Cyprus in June 2024. In order to combat transplantation stress, first treatment application was performed in the root zone with 20 mL of respective formulation, three days prior to the placement of plantlets under field conditions into 10 L pots with coco coir substrate (June 18, 2024). Three foliar applications were followed after transplantation in the field at different intervals [27, 46 and 74 days after planting (DAP)]. The last application took place just before flowering.

In total, the experiment consisted of the following treatments: (1) NOSH-Acetylsalicylic acid (NOSH-A, 100 μ M), (2) DMSO (0.1 % v/v) (solvent for NOSH-A) (3) melatonin (Mel, 100 μ M), (4) sodium alginate (NaA, 0.5 % w/v), (5) sodium alginate/melatonin (NaA/Melatonin, 100 μ M/0.5 % w/v), (6) glycine-betaine (GB, 10 mM) and (7) hydro-primed which acted as the control. All treatment solutions contained 0.1 % v/v Tween-20 surfactant to facilitate the wetting and spreading of the priming agents onto the leaves. Application volumes per plant per application were 20 mL at root zone and 24 mL, 38 mL and 46 mL for the three successive sprayings, respectively.

The potted plants were used to set up a randomized complete block design (RCBD) to account for the spacial variation observed across the experimental area, particularly related to the sun exposure at different times during the day due to the topography. Experimental set-up consisted of four blocks in which treatments were randomly assigned in each block and were present only once per block. Each treatment replicate consisted of six raspberry plants serving as one biological replicate. Plants were kept under black netting of 30 % shadow and atmospheric temperature during midday constantly reach 30 °C over the period of vegetative growth of the plants. Plants were irrigated during 4 intervals within the day to collect 20–25 % drainage with fertigated values of EC (μ S/cm) of no more than 1500 and pH value of 6.7.

2.3. Agronomic and physiological attributes

A set of agronomic attributes, namely sucker development, branching of laterals on ground level (Bottom Laterals-BL), total laterals (TL) per cane and of inflorescence laterals (IL: TL-BT), cane height (cm) and leaf number were assessed. Additionally, yield per plant was quantified starting on September 30 (101 DAP) and ending November 18 (150 DAP). Specifically, 13 harvests were carried out at 101, 104, 112, 116, 118, 123, 126, 130, 133, 137, 140, 143, 150 DAP. Cumulative yield was expressed in weight (g) per plant; in addition, the number of berries per plant to generate the index of average berry weight (g) was determined.

LI-600 Porometer/Fluorometer (LI-COR, USA) was used to measure gas stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) and transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$). Fluorescence measurements were conducted during light

conditions to represent photosystem II chlorophyll fluorescence, while electron transport rate was additionally measured ($\mu\text{mol m}^{-2} \text{s}^{-1}$). LI-600 was also used to measure parameters quantifying the heat stress, such as Vapor Pressure Deficit (kPa), Leaf Temperature ($^{\circ}\text{C}$) and Light Intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$). The physiological responses of the plants were measured weekly during the vegetative stage and biweekly, during the harvest phase (chlorophyll fluorescence, stomatal conductance), along with Normalized Difference Vegetation Index (NDVI) (Molina-Bravo et al., 2011; Rungrat et al., 2016).

Data from canopy reflectance were collected twice a month starting in August, when the vegetation indices were adequate to start recording the NDVI and flowering induction has initiated. Crop vigour, represented by NDVI, was assessed with handheld proximal crop sensor Greenseeker (TRIMBLE Inc. Sunnyvale, CA, USA) at a height of ca. 0.3 m from plant canopy and at a different height from the soil according to the developmental stage. Both, proximal sensors including NDVI and LI-600 measurements were collected starting in July 26 (35 DAP) and ending in November 1 (133 DAP), 2024. Overall, measurements took place at leaf level during 35, 41, 51, 62, 72, 82, 91, 112, 118 and 133 DAP, respectively.

2.4. Cellular damage indicators

In order to assess any cellular damage induced by variations in temperature prior to flowering, the degree of oxidative stress was assessed through the quantification of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content. Six fully expanded raspberry leaves per biological replication per treatment were collected once-off before flowering and immediately placed in dry ice in the field, before stored in -80°C until further biochemical analysis. The extent of lipid peroxidation was determined by measuring MDA content resulting from the thiobarbituric acid (TBA) reaction (Filippou et al., 2011). The absorption was measured at 532 and 600 nm (TECAN, Infinite 200[®] PRO) and MDA was estimated using the Lambert-Beer law, with extinction coefficient of $155 \text{ mM}^{-1}\text{cm}^{-1}$ and expressed as nmol g^{-1} fresh weight (FW).

Hydrogen peroxide (H_2O_2) content was calculated spectrophotometrically based on the oxidation of iodide (I^{-1}) to iodine (I), after the reaction of H_2O_2 with potassium iodide (SSKI oral solution), using the procedure described by Loreto and Velikova, 2001. The content of H_2O_2 was measured at 390 nm and was estimated based on a standard curve of known concentrations of H_2O_2 and expressed as $\mu\text{mol g}^{-1}$ F.W.

2.5. Fruit quality attributes

2.5.1. Soluble solid content (SSC), titratable acidity (TA) and sugar content

During harvest peak (133 DAP), approximately 200 g of raspberries were collected per biological replicate from each treatment and directly transferred to the laboratory. One-half of the amount (100 g) was frozen in liquid nitrogen and stored in -80°C until further analysis while the other half was homogenized using IKA[®] T25 digital ULTRA-TURRAX[®] for 1–2 min and transferred into 50 mL falcon tubes. The homogenate was then centrifuged (Sigma 4K15, Germany, 21,190 g, 10 min, 4°C) and the supernatant was collected. The supernatant was initially used to assess soluble solid content (SSC) and total acidity (TA) and then stored in -20°C until further analysis. SSC was quantified with the employment of a refractometer (Atago, PR-32 α , Japan) and results expressed as $^{\circ}\text{Brix}$. TA was determined using an automatic titrator with multiple positions (862 Compact Titrosampler, Metrohm AG, Switzerland). For each measurement, 1 mL of diluted juice in 49 mL distilled H_2O was used for titrating with 0.1 N NaOH to a pH end point of 8.1. Results were expressed as % citric acid, and the ripening index (RI) was calculated as the SSC/TA ratio.

Fructose, glucose and sucrose contents were quantified using the homogenate samples which were thawed before the analysis. From the homogenate, 2 mL were transferred into an Eppendorf tube and

centrifuged at 6163 g speed for 10 min and 1 mL of the supernatant was transferred to a new Eppendorf tube. Following this step, 1 mL of the mixture solvent mixture (acetonitrile/water, 80:20) was added and vortexed. The mixture was then filtered through PVDF $0.45 \mu\text{m}$ filters. Glucose, fructose and sucrose contents were quantified using a high-performance liquid chromatography system (Waters 1525–2707) coupled with a dual λ absorbance detector (Waters 2487) following a method by Jalaludin and Kim (2021) with some modifications. Briefly, the chromatographic separation of the analytes was achieved on a Waters SPHERISORB[®] NH_2 column ($4.6 \times 150 \text{ mm}$, $3 \mu\text{m}$), and the mobile phase consisted of water:acetonitrile (80:20). The flow rate was set at 1.0 mL/min, and the elution was isocratic. The injection volume was 15 μL and the column temperature set at 25°C . The detection wavelength was set at 190 nm.

The estimation of sugar concentrations was achieved by constructing a calibration curve for each analyte within the concentration range of 0.3–15 mg/mL. All measurements were performed in triplicate. Method validation included evaluation of linearity (correlation coefficients, slopes, and intercepts), limits of detection (LOD) and quantification (LOQ), calculated as $\text{LOD} = \frac{3.3\sigma}{S}$ and $\text{LOQ} = \frac{10\sigma}{S}$, where σ is the standard deviation of response, and S is the slope of calibration curve. The precision of an analytical method is expressed as the relative standard deviation, %RSD of the repeatability (intra-day) and intermediate precisions (inter-day) of three analyses ($n = 3$) during the same day and over three days studied, respectively. Precision was assessed at a concentration of 5 mg/mL of each analyte standard. The method was considered acceptable when the %RSD value was lower than 15 %. For recovery evaluation, a concentration of 6.25 mg/mL of each sugar standard was spiked into a raspberry sample matrix. The results of the validation parameters are presented in **Supplementary Table 3**. Additionally, quality control (QC) standards (10 mg/mL) analyzed between sample runs showed percent errors of 0.3–5.4 % for fructose, 1.9–4.7 % for glucose, and 2.4–5.1 % for sucrose.

2.5.2. Quantification of total phenolic, reduced ascorbic acid, anthocyanin and flavonoid content

Previously flash-frozen raspberry fruit samples were ground into fine powder using the basic A11 analytical mill (IKA Mills) and liquid nitrogen. Extraction procedure of total phenolic compounds was followed as described by Shehata et al. (2020) with some alterations: 1.5 mL of 50 % v/v methanol was added to 0.05 g of ground raspberry fruit and vortexed. Next, the mixtures were placed at -20°C for 24 h. Subsequently, samples were centrifuged for 10 min at 16 000 g at 4°C (Eppendorf Centrifuge 5415 R), and the supernatant was stored at -20°C . The total phenolic content was estimated by the method of Georgiadou et al. (2018) with slight alterations. The reaction mixture consisted of 100 μL of the diluted 50 % v/v methanol extract, 0.5 mL of distilled water, and 0.05 mL of the Folin-Ciocalteu reagent. After 3 min, 0.1 mL of saturated sodium carbonate solution was added, and the mixture was made up to 1 mL with distilled water. The mixture was thoroughly mixed, left to stand for 1 h in the dark at room temperature, and the absorbance was measured at 765 nm. Each measurement was repeated three times, and the results were expressed as mg gallic acid equivalent (GAE) 100 g^{-1} F.W.

Reduced ascorbic acid content was quantified in accordance to Habibzadeh et al. (2019) with some modifications. In short, 0.2 g was vortexed with 1.5 mL 2 % w/v metaphosphoric acid. Afterward, samples were centrifuged for 1 min at 16 000 g at 4°C (Eppendorf Centrifuge 5415 R), and the supernatant was used for the analysis. The reduced ascorbic acid was estimated by the method of Georgiadou et al. (2018) with some alterations. First, 500 μL of the diluted 2 % w/v metaphosphoric acid extract was added to 900 μL of 50 mmol L^{-1} 2,6-dichloroindophenol and the absorbance was monitored at 520 nm. Ascorbic acid (AsA) content was quantified using a standard curve and expressed as mg 100 g^{-1} F.W.

Extraction of total anthocyanin content was performed following the procedure of Bal & Ürün (2021) with some modifications: 1 mL of 95 % v/v ethanol: 0.1 N HCl (85:15) was added to 0.1 g of ground frozen raspberry fruit and vortexed. Successively, the mixtures were placed at -20°C for 24 h, then centrifuged for 10 min at 16 000 g at 4°C (Eppendorf Centrifuge 5415 R), and the supernatant was stored at -20°C . Total anthocyanin content was estimated by the pH-differential assay, using two buffer systems: potassium chloride buffer (0.025 M) at pH 1.0 and sodium acetate buffer (0.4 M) at pH 4.5 (Georgiadou et al., 2018). Samples were diluted in pH 1.0 and pH 4.5 buffers and their absorbances were subsequently measured at 510 and 700 nm. Anthocyanin concentration was computed as mg cyanidin-3-glucoside 100 g^{-1} of F.W.

Total flavonoid content was estimated according to the method described by Meyers et al. (2003) with minor modifications. 10 mL of acetone was added to 1 g of ground frozen raspberry fruit and vortexed. Next, the mixtures were placed at -20°C for 24 h. Subsequently, samples were centrifuged for 10 min at 16 000 g at 4°C (Eppendorf Centrifuge 5415 R), and the supernatant was stored at -20°C . Total flavonoids content was estimated by the method of Chang et al. (2020) with slight modifications. The reaction mixture consisted of 0.5 mL plant extract, 1.5 mL of 95 % v/v ethanol, 0.1 mL of 10 % w/v aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The absorbance of the reaction mixture was measured at 415 nm after incubation at room temperature for 30 min. The results were expressed as mg quercetin 100 g^{-1} F.W.

2.6. Polyphenolic compound analysis

The polyphenolic compound analysis by HPLC-DAD-ESI-MS/MS was performed according to Salazar-Orbea et al. (2022). Phenolics identification and quantification were carried out on an Agilent 1100 HPLC system equipped with a photodiode array detector (G1315D) and coupled in series to an HCT Ultra Bruker Daltonics ion trap mass spectrometer through an electrospray ionization (ESI) interface HPLC-DAD-ESI-MS/MS. The chromatographic separation was performed using a Poroshell 120 EC column ($3 \times 100\text{ mm}$, $2.7\text{ }\mu\text{m}$) from Agilent Technologies (Waldbronn, Germany). Phenolic compounds were identified by their UV spectra, retention time, molecular weight, and MS/MS fragmentation pattern. Phenolic compounds quantification was performed using the authentic standards of castalagin (280 nm), catechin (280 nm), *p*-coumaric acid (320 nm), pelargonidin (520 nm), ellagic acid (360 nm) and quercetin (360 nm) to quantify ellagitannins, flavan-3-ols, hydroxycinnamic acids, anthocyanins, ellagic acid conjugates and flavonols respectively.

2.7. Melatonin content

Melatonin extraction and quantification was carried out using a Melatonin ELISA Kit following the manufacturer's instructions (Enzo Life Sciences, Farmingdale, NY, USA).

2.8. Volatile organic compounds

Five grams of frozen powdered raspberry fruit tissue was placed in 50 mL tube and 5 mL of 1 M NaCl solution was added. The mixture was homogenized using the IKA® T25 digital ULTRA-TURRAX® (IKA Mills) and samples were stored at -80°C until further analysis. Prior to the analysis, samples were transferred to a headspace vial (20 mL) and incubated in a water bath (40°C) for 35 min. After the incubation, the volatile compounds were extracted by SPME fiber (50/30 μm DVB/CAR/PDMS, Stableflex (2 cm), 23Ga) for 40 min. Immediately after the 40 min of extraction, the SPME fibre was injected manually into the inlet of the GC-MS.

A Shimadzu GCMS-QP2010 Plus System was used for the determination of the VOC composition of raspberries aroma, following the

protocol described by Vandendriessche et al. (2013) with some modifications. GL Sciences InertCap 5MS 30 m - 0.25 mm - $0.25\text{ }\mu\text{m}$ film thickness capillary column was used. Helium (He) was used as carrier gas (1.2 mL/min) at high purity. The samples were injected manually in splitless mode. The injection temperature was set at 250°C . The oven was programmed as follows: hold at 35°C for 5 min, increase to 150°C in 4°C/min increments, then to 240°C in 50°C/min increments. The temperature remained at 240°C for 5 min. The MS Detector operated in EI mode (full scan, *m/z* range: 30–350). The EI-mass spectra were compared with the NIST library spectra.

2.9. Statistical analysis

Statistical analysis for quality attributes involved first one-way ANOVA analysis and then Tukey-HSD post-hoc pairwise comparison test at $p \leq 0.05$ using SPSS v.25 (SPSS Inc., Chicago, IL, USA). Biochemical data were statistically analysed using one-way ANOVA followed by Tukey-HSD post-hoc test ($p \leq 0.05$) both performed in GraphPad version 10.4.1 (GraphPad Software, San Diego, CA, USA). Principal component analysis (PCA) and heatmap were created using ClustVis 2.0 according to Metsalu and Vilo (2015). For metabolites the data were normalized to the control. For VOCs the data matrices of the original component data (VOCs concentrations versus treatment) were standardized in order to present (via a hierarchical clustering analysis heatmap) differences in the relative VOCs content. Euclidean distance was used as the clustering distance metric.

3. Results and discussion

3.1. Morphophysiological responses

The effect of PAs on plant architecture was assessed in this study to evaluate the impact of priming on canopy size. Canopy size as well as total number of laterals determines the yield efficiency of primocane raspberry plants. The priming agents tested did not significantly influence canopy development and plant architecture when compared with control samples (Supplementary Figure 1). Over the 5-month experimental period, physiological measurements did not show any striking differences among treatments applied, although the pattern was variable (Fig. 1). Leaf temperature and leaf vapour pressure deficit play an important role on the stomatal conductance and transpiration rate. Transpiration as well as stomatal conductance were slightly higher at 35 DAP and decreased along the developmental period and closer to the flowering period (Fig. 1).

Leaf vapour pressure deficit (VPD) can be defined as the difference between the moisture holding capacity of the atmosphere (saturation vapor pressure) and moisture currently present in the atmosphere (vapor pressure). This is highly influenced by changes in temperature as higher temperatures increase the saturation capacity of the atmosphere at a faster rate compared to actual vapor pressure, leading to higher VPD (Grossiord et al., 2020). Higher values of VPD negatively influence stomatal conductance and transpiration as plants activate water-conservation mechanisms to minimize water loss. Therefore, fluctuations observed in stomatal conductance and transpiration are highly driven by temperature and VPD as well as the transition along the different developmental stages.

Biochemical analysis of cellular damage indicators from leaves harvested prior to flowering revealed no significant differences among treatments tested. Such results can be considered as an indicator that no harmful effect due to the priming treatments on leaves occurred (Supplementary Figure 2). Yet, additionally time points need to be tested to confirm this hypothesis.

3.2. Yield performance

Significant improvements due to melatonin pre-treatment were

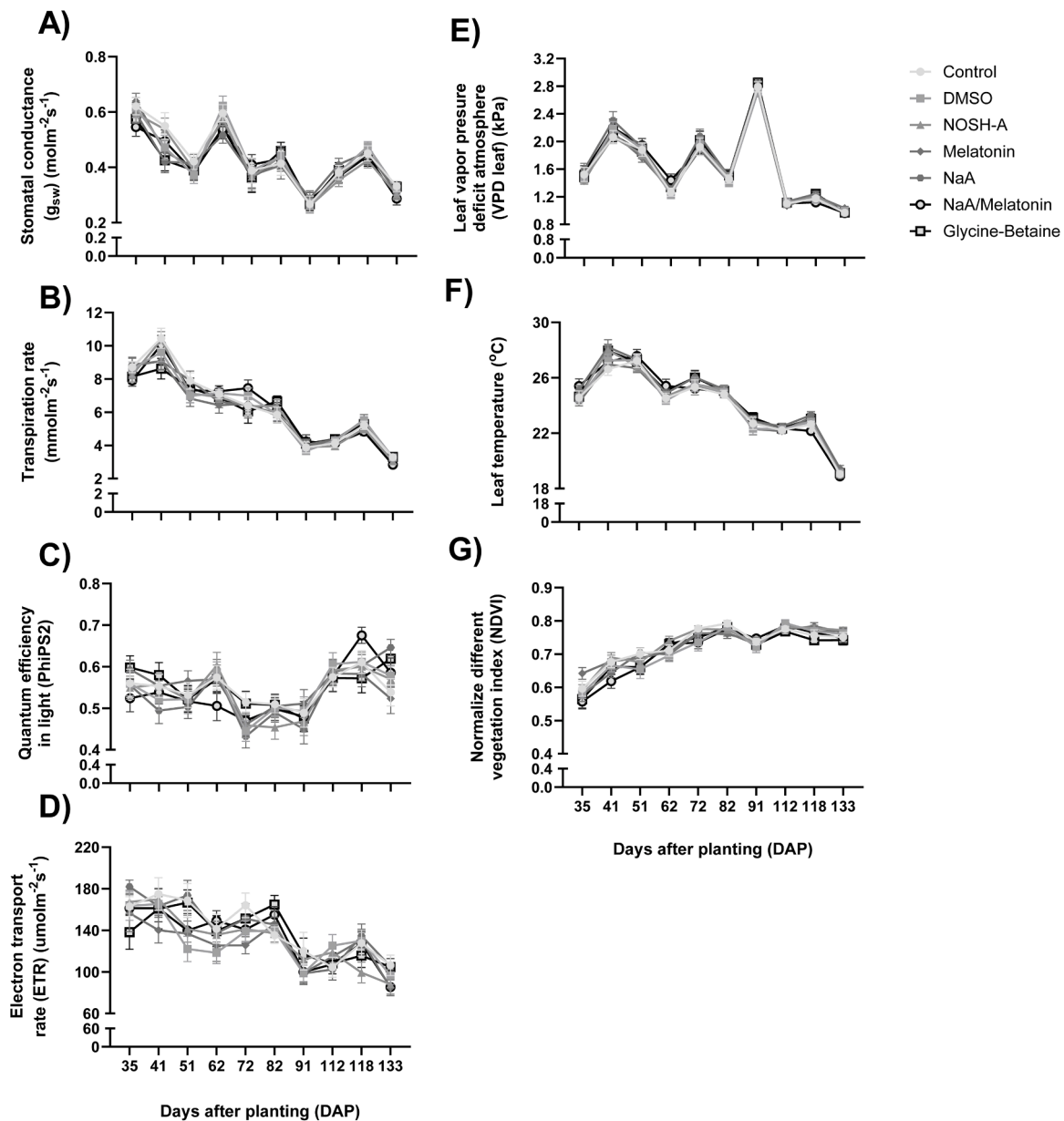


Fig. 1. The effect of pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/melatonin and glycine-betaine on A) stomatal conductance (g_{sw}), B) transpiration rate, C) quantum efficiency in light (PhiPS2), D) electron transport rate (ETR), E) Leaf vapor pressure deficit atmosphere (VPD leaf), F) Leaf temperature, G) Normalized difference vegetation index (NDVI) of raspberry leaves (cv. 'Vica Abril').

observed in the yield performance of the primocane raspberry 'Vica Abril' (Fig. 2). Early in the harvest period, at 118 DAP, melatonin primed plants showed pronounced differences in cumulative yield (ca. a 4-fold increase) compared with the hydro-primed control (Fig. 2C). By the end of the harvesting period (150 DAP), cumulative yield in melatonin-treated plants maintained a significant higher yield compared with the hydro-primed plants (Fig. 2D). Notably, yield improvements observed due to melatonin declined over time suggesting that the effectiveness of priming was transient (Fig. 2B). Overall, melatonin can serve as a sustainable strategy to improve raspberry productivity early in the harvesting period, which is particularly important during periods of limited fruit availability. In another soft fruit crop, strawberry, the melatonin application mitigated the negative effects of salinity stress, leading to increased fruit yield and quality. This was achieved by enhancing the plant's antioxidant defense systems and improving photosynthetic efficiency (Zahedi et al., 2020).

3.3. Fruit quality attributes

Titrateable acidity (TA) of fruits refers to the concentration of all organic acids present and significantly impacts fruit's taste, texture, and overall quality. In the present study, plants pre-treated with melatonin, NOSH-A and NaA, exhibited significantly higher TA compared with the hydro-primed controls (Table 1). Soluble solids content (SSC) ranged from 8.5–9.7 % which is consistent with other studies (Ancos et al., 1999; Pantelidis et al., 2007). Current findings showed no significant impact of priming on SSC of raspberries, aligning with previous reports where the pre-harvest application of melatonin and glycine betaine on raspberries and cherries, respectively, did not result to any significant change in SSC (Li et al. 2019; Shah et al., 2024).

While no significant differences were observed in SSC among treatments, fructose, sucrose and glucose content were quantified to assess potential differences in sugar composition which may have a significant impact on fruit flavour (Fig. 3E, F, G). Concentration of the sugars in

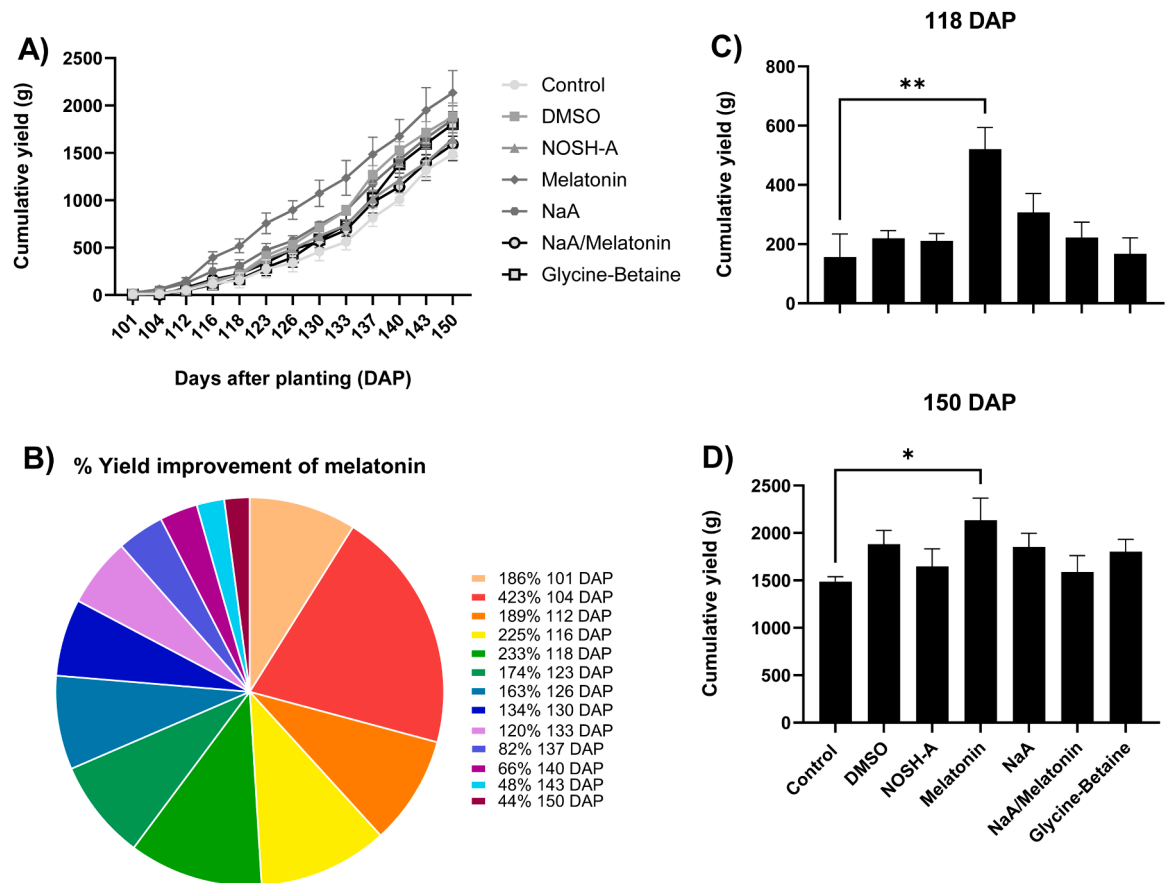


Fig. 2. A) Average cumulative weight (g) for the thirteen harvests along the harvest season, B) % yield improvement of melatonin per growing season, C) average cumulative weight (g) for the first five harvests per treatment and D) average cumulative weight (g) for the thirteen harvests per treatment of harvested raspberry fruits (cv. ‘Vica Abril’). ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

Table 1
The effect of pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/Mel and GB on fruit quality attributes [soluble solid content (SSC), total acidity (TA) and RI (SSC/TA)] of raspberry fruits (cv. ‘Vica Abril’).

Treatment	SSC (° Brix)	TA (% citric acid)	RI (SSC/TA)
Control	9.25 ± 0.31 a	2.47 ± 0.07 b	3.76 ± 0.19 a
DMSO	8.45 ± 0.17 a	2.70 ± 0.04 ab	3.13 ± 0.07 a
NOSH-A	8.90 ± 0.27 a	2.77 ± 0.04 ab	3.22 ± 0.10 a
Melatonin	9.01 ± 0.36 a	2.91 ± 0.11 a	3.14 ± 0.20 a
NaA	8.70 ± 0.17 a	2.88 ± 0.11 a	3.03 ± 0.11 a
NaA/Melatonin	9.70 ± 0.53 a	2.56 ± 0.06 ab	3.80 ± 0.26 a
Glycine-Betaine	8.78 ± 0.23 a	2.58 ± 0.04 ab	3.40 ± 0.05 a

raspberry juice can be highly variable ranging from 1.0–6.0, 0.8–4.7, 0.1–3.0 g 100 mL⁻¹ for fructose, glucose, and sucrose, respectively and is dependent on the cultivar as well as mesoclimate conditions (Spanos and Wrolstad, 1987; Viljakainen et al., 2002). Results presented herein, showed that NOSH-A significantly improved sucrose content up to 35 %, while NaA treatment resulted in lower sucrose contents compared with the hydro-primed (control) samples. Furthermore, NaA alone or in combination with melatonin treatments exhibited lower levels of fructose and glucose. Observed increases in sucrose content following NOSH-A treatment could be attributed to altered sucrose metabolism levels, as individual components of the donor (i.e. NO, H₂S, and aspirin) have all been shown to increase activity levels of sucrose synthase and sucrose phosphate synthase (Jiang et al., 2007; Huang et al., 2020; Gao et al., 2024). In general, the effect of priming on sugar content can be variable in different crops and conditions. Okatan et al. (2022) investigated the effect of melatonin on four strawberry cultivars with no

significant effect being reported on sugar content, while reports on priming with melatonin in grapevine and plum trees, indicated enhanced sucrose content, without however the exact mechanism to be clear (Xia et al. 2021; Xiao et al., 2024). Some studies indicated that melatonin increases sucrose content by promoting the activity of sucrose phosphate synthase, while others propose that melatonin increases the activities of sucrose synthase (in the catabolic direction), sucrose phosphate synthase, glucokinase, and fructokinase (Xiao et al., 2024). However, in the present study, melatonin pre-treatment did not influence sugar content in raspberries. Nevertheless, a more in-depth understanding of the effects of different priming agents on sugar biosynthesis across different crops is needed.

3.4. Total phenols, flavonoids, anthocyanins and reduced ascorbic acid content

Raspberry fruits are known for their high content of polyphenolic compounds including phenolic acids, flavonoids, and anthocyanins (Sariburun et al., 2010). In the present study, priming application did not result in any significant difference on the total phenolic content with the average concentration of total phenolics being 160.7 mg gallic acid equivalents 100 g⁻¹ FW (Fig. 3A). Contradictory results have been reported in a recent study, where exogenous melatonin application (50 and 200 µmol L⁻¹) led to improved TPC in raspberries (Shah et al., 2024). Overall, total phenolic content (TPC) may vary significantly depending on the cultivar and the harvest period (Liu et al., 2002; Bobinaite et al., 2012). To what extent the cultivar under study may possessed different TPC if harvested under different microclimate conditions or at other time points need to be further elucidated. This is also

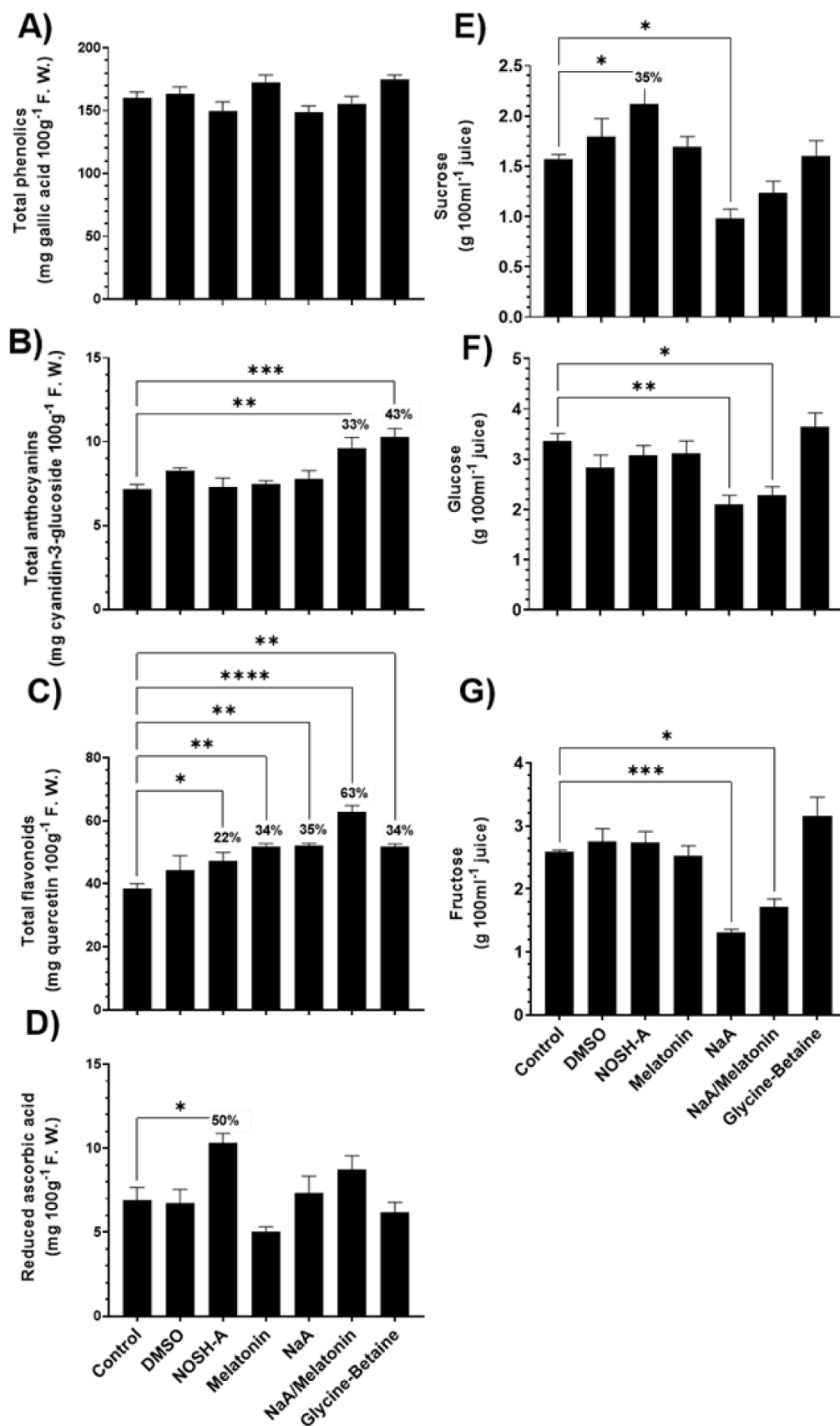


Fig. 3. The effect of pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/melatonin and glycine-betaine on A) total phenolics, B) total anthocyanins, C) total flavonoids, D) reduced ascorbic acid, E) sucrose, F) glucose, G) fructose of raspberry fruits (cv. 'Vica Abril'). ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

the case if analysis were conducted among different raspberry cultivars in order to explore the effect of the genetic background.

Anthocyanin content of Mel/NaA conjugate and GB priming applications led to modest, yet significant increase (Fig. 3B). Specifically, Mel/NaA treatment led to 33 % increase in total anthocyanins, while GB treatment resulted in 43 % improvement in anthocyanin content (Fig. 3B). Raspberries contain significant levels of anthocyanins, often exceeding 35 mg cyanidin-3-glucoside equivalents 100 g⁻¹ FW (Pantelidis et al., 2007), with major compounds including cyanidin-3-sophoroside, cyanidin-3-glucoside, and pelargonidin-3-sophoroside (Ancos et al., 1999).

While all priming applications resulted in >22 % of improvement in total flavonoids, the impact of Mel/NaA conjugate was most significant with improvements of up to 63 % compared with the hydro-primed control (Fig. 3C). A recent study demonstrated the positive impact of Mel pre-treatment in total flavonoid content (Shah et al., 2024). Flavonoids are a major group of polyphenols present in raspberries, known for their antioxidant, anti-inflammatory, and cardiovascular health benefits (Yao et al., 2004; Sariburun et al., 2010). Among them, quercetin and kaempferol are particularly dominant (Bradish et al., 2012).

A further notable result identified, was the effect of NOSH-A treatment in reduced ascorbic acid content which resulted in a 50 % increase (Fig. 3D). Zhang et al. (2024), demonstrated that salicylic acid impacts ascorbate-glutathione cycle in prune fruits, improving ascorbic acid content by up-regulating the activity of key metabolic enzymes. Given the presence of an acetylsalicylic acid moiety in the NOSH-A chimera, it is plausible to employ similar mechanistic action.

Raspberries are known for their high ascorbic acid (vitamin C) content which is recognized for its high antioxidant potential and ability to counteract oxidative stress (Ponder and Hallmann, 2020). Ascorbic acid content is influenced by different factors such as cultivar and harvest period, with reported levels typically exceeding 20 mg per 100 g of fresh fruit (Pantelidis et al., 2007; Ancos et al., 1999; Ponder and Hallmann, 2020). Our study demonstrates the importance of NOSH-A in the improvement of ascorbic acid content, especially in cultivars which tend to demonstrate lower ascorbic acid content.

3.5. Polyphenolic compound analysis

Polyphenol analysis conducted in this study, led to the identification and quantification of 13 polyphenolic compounds in raspberry fruits, following the application of different priming agents along plant development. Significant improvements due to priming application were observed in caffeoylglucose, cyanidin 3-O-sophoroside, cyanidin 3,5-diglucoside, kaempferol derivative, cyanidin-3-O-glucoside, ellagic acid pentoside and ellagic acid acetyl pentoside (Fig. 4). The compound corresponding to either Cyanidin 3-O-sophoroside or Cyanidin 3,5-diglucoside could not be fully determined as both metabolites share the same mass and fragmentation pattern. In addition, some ellagic acid and conjugated derivatives could not be fully distinguished due to the similarity of the spectra between them. Furthermore, various ellagitannins were also present in the samples but were below the threshold of detection and thus are not included in the metabolomics analysis. The most significant effect due to priming application was observed in the content of kaempferol derivative, with NaA and NaA/Mel treatments resulting in a three-fold increase in its content compared with the control (Fig. 4E). Additionally, elevated levels of kaempferol derivative were also observed as a result of Mel and GB treatments ($p < 0.01$). Among all priming applications, NaA and NaA/Mel treatments had the most significant effects on enhancing the content of different polyphenolic compounds identified in the present study (Fig. 4).

In order to evaluate the patterns observed in the metabolomics data and assess whether these patterns are associated with priming application, a principal component analysis (PCA) was performed. The approach visualizes the data in a two-dimensional space based on the directions of maximum variance. Distinct separation of different priming treatments suggests differential effects on the polyphenolic

composition of raspberry fruits. The first two principal components summarize 80.5 % of total variance (PC1=58.8 %; PC2=21.7 %) (Fig. 5A). PCA loading values indicate the contribution of each polyphenolic compound to the principal components. Significant impact of kaempferol derivative on both PC1 and PC2 was observed. PC1 is largely influenced by kaempferol derivative (0.581), cyanidin-3-O-sophoroside/Cyanidin 3,5-diglucoside (0.437), and cyanidin-3-O-glucoside (0.423), suggesting that variation of PC1 is driven by the differences observed in anthocyanins and flavonols between treatments. Kaempferol derivative (0.581) and catechin (0.351) strongly influence the variability observed in PC2. Separation of priming agents in PCA is strongly influenced by the variation observed in the flavonol and anthocyanin content (Fig. 5A,B). Due to the lack of authentic standards of the cyanidin glycosides, and the almost identical fragmentation patterns, it was not possible to distinguish between cyanidin 3-O-sophoroside and cyanidin 3,5-diglucoside and thus both are considered when dealing with the cyanidin dihexosides present in raspberries. The same happens with some isomers of ellagic acid acetyl-pentoside and ellagic acid pentosides, in which MS spectrometry fragmentation does not allow the differentiation between isomers, and authentic standards are not available.

An overview of the results of the analysis of all detected anthocyanin, catechins, flavonols and phenolic acids are shown in the form of heatmap (Fig. 5C). Strong impact of priming is observed in the anthocyanin group as well as flavonoid group, highlighting the significant impact of melatonin, NaA, NaA/Mel conjugate and GB betaine on polyphenolic compound content (Fig. 5C).

Polyphenolic compounds identified through the metabolomic analysis in this study were in accordance with other publications investigating raspberry secondary metabolites (Carvalho et al., 2013; Renai et al., 2021; Zhang et al., 2018). Interestingly, NaA and NaA/Mel treatments had a significant effect on kaempferol derivative content. Kaempferol is a known flavonoid recognized for its high antioxidant and health promoting-effects, like anti-inflammatory, anticancer, antidiabetic, and neuroprotective properties (Calderon-Montano et al., 2011; Parveen et al., 2023). Similarly, NaA/Mel treatment resulted in increased cyanidin-3-O-glucoside and cyanidin 3,5-diglucoside contents. Given that NaA/Mel treatment led to a 33 % increase in total anthocyanins, it is very likely that increase is driven by these compounds (Fig. 3B). These two compounds belong to the anthocyanin group and have high antioxidant potential (Zhang et al., 2023). PCA further supports these trends as both kaempferol derivative and cyanidin glycosides had the biggest contribution to the principal components associated with treatment differences (Fig. 5A,B).

These results indicate that priming agents have the potential of influencing not only the total flavonoid and anthocyanin content, but also affect the composition of key bioactive compounds improving fruit functional value. Interestingly, a recent report on the direct pre-harvest application of NaA/Mel conjugates on strawberry fruit at three successive developmental stages, namely large green (LG), small white (SW) and large white (LW), revealed similar trends in increased contents in ellagitannins, flavan-3-ols and ellagic acid and their conjugates in fully-ripe harvested fruit (Georgiadou et al., 2025). The present study highlights the promising potential involving novel priming agents like NaA/Mel in enhancing the nutritional value of raspberry fruits through the targeted increase in the content of health promoting polyphenolic compounds.

3.6. Endogenous melatonin content

Plants treated with Mel and NaA/Mel showed that harvested fruits contained higher levels of melatonin compared with the hydro-primed control, supporting their successful donation (Supplementary Figure 3). These results further support the stability of melatonin within plant structure, suggesting its potential integration into different metabolic processes (Antonίου et al., 2017). Elevated levels of melatonin

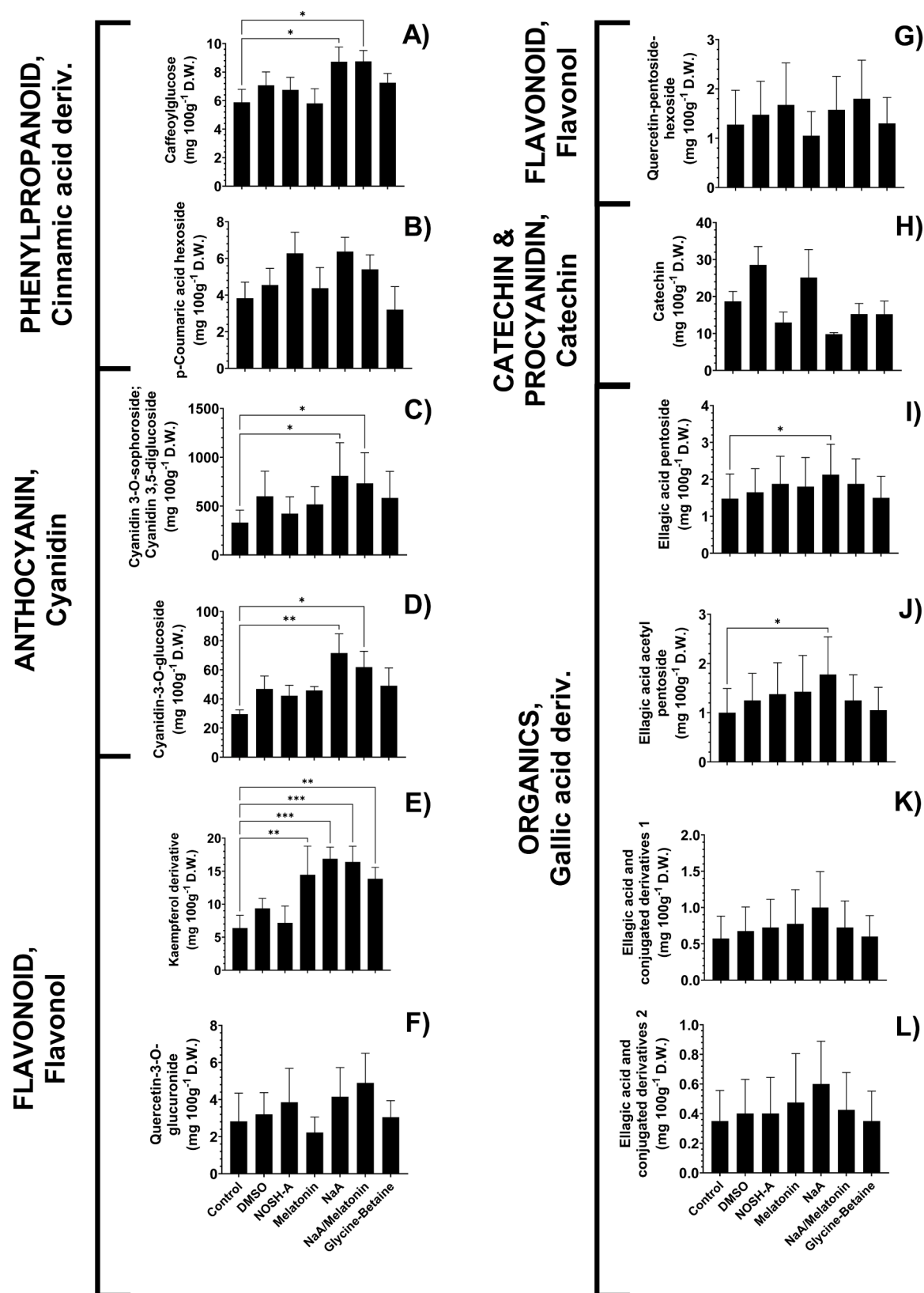


Fig. 4. Content of individual classes (phenylpropanoid, anthocyanin, flavonoid, catechin and procyanidine, organics,) of metabolomics data in raspberry fruits (cv. 'Vica Abril') after the pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/melatonin and glycine-betaine. ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, **** = $p \leq 0.0001$.

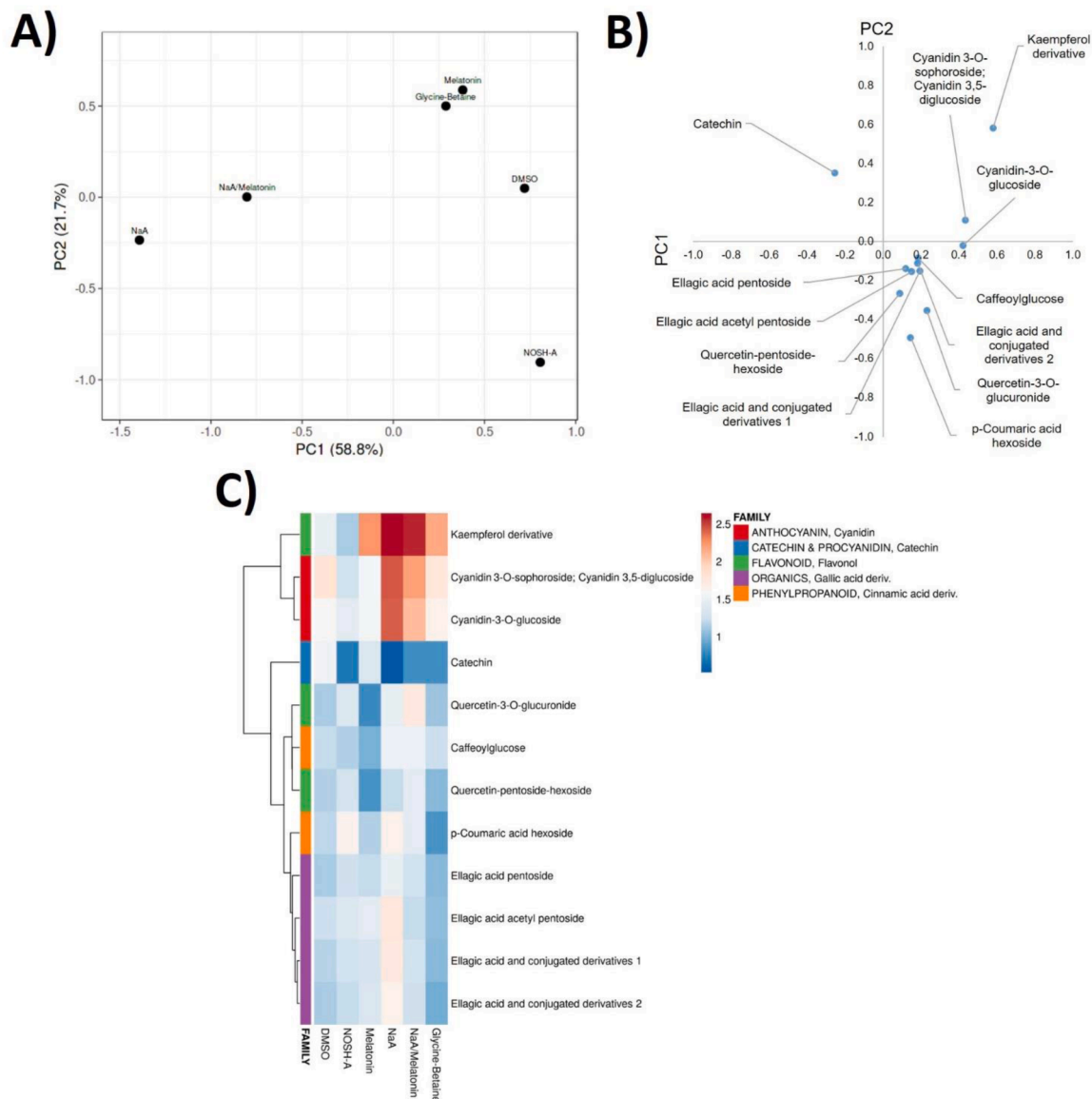


Fig. 5. A) Principal component analysis (PCA), B) xy-scatter plot graph of metabolites and C) heatmap representing the fold changes of metabolomics data of raspberry fruits (cv. 'Vica abril') after the pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/melatonin and glycine-betaine. The data were normalized to control.

content may also indicate the induction of the transcript levels of key enzymes involved in melatonin biosynthesis following exogenous melatonin application, leading to *de novo* synthesis (Priti et al., 2024).

3.7. Volatile compounds in raspberry fruits

The analysis of VOCs in raspberry fruits identified a total of 98 distinct compounds (Supplementary Table 1). Among all the samples analysed, the most abundant compounds detected included β -ionone, ethyl acetate, ethanol, α -ionone, 2-hexenal, n-hexanal, 3-hexenal, and acetaldehyde. These compounds were consistently present across all the samples, highlighting their prominence in the volatile profile of raspberry fruits.

Volatile compounds are typically associated with scents detectable by the human nose, often contributing to pleasant aromas and flavours. However, volatile compounds in plants also have diverse ecological and functional roles: they attract pollinating insects, signal the ripeness of fruits for seed dispersal, and alleviate the effects of abiotic stress (Aprea et al., 2015). Most of the compounds that have been identified in this

study have been reported in previous studies of volatile compounds in raspberries (Aprea et al., 2015; Gu et al., 2022; Zhang et al., 2021).

The PCA score plot illustrates the distribution of the replicate treatments based on their principal component scores (Supplementary Figure 4A). The first two principal components, PC1 and PC2, accounted for 43.7 % of the total variance in VOCs. One of the NOSH-A replicate samples showed to be a clear outlier while the scores for all other treated samples were mixed up, showing no clear separation between the treatments. If any effect was visible this would be the slight separation of the control treatment from all other treatments along PC2. However, further analyses using PLS did not reveal any consistent differences (data not shown).

VOCs with an average contribution greater than 0.5 % across all treatments were considered as main components contributing to the raspberry aroma and their characteristic fragrance is represented in the clustering analyses of Fig. 6. Compounds, such as acetone and acetaldehyde are typically associated with oxidation products. Acetaldehyde is connected with the fermentation, fruit ripening and senescence, while acetone has been associated with fermentative degradation of sugars

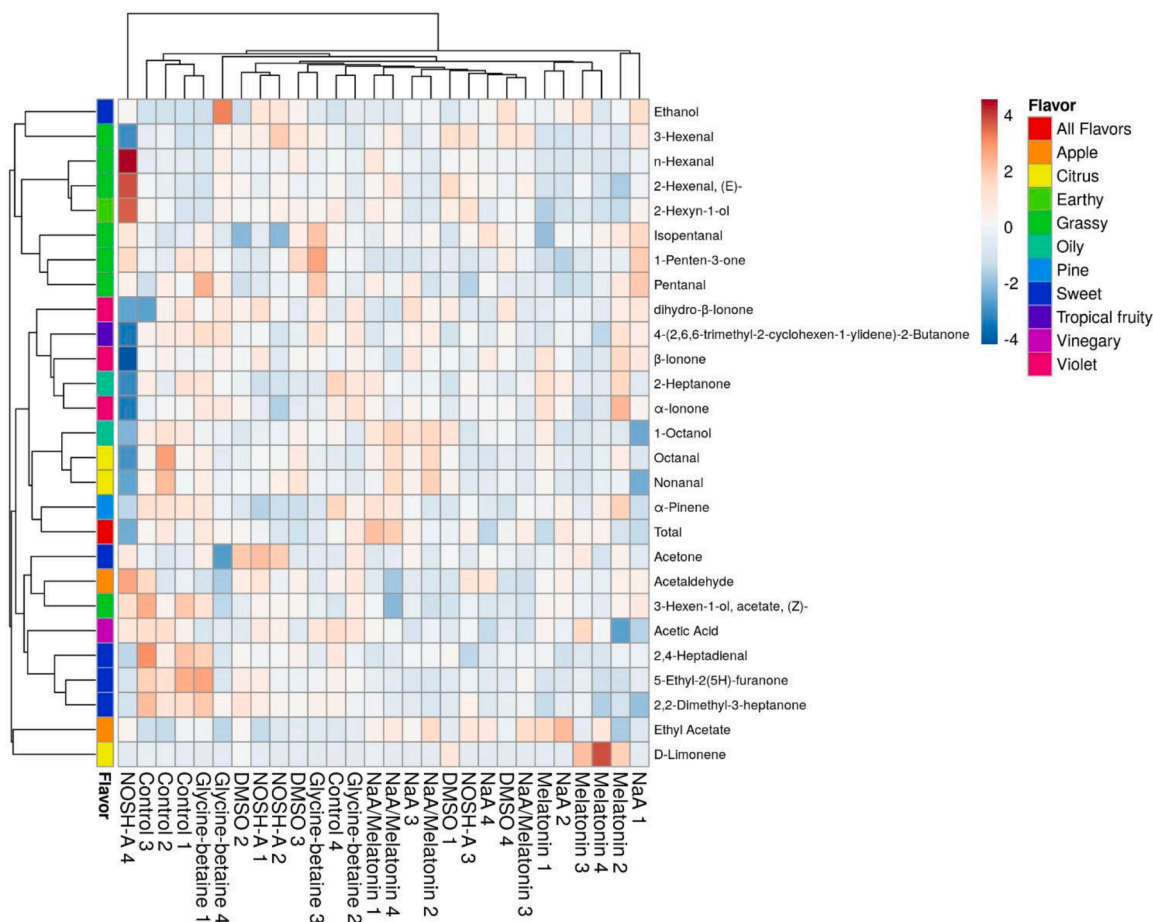


Fig. 6. Heatmap representing the fold changes of VOCs data of raspberry fruits (cv. 'Vica Abril') after the pre-harvest application of DMSO, NOSH-A, melatonin, NaA, NaA/melatonin and glycine-betaine. The data were standardized as described in Section 2.9 Statistical analysis.

and lipids (Strommer and Garabagi, 2009; Chierici et al., 2015; Mus et al., 2020). Moreover, compounds like n-hexanal, (E)-2-hexenal and 3-hexenal represent a distinct chemical sub-group, often associated with grassy notes (Akkad et al., 2023; Su et al., 2020; Yang et al., 2022).

Compounds such as isopentanal, α -pinene, pentanal, 2-heptanone and the α - and β -ionone suggest a contribution from fruity and floral aromas (Amanpour et al., 2019; Aprea et al., 2015; Ma et al., 2022). Ionones (α - and β -) are significant compounds, derived from the same carotenoid-based biosynthetic pathway (Aprea et al., 2015), and both linked to floral notes, which are characteristic of raspberry fruit.

D-limonene was high in three of the four Mel-treated samples corresponding to a citrus-like aroma in raspberry fruits (Ibáñez et al., 2020). An increase in limonene content can enhance the fruity and fresh sensory attributes of the fruit, making it more appealing to consumers (Gu et al., 2022; Paterson et al., 2013). Moreover, this terpene has been associated with various health benefits, such as anti-inflammatory and anti-obesity effects, suggesting that higher limonene content could potentially enhance the health-promoting properties of raspberry fruits (Gu et al., 2022). Arnao et al. (2022) reported that melatonin can increase the content of compounds such as limonene and β -caryophyllene.

Subsequently individual compounds were grouped based on their chemical classes to reveal a possible overall shift in the VOC pathways (Fig. 7). The main shift was observed in terms of total aldehydes and terpenoids. Mel-treated samples exhibited the lowest concentrations of aldehydes and the highest concentrations of terpenoids compared with all the other treated and control samples. Aldehydes are generally associated with not only secondary (Gutensohn et al., 2011) but also

primary metabolism, as they are involved in lipid peroxidation, carbohydrate metabolism, and amino acid metabolism (O'Brien et al., 2005; Rizzo, 2014). In contrast, terpenoids are primarily linked to secondary metabolism (Chen et al., 2011). This pattern indicates that melatonin may enhance the activity of enzymes involved in secondary metabolic pathways, particularly those related to terpenoid biosynthesis. A recent study by Eghlima et al. (2025) confirmed that melatonin enhances the essential oil yield of *Thymus vulgaris* under water-deficit conditions. This increase in secondary metabolites was attributed to stimulated terpenoid biosynthesis, driven by enhanced meristem activity and the activation of enzymatic pathways. These findings align with the increased concentration of the terpenoid d-limonene observed in the Mel-treated samples of the present study. Similarly, Arnao et al. (2022) reported that exogenous melatonin positively influences the expression of genes involved in terpenoid biosynthesis, thereby contributing to the increased accumulation of terpenes, including limonene, as previously discussed. Interestingly, the direct pre-harvest application of Mel and NaA/Mel on strawberry fruit during different developmental stages prior to fully ripe stage did not lead to a significantly altered aroma profile in comparison with hydro-primed control samples (Georgiadou et al., 2025), highlighting the importance of the timing of application for potential aroma effects.

In spite of the observed shift from aldehydes to terpenoids in Mel treated fruit (Fig. 7), largely induced by the change in d-limonene, given the lack of a consistent sample clustering (Fig. 6 and Supplementary Figure 4A) no clear discrimination was observed between priming treatments.

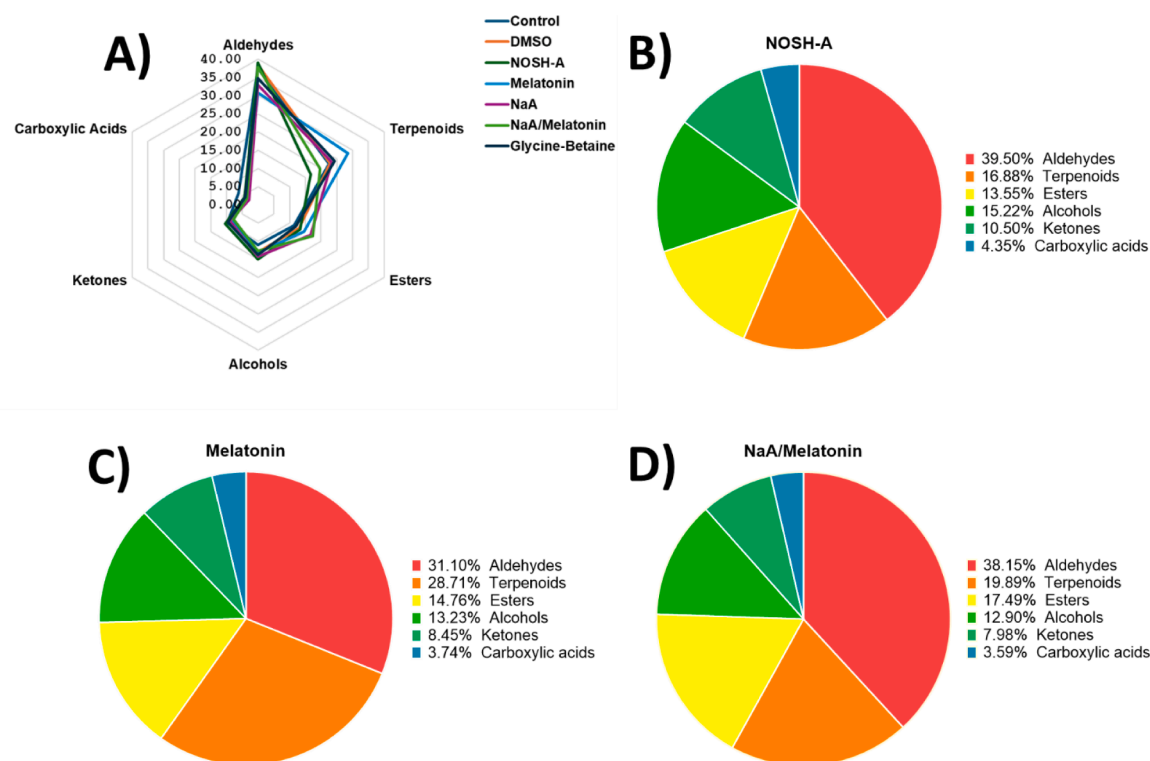


Fig. 7. A) Radar chart of the higher average percentage (%) of chemical groups of VOCs across the different treatments and pie chart of the B) NOSH-A, C) melatonin and D) NaA/melatonin treatments with the higher percentage (%) of chemical groups of VOCs of raspberry fruits (cv. 'Vica abril').

4. Conclusions

The present study provides new knowledge on the impact of selected priming agents on vegetative growth, physiological performance, yield efficiency and fruit primary and secondary metabolites of a primocane raspberry cultivar. While priming did not affect vegetative growth and physiological parameters, significant improvements were observed in yield and/or fruit quality attributes in a priming agent-dependent manner. Notable transient improvements in early harvests were observed in melatonin-primed plants, suggesting time-dependent yield improvement which is particularly important in periods of low fruit availability. Additionally, pre-treatment with NaA and NaA/Mel along the developmental period led to alterations on fruit sugar composition and polyphenolic content, with special reference to enhanced contents in kaempferol derivatives, total flavonoids, and anthocyanins, compounds with potent antioxidant properties. NOSH-A treatment demonstrated particularly promising increases in ascorbic acid and sucrose contents, while GB treatment resulted in an increase in total anthocyanins. Beside some changes in d-limonene content the observed improvements in yield and/or fruit quality attributes were not at the expense of any drastic consistent changes in aroma composition. Overall, this study indicates that chemical priming with Mel, alone or in combination with sodium alginate, can be further exploited as a sustainable strategy to enhance fruit productivity and nutritional value of raspberry fruit, without compromising plant growth. Further investigation of the mechanistic action of these compounds is needed to identify the exact biochemical pathways involved and optimize application protocols across variable environmental conditions.

Supporting information

Supporting information may be found in the online version of this article.

CRediT authorship contribution statement

Nicolas Valanides: Methodology, Investigation. **Egli C. Georgiadou:** Writing – original draft, Supervision, Methodology, Formal analysis, Data curation. **Eleni D. Myrtsi:** Writing – original draft, Methodology, Formal analysis. **Carlos Javier Garcia Hernandez Gil:** Methodology, Formal analysis, Data curation. **Anna Maria Taliadorou:** Methodology, Investigation. **Sofia Torrado:** Methodology, Investigation. **Maarten L.A.T.M. Hertog:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Francisco Tomás-Barberán:** Writing – review & editing, Validation, Data curation. **Vasileios Fotopoulos:** Writing – original draft, Visualization, Project administration, Methodology, Conceptualization. **George A. Manganaris:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

V.F. is a coinventor of patent WO/2015/123,273 dealing with the use of NOSH-A in plants, and a coinventor of patent #WO/2023/099627A1 pending dealing with the use of sodium alginate conjugates with priming agents in plants. The remaining authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.scienta.2025.114465](https://doi.org/10.1016/j.scienta.2025.114465).

Data availability

Data will be made available on request.

References

- Agathokleous, E., Zhou, B., Xu, J., Ioannou, A., Feng, Z., Saitanis, C.J., Frei, M., Calabrese, E.J., Fotopoulos, V., 2021. Exogenous application of melatonin to plants, algae, and harvested products to sustain agricultural productivity and enhance nutritional and nutraceutical value: a meta-analysis. *Env. Res* 200, 111746. <https://doi.org/10.1016/j.envres.2021.111746>.
- Akkad, R., Buchko, A., Soladoye, P.O., Han, J., Curtis, J.M., 2023. A study of the sensory attributes of flours and crackers made from sprouted and unsprouted faba beans. *LWT* 179, 114650. <https://doi.org/10.1016/j.lwt.2023.114650>.
- Amanpour, A., Guclu, G., Kelebek, H., Selli, S., 2019. Characterization of key aroma compounds in fresh and roasted terebenth fruits using aroma extract dilution analysis and GC-MS-olfactometry. *Microchem. J.* 145, 96–104. <https://doi.org/10.1016/j.microc.2018.10.024>.
- Ancos, B.de, Gonzalez, E.M., Pilar, M., C., 1999. Differentiation of raspberry varieties according to anthocyanin composition. *Eur. Food. Res. Technol* 208, 33–38.
- Antoniou, C., Chatzimichail, G., Xenofontos, R., Pavlou, J.J., Panagiotou, E., Christou, A., Fotopoulos, V., 2017. Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. *J. Pineal. Res* 62. <https://doi.org/10.1111/jpi.12401>.
- Antoniou, C., Xenofontos, R., Chatzimichail, G., Christou, A., Kashfi, K., Fotopoulos, V., 2020. Exploring the potential of nitric oxide and hydrogen sulfide (NOSH)-releasing synthetic compounds as novel priming agents against drought stress in *Medicago sativa* plants. *Biomolecules* 10, 120. <https://doi.org/10.3390/biom10010120>.
- Apra, E., Biasioli, F., Gasperi, F., 2015. Volatile compounds of raspberry fruit: from analytical methods to biological role and sensory impact. *Molecules* 20, 2445–2474. <https://doi.org/10.3390/molecules20022445>.
- Arnao, M.B., Cano, A., Hernández-Ruiz, J., 2022. Phytomelatonin: an unexpected molecule with amazing performances in plants. *J. Exp. Bot* 73, 5779–5800. <https://doi.org/10.1093/jxb/era009>.
- Bal, E., Ürün, B.A., 2021. Effects of chitosan coating with putrescine on bioactive compounds and quality of strawberry cv. San Andreas during cold storage. *Erwerbs-Obstbau* 63, 7–14. <https://doi.org/10.1007/s10341-020-00531-9>.
- Bobinaite, R., Viskelis, P., Venskutonis, P.R., 2012. Variation of total phenolics, anthocyanins, ellagic acid and radical scavenging capacity in various raspberry (*Rubus* spp.) cultivars. *Food. Chem* 132, 1495–1501. <https://doi.org/10.1016/j.foodchem.2011.11.137>.
- Bradish, C.M., Perkins-Vecz, P., Fernandez, G.E., Xie, G., Jia, W., 2012. Comparison of flavonoid composition of red raspberries (*Rubus idaeus* L.) grown in the southern United States. *J. Agric. Food. Chem* 60, 5779–5786. <https://doi.org/10.1021/jf203474e>.
- Carvalho, E., Franceschi, P., Feller, A., Palmieri, L., Wehrens, R., Martens, S., 2013. A targeted metabolomics approach to understand differences in flavonoid biosynthesis in red and yellow raspberries. *Plant. Physiol. Biochem* 72, 79–86. <https://doi.org/10.1016/j.plaphy.2013.04.001>.
- Chang, C.-C., Yang, M.-H., Wen, H.-M., Chern, J.-C., 2020. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food. Drug. Anal* 10. <https://doi.org/10.38212/2224-6614.2748>.
- Chen, F., Tholl, D., Bohlmann, J., Pichersky, E., 2011. The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant. J* 66, 212–229. <https://doi.org/10.1111/j.1365-3113.2011.04520.x>.
- Chierici, S., Bugoni, S., Porta, A., Zanoni, G., Vidari, G., 2015. The importance of the 5-alkyl substituent for the violet smell of ionones: synthesis of racemic 5-demethyl- α -ionone. *Nat. Prod. Commun* 10 (6). <https://doi.org/10.1177/1934578X1501000612>.
- Demchak, K., Hanson, E.J., 2013. Small fruit production in high tunnels in the US. *Acta Hort* 41–44. <https://doi.org/10.17660/ActaHortic.2013.987.4>.
- Eghlima, G., Aghamir, F., Hajizadeh, H.S., Zarbakhsh, S., 2025. Role of melatonin in promoting growth attributes, thymol, rosmarinic acid and biochemical properties in *Thymus vulgaris* L. under water deficiency. *BMC. Plant. Biol* 25 (1). <https://doi.org/10.1186/s12870-025-06667-8>.
- Filippou, P., Antoniou, C., Fotopoulos, V., 2011. Effect of drought and rewetting on the cellular status and antioxidant response of *Medicago truncatula* plants. *Plant. Signal. Behav* 6, 270–277. <https://doi.org/10.4161/psb.6.2.14633>.
- Gao, Z., Zhao, K., Zhang, Z., Nizamani, M.M., Li, S., Li, M., Gong, D., Wang, J., Hu, M., 2024. Hydrogen sulfide alleviates pericarp browning in lichi fruit by modulating energy and sugar metabolisms. *Front. Plant. Sci* 15, 1421203. <https://doi.org/10.3389/fpls.2024.1421203>.
- Georgiadou, E.C., García, C.J., Taliadorou, A.M., Gedeon, S., Valanides, N., Valado, A., Gohari, G., Balsells-Llauro, M., Alcázar, R., Hertog, M.L.A.T.M., Tomás-Barberán, F.A., Manganaris, G.A., Fotopoulos, V., 2025. Pre-harvest application of sodium alginate functionalized with melatonin enhances secondary metabolism in strawberry fruit. *Curr. Plant. Biol* 43, 100515. <https://doi.org/10.1016/j.cpb.2025.100515>.
- Georgiadou, E.C., Goulas, V., Majak, I., Ioannou, A., Leszczynska, J., Fotopoulos, V., 2018. Antioxidant potential and phytochemical content of selected fruits and vegetables consumed in Cyprus. *Biotechnol. Food. Sci* 3–14. <https://doi.org/10.34658/BFS.2018.82.1.3-14>.
- Gohari, G., Jiang, M., Manganaris, G.A., Zhou, J., Fotopoulos, V., 2024. Next generation chemical priming: with a little help from our nanocarrier friends. *Trends Plant Sci* 29, 150–166. <https://doi.org/10.1016/j.tplants.2023.11.024>.
- Grossiord, C., Buckley, T.N., Cernusak, L.A., Novick, K.A., Poulter, B., Siegwolf, R.T.W., Sperry, J.S., McDowell, N.G., 2020. Plant responses to rising vapor pressure deficit. *New. Phytol* 226, 1550–1566. <https://doi.org/10.1111/nph.16485>.
- Gu, I., Howard, L., Lee, S.-O., 2022. Volatiles in berries: biosynthesis, composition, bioavailability, and health benefits. *Appl. Sci.* 12, 10238. <https://doi.org/10.3390/app122010238>.
- Gutensohn, M., Klempien, A., Kaminaga, Y., Nagegowda, D.A., Negre-Zakharov, F., Huh, J., Luo, H., Weizbauer, R., Mengiste, T., Tholl, D., Dudareva, N., 2011. Role of aromatic aldehyde synthase in wounding/herbivory response and flower scent production in different *Arabidopsis* ecotypes. *Plant. J* 66, 591–602. <https://doi.org/10.1111/j.1365-3113.2011.04515.x>.
- Guzmán, M.G., Cellini, F., Fotopoulos, V., Balestrini, R., Arbona, V., 2022. New approaches to improve crop tolerance to biotic and abiotic stresses. *Physiol. Plan.* 174, e13547. <https://doi.org/10.1111/ppl.13547>.
- Habibzadeh, F., Hazrati, S., Gholamhosseini, M., Khodaei, D., Habashi, D., 2019. Evaluation of quantitative and qualitative characteristics of strawberry in response to bio- and chemical fertilizers. *Gesunde. Pflanz* 71, 103–111. <https://doi.org/10.1007/s10343-019-00455-9>.
- Huang, J., Zhu, C., Hussain, S., Huang, J., Liang, Q., Zhu, L., Cao, X., Kong, Y., Li, Y., Wang, L., Li, J., Zhang, J., 2020. Effects of nitric oxide on nitrogen metabolism and the salt resistance of rice (*Oryza sativa* L.) seedlings with different salt tolerances. *Plant Physiol. Biochem* 155, 374–383. <https://doi.org/10.1016/j.plaphy.2020.06.013>.
- Ibáñez, M.D., Sanchez-Ballester, N.M., Blázquez, M.A., 2020. Encapsulated limonene: a pleasant lemon-like aroma with promising application in the agri-food industry. *Rev. Mol.* 25 (11), 2598. <https://doi.org/10.3390/molecules25112598>.
- Jalaludin, I., Kim, J., 2021. Comparison of ultraviolet and refractive index detections in the HPLC analysis of sugars. *Food Chem.* 365, 130514. <https://doi.org/10.1016/j.foodchem.2021.130514>.
- Jiang, J., Li, T.L., Lu, S.W., Guo, J.M., 2007. Effects of acetylic salicylic acid on the activities of sucrose metabolism related enzymes during fruit development of tomato (*Lycopersicon esculentum* Mill.). *Plant. Physiol. Commun* 43, 649–652.
- Li, M., Zhi, H., Dong, Y., 2019. Influence of preharvest and postharvest applications of glycine betaine on fruit quality attributes and storage disorders of 'Lapins' and 'Regina' Cherries. *HortScience* 54, 1540–1545. <https://doi.org/10.21273/HORTSCI14188-19>.
- Liu, M., Li, X.Q., Weber, C., Lee, C.Y., Brown, J., Liu, R.H., 2002. Antioxidant and antiproliferative activities of raspberries. *J. Agric. Food. Chem* 50, 2926–2930. <https://doi.org/10.1021/jf0111209>.
- Loreto, F., Velikova, V., 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant. Physiol* 127, 1781–1787. <https://doi.org/10.1104/pp.010497>.
- Calderon-Montano, M., Burgos-Moron, J., Perez-Guerrero, E., Lopez-Lazaro, C., M., 2011. A review on the dietary flavonoid kaempferol. *Mini-Rev. Med. Chem* 11, 298–344. <https://doi.org/10.2174/138955711795305335>.
- Ma, N., Guan, R., Zhao, R., Geng, Y., 2022. GC-IMS-based preliminary analysis of volatile flavor compounds in Ejiao at different processing stages. *J. Food. Qual* 2022, 1–12. <https://doi.org/10.1155/2022/3961593>.
- Manganaris, G.A., Valanides, N., Gohari, R., Milivojevic, J., DeVetter, L.W., Fotopoulos, V., 2024. Exploring the potential of priming agents towards enhanced performance of *Rubus* species. *Acta Hort* 1388, 7–16. <https://doi.org/10.17660/ActaHortic.2024.1388.2>.
- Metsalu, T., Vilo, J., 2015. ClustVis: a web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. *Nucleic. Acids. Res* 43, W566–W570. <https://doi.org/10.1093/nar/gkv468>.
- Meyers, K.J., Watkins, C.B., Pritts, M.P., Liu, R.H., 2003. Antioxidant and antiproliferative activities of strawberries. *J. Agric. Food. Chem* 51, 6887–6892. <https://doi.org/10.1021/jf034506n>.
- Molina-Bravo, R., Arellano, C., Sosinski, B.R., Fernandez, G.E., 2011. A protocol to assess heat tolerance in a segregating population of raspberry using chlorophyll fluorescence. *Sci. Hort* 130, 524–530. <https://doi.org/10.1016/j.scienta.2011.07.022>.
- Mus, F., Wu, H.-H., Alleman, A.B., Shisler, K.A., Zadovnyy, O.A., Bothner, B., Dubois, J. L., Peters, J.W., 2020. Insights into the unique carboxylation reactions in the metabolism of propylene and acetone. *Biochem. J.* 477, 2027–2038. <https://doi.org/10.1042/BCJ20200174>.
- O'Brien, P.J., Siraki, A.G., Shangari, N., 2005. Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. *Crit. Rev. Toxicol* 35, 609–662. <https://doi.org/10.1080/10408440591002183>.
- Okatan, V., Aşkin, M.A., Polat, M., Bulduk, I., Çolak, A.M., Güçlü, S.F., Kahramanoğlu, İ., Tallarita, A.V., Caruso, G., 2022. Effects of melatonin dose on fruit yield, quality, and antioxidants of strawberry cultivars grown in different crop systems. *Agriculture* 13, 71. <https://doi.org/10.3390/agriculture13010071>.
- Pantelidis, G., Vasilakakis, M., Manganaris, G., Diamantidis, G., 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries,

- blackberries, red currants, gooseberries and Cornelian cherries. Food. Chem 102, 777–783. <https://doi.org/10.1016/j.foodchem.2006.06.021>.
- Parveen, S., Bhat, I.U.H., Bhat, R., 2023. Kaempferol and its derivatives: biological activities and therapeutic potential. Asian. Pac. J. Trop. Biomed 13, 411. <https://doi.org/10.4103/2221-1691.387747>.
- Paterson, A., Kassim, A., McCallum, S., Woodhead, M., Smith, K., Zait, D., Graham, J., 2013. Environmental and seasonal influences on red raspberry flavour volatiles and identification of quantitative trait loci (QTL) and candidate genes. Theor. Appl. Genet 126, 33–48. <https://doi.org/10.1007/s00122-012-1957-9>.
- Ponder, A., Hallmann, E., 2020. The nutritional value and vitamin C content of different raspberry cultivars from organic and conventional production. J. Food. Compos. Anal 87, 103429. <https://doi.org/10.1016/j.jfca.2020.103429>.
- Priti, Kapoor, P., Mali, S., Verma, V., Katoch, M., Zinta, G., Bhargava, B., 2024. Genome-wide characterization of melatonin biosynthetic pathway genes in carnation (*Dianthus caryophyllus* L.) and their expression analysis in response to exogenous melatonin. Sci. Hortic 338, 113776. <https://doi.org/10.1016/j.scienta.2024.113776>.
- Renai, L., Scordo, C.V.A., Chiuminatto, U., Ulaszewska, M., Giordani, E., Petrucci, W.A., Tozzi, F., Nin, S., Del Bubba, M., 2021. Liquid chromatographic quadrupole time-of-flight mass spectrometric untargeted profiling of (Poly)phenolic compounds in *Rubus idaeus* L. and *Rubus occidentalis* L. Fruits and their comparative evaluation. Antioxidants 10, 704. <https://doi.org/10.3390/antiox10050704>.
- Rizzo, W.B., 2014. Fatty aldehyde and fatty alcohol metabolism: review and importance for epidermal structure and function. Biochim. Biophys. Acta. BBA. - Mol. Cell. Biol. Lipids 1841, 377–389. <https://doi.org/10.1016/j.bbalip.2013.09.001>.
- Rungrat, T., Awlia, M., Brown, T., Cheng, R., Sirault, X., Fajkus, J., Trtilek, M., Furbank, B., Badger, M., Tester, M., Pogson, B.J., Borevitz, J.O., Wilson, P., 2016. Using phenomic analysis of photosynthetic function for abiotic stress response gene discovery. Arab. Book 14, e0185. <https://doi.org/10.1199/tab.0185>.
- Salazar-Orbea, G., García-Villalba, R., Sánchez-Siles, L.M., Tomás-Barberán, F.A., García, C.J., 2022. Untargeted metabolomics reveals new markers of food processing for strawberry and apple purees. Molecules 27, 7275. <https://doi.org/10.3390/molecules27217275>.
- Sariburun, E., Şahin, S., Demir, C., Türkben, C., Uylaşer, V., 2010. Phenolic content and antioxidant activity of raspberry and blackberry cultivars. J. Food. Sci 75. <https://doi.org/10.1111/j.1750-3841.2010.01571.x>.
- Savvides, A., Ali, S., Tester, M., Fotopoulos, V., 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? Trends Plant Sci. 21, 329–340. <https://doi.org/10.1016/j.tplants.2015.11.003>.
- Shah, H.M.S., Singh, Z., Hasan, M.U., Kaur, J., Afrifa-Yamoah, E., Woodward, A., 2024. Melatonin application suppresses oxidative stress and maintains fruit quality of cold stored 'Esperanza' raspberries by regulating antioxidant system. Postharvest. Biol. Technol 207, 112597. <https://doi.org/10.1016/j.postharvbio.2023.112597>.
- Shehata, S.A., Abdeldaym, E.A., Ali, M.R., Mohamed, R.M., Bob, R.I., Abdelgawad, K.F., 2020. Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage. Agronomy 10, 1466. <https://doi.org/10.3390/agronomy10101466>.
- Sønsteby, A., Heide, O.M., 2008. Environmental control of growth and flowering of *Rubus idaeus* L. cv. Glen. Ample. Sci. Hortic 117, 249–256. <https://doi.org/10.1016/j.scienta.2008.05.003>.
- Sønsteby, A., Stavang, J.A., Heide, O.M., 2013. Production of high-yielding raspberry long canes: the way to 3 kg of fruit per cane. J. Hortic. Sci. Biotechnol 88, 591–599. <https://doi.org/10.1080/14620316.2013.11513011>.
- Spanos, G.A., Wrolstad, R.E., 1987. Anthocyanin pigment, nonvolatile acid, and sugar composition of red raspberry juice. J. AOAC. Int 70, 1036–1046. <https://doi.org/10.1093/jaoac/70.6.1036>.
- Strommer, J., Garabagi, F., 2009. ADH and PDC: key roles for enzymes of alcoholic fermentation (Eds.). In: Gerats, T., Strommer, J. (Eds.), Petunia. Springer, New York, pp. 71–84. https://doi.org/10.1007/978-0-387-84796-2_4.
- Su, C., Zheng, X., Zhang, D., Chen, Y., Xiao, J., He, Y., He, J., Wang, B., Shi, X., 2020. Investigation of sugars, organic acids, phenolic compounds, antioxidant activity and the aroma fingerprint of small white apricots grown in Xinjiang. J. Food. Sci 85, 4300–4311. <https://doi.org/10.1111/1750-3841.15523>.
- Vandendriessche, T., Nicolai, B.M., Hertog, M.L.A.T.M., 2013. Optimization of HS SPME fast GC-MS for high-throughput analysis of strawberry aroma. Food. Anal. Methods 6, 512–520. <https://doi.org/10.1007/s12161-012-9471-x>.
- Viljakainen, S., Visti, A., Laakso, S., 2002. Concentrations of organic acids and soluble sugars in juices from Nordic berries. Acta. Agric. Scand. B. —. Soil. Plant. Sci 52, 101–109. <https://doi.org/10.1080/090647102321089846>.
- Xia, H., Shen, Y., Deng, H., Wang, J., Lin, L., Deng, Q., Lv, X., Liang, D., Hu, R., Wang, Z., Xiong, B., 2021. Melatonin application improves berry coloration, sucrose synthesis, and nutrient absorption in 'Summer Black' grape. Food. Chem 356, 129713. <https://doi.org/10.1016/j.foodchem.2021.129713>.
- Xiao, Y., Wu, Y., Huang, Z., Guo, M., Zhang, L., Luo, X., Xia, H., Zhang, X., Liang, D., Lv, X., Wang, J., Lin, L., 2024. Mechanism of induced soluble sugar accumulation and organic acid reduction in plum fruits by application of melatonin. BMC. Plant. Biol 24, 1208. <https://doi.org/10.1186/s12870-024-05949-x>.
- Yang, P., Yu, M., Song, H., Xu, Y., Lin, Y., Granvogl, M., 2022. Characterization of key aroma-active compounds in rough and moderate fire Rougui Wuyi Rock tea (*Camellia sinensis*) by sensory-directed flavor analysis and elucidation of the influences of roasting on aroma. J. Agric. Food. Chem 70, 267–278. <https://doi.org/10.1021/acs.jafc.1c06066>.
- Yao, L.H., Jiang, Y.M., Shi, J., TomaS-Barbera, F.A., Datta, N., Singanusong, R., Chen, S. S., 2004. Flavonoids in food and their health benefits. Plant. Foods. Hum. Nutr 59, 113–122. <https://doi.org/10.1007/s11130-004-0049-7>.
- Zahedi, S.M., Hosseini, M.S., Abadía, J., Marjani, M., 2020. Melatonin foliar sprays elicit salinity stress tolerance and enhance fruit yield and quality in strawberry (*Fragaria × ananassa* Duch.). Plant. Physiol. Bioch 49, 313–323. <https://doi.org/10.1016/j.plaphy.2020.02.021>.
- Zhang, M.-Q., Zhang, J., Zhang, Y.-T., Sun, J.-Y., Prieto, M.A., Simal-Gandara, J., Putnik, P., Li, N.-Y., Liu, C., 2023. The link between the phenolic composition and the antioxidant activity in different small berries: a metabolomic approach. LWT 182, 114853. <https://doi.org/10.1016/j.lwt.2023.114853>.
- Zhang, W., Lao, F., Bi, S., Pan, X., Pang, X., Hu, X., Liao, X., Wu, J., 2021. Insights into the major aroma-active compounds in clear red raspberry juice (*Rubus idaeus* L. cv. Heritage) by molecular sensory science approaches. Food. Chem 336, 127721. <https://doi.org/10.1016/j.foodchem.2020.127721>.
- Zhang, X., Liu, Y., Zhang, W., Yang, W., An, S., Guo, M., Chen, G., 2024. Salicylic acid treatment ameliorates postharvest quality deterioration in 'France' Prune (*Prunus domestica* L. 'Xime') fruit by modulating the antioxidant system. Foods 13, 2871. <https://doi.org/10.3390/foods13182871>.
- Zhang, X., Sandhu, A., Edirisinghe, I., Burton-Freeman, B., 2018. An exploratory study of red raspberry (*Rubus idaeus* L.) (poly)phenols/metabolites in human biological samples. Food. Funct 9, 806–818. <https://doi.org/10.1039/C7FO00893G>.

Exploring the potential of priming agents towards enhanced performance of *Rubus* species

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Abstract

The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of fruit crops is relatively novel. The process of priming involves prior exposure to biotic or abiotic stress factors rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: i) chemicals (i.e., hormones, reactive oxygen nitrogen and sulphur species (RONSS), and small organic molecules), ii) microorganisms (i.e., arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPR)), and iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops that have high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. To our knowledge, the concept of priming in soft fruits is relatively new with scarce information available. The aim of the current report is dual. Initially, this report provides information regarding the prospects of priming agents as a novel agricultural and technological approach to improve stress tolerance for a range of *Rubus* species, namely red raspberry, blackberry, boysenberry, cloudberry, loganberry and black raspberry. Additionally, it describes the challenges and constraints of raspberry production within a global context, providing examples and case studies from the United States and Europe, two industries with striking differences in their production models.

Keywords: soft fruits, berries, *Rubus idaeus*, agricultural biostimulants, stress conditions, melatonin, putrescine, sodium alginate, raspberry production model

INTRODUCTION

Small (or soft) fruits are an excellent natural source of biologically active components that provide significant health-promoting benefits and this has led to an exponential growth in their production, including *Rubus* species (Pantelidis et al., 2007; Manganaris et al., 2014; Fotirić Akšić et al., 2019; Milosavljević et al., 2020). Most *Rubus* species used for commercial production are native to Europe and belong to the subgenus *Idaeobatus* within the genus *Rubus*, which contains approximately 740 species (Hummer, 2010). Given the global distribution and scale of production of *Rubus* species, they are used both commercially and in niche markets.

Red raspberry (*Rubus idaeus* L.) has a predominant position among *Rubus* species with a diversity and richness in the content of phenolic compounds, such as anthocyanins, flavonoids, and phenolic acids. Raspberry fruits have been reported as an important source of antioxidants with differences noted between tested florican- and primocane-fruiting cultivars (Dragišić Maksimović et al., 2013; Milivojević et al., 2011). Besides genotypic (cultivar) effects, other factors such as the environment, ripening stage, cultivation techniques, postharvest treatments and storage conditions may affect their content (Di Vittori et al., 2018).

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However, soft fruits severely suffer from exogenous stress conditions, such as drought or heat stress. The application of priming agents can minimize the effects of biotic and abiotic stress, resulting in increased productivity and improved fruit physicochemical characteristics. Priming can be achieved by applying natural or synthetic compounds which act as signaling transducers, 'activating' the plant's defence system. Exposure to a stimulus allows a plant to respond in a more rapid and effective way to a later stimulus (the same or equivalent) compared with a non-primed plant. However, the effects of priming agents have been poorly characterized in *Rubus* species.

The current report provides information on the prospects of applying priming agents as a novel agricultural and technological approach from a range of perspectives in raspberries and, moreover, in the other *Rubus* species about which little information is available. It additionally provides an up-to-date overview of the current challenges and perspectives of raspberry production by examining main producing areas: the United States in the Americas and the Republic of Serbia in Europe, two industries that apply different production models.

DESCRIPTION OF PRIMING AGENTS FOR POTENTIAL USE ON SOFT FRUITS

Plants suffer from abiotic (salinity, drought, heavy metals, etc.) and biotic (pathogenic microorganisms and pests) stress conditions. Reception of multiple environmental stimuli causes several metabolic pathways to become 'switched on' in response to accumulation of signaling molecules. The process of priming involves prior exposure to biotic or abiotic stress factors making a plant more resistant/tolerant to future exposure. A first encounter with a particular stress factor can trigger the establishment of a molecular memory that primes or acclimates the plant (Savvides et al., 2016; Sherin et al., 2022). Priming agents applied in agriculture simulate this process.

Priming agents can be classified into the following classes: 1) chemicals (including natural and synthetic molecules) such as i) hormones (i.e., salicylic acid, jasmonic acid, strigolactones), ii) reactive oxygen nitrogen and sulphur species (RONSS: NO, H₂S, H₂O₂) and iii) small organic molecules (i.e., melatonin, putrescine); 2) microorganisms such as i) arbuscular mycorrhizal fungi (AMF, including *Funneliformis mosseae* and *Rhizophagus irregularis*), and ii) plant growth-promoting bacteria (PGPR, soil bacteria living in the rhizosphere that are involved in promoting plant growth and development); and 3) nanomaterials, which can include organic nanoparticles, inorganic nanoparticles and polymers (Savvides et al., 2016; Ioannou et al., 2020; Sherin et al., 2022). This work focuses on the effect of polyamines (with special reference to putrescine), melatonin and sodium alginate as potential agents that can be successfully used in *Rubus* crop production to increase yield performance and improve plant adaptability to stress conditions.

Polyamines

These compounds play a pivotal role in shaping the intricate web of physiological processes within plants that impact the quality, yield, and nutritional properties of fruits. As essential regulators of growth and development, these organic compounds are synthesized through the intricate pathways of amino acid metabolism. In addition to the well-known polyamines (putrescine, spermidine, and spermine), other variations are found in different plant species, which may contribute to the diversity of functions of these compounds (Minocha et al., 2014). One of the primary functions of polyamines lies in their influence on cell division and differentiation. During plant development, the controlled and precise division of cells is crucial for the formation of tissues and organs. Polyamines act as crucial modulators in this process, ensuring the proper organization and differentiation of various cell types (Chen et al., 2019). Moreover, they participate in the regulation of the cell cycle, ensuring that cell division occurs at the right time and in the appropriate context. As plants progress through their life cycle, they eventually undergo senescence, a natural ageing process. Polyamines are intricately involved in this phase, orchestrating the breakdown of cellular components and facilitating nutrient remobilization, which is vital for the overall health and resource utilization of the plant (Alcázar et al., 2010, 2020).

The impact that polyamines have on plant growth, development, and stress response

has led to the exploration of their potential application in agriculture, particularly in enhancing pre- and postharvest fruit quality (Chen et al., 2019). As priming agents, polyamines have demonstrated their ability to prepare fruits to better withstand various stresses they may encounter during growth and storage, thereby enhancing overall fruit quality and plant resilience (Gao et al., 2021). Preharvest treatments involve the application of polyamines to plants before fruit maturation and harvest. This treatment has been found to positively influence the fruit development and the ripening processes. By regulating gene expression and protein synthesis, polyamines can impact the production of ripening-related enzymes and signalling molecules, leading to improved fruit flavour, colour, and texture. Moreover, preharvest polyamine treatment has been shown to increase fruit yield by promoting cell division and differentiation in the growing fruits. This effect translates into larger and more abundant fruits, contributing to higher overall crop productivity (Fortes and Agudelo-Romero, 2018; Gao et al., 2021).

As a postharvest treatment, polyamines have proven valuable in extending the shelf life of fresh fruit. Postharvest stressors, such as temperature fluctuations, humidity changes, and mechanical damage during handling and transportation, can cause fruit deterioration. Polyamines, with their role in maintaining cell membrane stability and protecting against oxidative stress, help delay the onset of senescence and maintain the quality of harvested fruits for a longer duration (Aghdam and Bodbodak, 2014; Zhang et al., 2020). In addition to their direct effects on fruit quality, the role of polyamines as priming agents enhances the ability of fruits to cope with stressors during postharvest storage. By pre-conditioning the fruits with polyamines, their defence mechanisms are activated and primed, allowing them to respond more effectively to stress factors encountered after harvest. This includes better resistance to pathogens, reduced fruit decay, and a higher capacity to withstand physiological disorders (Gao et al., 2021).

In addition to improving pre- and postharvest fruit quality, polyamines can also improve yield components and the nutritional value of fruits by enhancing antioxidant capacity and essential nutrients. Putrescine is the most widely studied polyamine that impacts some of these variables. For example, several studies have demonstrated that putrescine treatment can significantly impact fruit size and weight in various crops. This effect is attributed to its ability to stimulate cell division and elongation (Khan and Singh, 2010; Shanbehpour et al., 2020). By promoting these crucial growth processes, putrescine contributes to the development of larger fruits, resulting in enhanced market appeal and overall crop yields (Gao et al., 2021). Furthermore, putrescine application exhibits a remarkable influence on fruit colour development. Through its role in stimulating the synthesis of pigments, such as anthocyanins and carotenoids, putrescine contributes to vibrant and attractive fruit hues. This enhancement in colour not only adds visual appeal to the fruits but also signifies potential health-promoting benefits associated with these pigments. Application of putrescine leads to a notable increase in the antioxidant capacity of fruits. Torrigiani et al. (2004) and Singh et al. (2022) have highlighted its positive effect on enhancing the activity of antioxidant enzymes and maintaining elevated levels of phytochemical compounds. This heightened antioxidant defence system provides fruits with enhanced protection against oxidative stress, which is crucial for extending postharvest shelf life and ensuring better fruit quality during storage and transportation. In addition to the impact on size, colour, and antioxidant capacity, putrescine application also positively influences the nutritional composition of fruit. Notably, it has been associated with higher levels of essential nutrients, including vitamins, minerals, and phenolic compounds (Mirdehghan and Rahimi, 2016; Singh et al., 2021). This nutritional enrichment further augments the value of the fruits, offering consumers with more nutrient-dense and health-promoting options. As research in this field continues, further insights into the molecular mechanisms behind polyamine action and their applications to food production will undoubtedly open new avenues for agricultural innovation (Valero et al., 2002; Fortes and Agudelo-Romero, 2018).

Melatonin

Melatonin, a natural plant hormone, acts as a potent antioxidant and free radical

scavenger, providing protection against oxidative stress induced by environmental factors like high temperature extremes, drought, and UV radiation. This protective effect contributes to the overall health and vigour of horticultural crops. Furthermore, melatonin has a pivotal role on various physiological processes in fruit trees, such as photosynthetic enhancement, circadian rhythm regulation, and hormone balance promotion, leading to improved nutrient assimilation and allocation, and ultimately resulting in better fruit development and quality (Agathokleous et al., 2021; Nawaz et al., 2016; Zeng et al., 2022; Zhang et al., 2020).

Postharvest application of melatonin involves treating harvested fruits with melatonin solutions or coatings to enhance their quality, extend shelf life, and reduce postharvest losses. Melatonin applied postharvest has been reported to delay ripening, preserve antioxidant capacity and reduce the intensity and severity of postharvest diseases (Ze et al., 2021; Zhang et al., 2020). Melatonin can also be incorporated into edible coatings or films applied to fruit surfaces. These coatings provide a protective barrier against moisture loss, gas exchange, and microbial contamination. Melatonin-infused coatings subsequently help to maintain fruit quality and prolong shelf life (Arnao and Hernández-Ruiz, 2020; Xu et al., 2019; Ze et al., 2021). A recent meta-analysis provides an overview of the effect of melatonin on postharvest performance and antioxidant properties of fresh fruits (Madebo et al., 2022).

The mode of action of melatonin is through activation of i) enzymatic (SOD, APX, CAT) and non-enzymatic antioxidant systems (ascorbate and glutathione, total phenols, anthocyanins, flavonoids), ii) the GABA-shunt pathway, iii) endogenous melatonin, as well as causing a decrease in lipid peroxidation (MDA) and hydrogen peroxide. It has been also reported to reduce tissue softening and enhance resistance to fruit fungal decay. Recent studies have shown that melatonin-primed raspberry plants exhibit increased resilience against diseases and pests, bolstering the plant's defence mechanisms and reducing pathogen infestations which leads into higher fruit yields (Arnao and Hernández-Ruiz, 2020; Zhang et al., 2020).

Sodium alginate

It is a biodegradable polymer that can be broken down and metabolized by microorganisms or degrades naturally in the environment when disposed of properly. This in turn reduces its impact on the ecosystem compared to non-biodegradable materials that persist in the environment. This inherent eco-friendly property makes it an attractive solution for a wide range of applications (Shit and Shah, 2014). Chemically, sodium alginate is a linear polysaccharide composed of repeating units of two monosaccharides: β -D-mannuronic acid (M) and α -L-guluronic acid (G). These monosaccharide units are linked together by 1,4-glycosidic linkages. The arrangement and composition of M and G units within the polymer chain play a crucial role in determining the physicochemical properties of sodium alginate. In its chemical structure, the M and G units alternate along the polymer chain, creating distinct patterns of M-G and G-M sequences (Yerramathi et al., 2021). The distribution and ratio of these units can vary, resulting in different alginate variants with diverse properties such as solubility, gelation capacity, and viscosity. This versatility allows sodium alginate to be tailored for specific applications, making it an ideal choice for a wide range of industrial and biomedical uses (Yerramathi et al., 2021; Karim et al., 2022). Overall, the viscosity of sodium alginate solutions can be fine-tuned by controlling the distribution and composition of M and G units, making it valuable for thickening and stabilizing applications in various industries (Yerramathi et al., 2021; Karim et al., 2022).

Sodium alginate can be applied via nano smart delivery systems in the following ways: i) encapsulation of active ingredients: it can be used to encapsulate active ingredients within alginate-based nanostructures; the encapsulation helps protect the active ingredient, control its release, and enhance its stability; ii) nanoparticle formulation: it can be utilized to form nanoparticles, either alone or in combination with other materials for targeted delivery; iii) hydrogel systems: it can form hydrogels when crosslinked with divalent cations like calcium ions. Alginate-based hydrogels have been used as smart delivery systems for the controlled release of growth factors or other bioactive agents; iv) bioactive scaffold material for tissue engineering applications (Nair et al., 2020; Yerramathi et al., 2021; Karim et al., 2022).

RASPBERRY FRUIT PRODUCTION: CHALLENGES AND CONSTRAINTS WITHIN A GLOBAL CONTEXT

The case of the United States (US)

The US ranked as the 5th largest global producer of raspberries in 2021 with a production area covering 6,578 ha (FAOSTAT, 2023). Within the US, Washington and California lead processed and fresh market production, respectively, with the value of utilized production in 2021 estimated at \$ 531.3 million US dollars (United Department of Agriculture, National Agricultural Statistics Service, 2022). Washington primarily grows florican-fruited raspberry that is machine harvested with the average yield at 8687 kg ha⁻¹. In contrast, California hand harvests primocane-fruited plants with average yields at 21296 kg ha⁻¹. 'Meeker' along with 'WakeField' and 'WakeHaven' are the most widely planted cultivars in Washington, while California growers often use private genetics from companies such as Driscoll's. Other states cultivate raspberry and the crop is used within local and regional foodsheds.

The US raspberry industry works cooperatively with public and private research and outreach programs to address prioritized production issues. Development of new and adapted cultivars with high yield potential, superior fruit quality, and tolerance or resistance to key pests and diseases is an important breeding aim addressed by public and private breeding programs. Management of pests such as spotted wing drosophila (*Drosophila suzukii*) and mites as well as pathogens and plant-parasitic nematodes are also high priority production constraints. Diseases of most concern include botrytis fruit rot, Phytophthora root rot, yellow rust, and cane blight, while root lesion nematode (*Pratylenchus penetrans*) and virus-transmitting nematodes are also of concern. Labour is yet another key constraint as the cost of labour continues to increase, while the availability of an agricultural workforce diminishes. Recently, adaptation to heat has emerged as a new challenge. High temperatures have been especially challenging in the Pacific Northwest region with some growers suffering complete or partial crop loss due to recent extremes in heat. California growers often use poly-tunnels, which can provide some protection from heat and UV damage depending on implementation, but this practice is not widely used in raspberry production in Washington and the greater Pacific Northwest region. Release of new cultivars with improved tolerance or resistance to key biotic and abiotic stressors will be important in helping the raspberry industry adapt to production challenges, but breeding is a slow process and needs to be complemented with other horticultural tactics. Priming agents are one potential avenue that can be rapidly employed to enhance or sustain production of this economically important crop. However, research is needed to inform the utilization of priming agents and needs to be paired with economic analyses to understand the implications of this new practice on profitability.

The case of Serbia

According to FAOSTAT (2023), the total cultivated area is 20,807 ha with an annual production of 110,589 t in 2021 that results on a relative low yield per hectare. The area of each farm is 0.25 ha and cultivation costs are estimated at 1.7 € kg⁻¹ of produced fruit with labour costs representing the largest share. More than 90% of raspberries produced are frozen in cold stores. The selling price varies significantly from 4.5 € kg⁻¹ (2022) to 1.7 € kg⁻¹ (2023) with the vast majority of fruits destined for freezing/processing (>90%) and exported to foreign markets as various frozen products (rollend, gris and block). The export of fresh raspberries is insignificant.

The predominant cultivar is 'Willamette' (90%), followed by 'Meeker' (3%) and 'Fertödi Zamos' (2%), while 'Tulameen' and 'Glen Ample', which are mainly used for fresh consumption, are rarely present due to domestic consumption of this fruit being very small and the export of fresh raspberries from Serbia quite limited (Nikolić and Tanović, 2012). The vertical trellis system and careful balancing of the vegetative and generative growth of biennial raspberries by removing the first series of primocanes have been employed towards enhanced crop efficiency (Milivojević et al., 2017). However, removing young canes is a very labour-intensive and expensive management practice in commercial raspberry plantations;

therefore, previous studies have highlighted the need to apply growth retardants (i.e., prohexadione-calcium, ProCa), which block the last steps of gibberellin biosynthesis and thus prevent the formation of active forms of gibberellins. In 'Willamette', ProCa treatment was effective at reducing vegetative growth, increasing the number of inflorescences cane⁻¹ and promoting the yield and nutritional fruit quality (Dragišić Maksimović et al., 2017).

Over the last decade, the production volume of primocane fruiting cultivars was slightly increased. Older cultivars such as 'Polka', 'Polana', 'Autumn Bliss', 'Himbo Top' and 'Heritage' are the most common in commercial plantations, which are mainly cultivated in a hedgerow system with additional trellises. In open-field production, all canes are mowed down after fruiting in early spring and the crop is only produced on newly developed primocanes from mid-July to fall. More recently, two newly-introduced cultivars ('Enrosadira' and 'Kwanza') have been extended into protected environments to meet the growing needs of producers and consumers. These cultivars continually set fruit on the current season's canes from mid-July to fall, and some of the previous season's canes are retained to produce early fruit from late May to late June in the following year, thus extending the harvest season and providing higher total yields for bearing twice a year. The introduction of new cultivars that are more productive and resistant or tolerant to pathogens and stress conditions is essential for the raspberry industry to overcome production constraints. The main constraints in the Serbian raspberry industry are: adverse effects of climatic factors; poor quality of planting material (mainly propagated in commercial plantations); use of inappropriate soils and sites for establishment of new plantations; lack of labour for harvesting; inability to use the same cultivar(s) for fresh and processed markets, and the stock of unsold frozen raspberries from the previous year depressing prices. As labour costs have increased, basic cultural practices should be mechanized. In Serbia, the daily harvest rate per worker is between 40 and 60 kg, which accounts for up to 70-80% of production costs, while mechanization of harvesting can reduce this value by 10-12 times and reduce direct operating costs by 50-70% (Kazakov et al., 2009).

New directions for the Serbian raspberry industry include machine harvesting of raspberries destined for freezing and processing, cultivation of raspberries in protected environments (anti-hail nets, rain shelters, and greenhouses) to prevent adverse effects of extreme climatic factors and the promotion of new, superior primocane fruiting raspberry cultivars. For example, 'Enrosadira' (Sant Orsola, Italy) and 'Kwanza' (Advanced Berry Breeding, The Netherlands, etc.) have been reported to yield more than 30 t ha⁻¹ and have very large fruit size with good flavor and excellent shelf life. The development of new cultivation techniques, such as use of long canes and soilless culture, are also new directions to produce year-round raspberries for fresh consumption. The ability to produce year-round raspberries for fresh consumption through a combination of superior cultivars and new production techniques with a gap during July and August is a realistic scenario under Mediterranean conditions (Evangelos Tsormpatsidis, pers. commun.). The application of priming agents may have an important role as a new production technique that expands the resiliency and viability of this evolving raspberry industry in Europe.

CONCLUSIONS

The following conclusions can be drawn from this report:

- Expanding global production of soft fruits, including *Rubus* species, is challenged by biotic and abiotic stress factors. Climate change and extremes in weather necessitate new tools that can rapidly and economically reduce crop loss due to abiotic stress and should complement other breeding and horticultural research efforts;
- In the context of the high demand for fresh and processed raspberries in the world market, there is a great need to increase the profitability of cultivation through the use of innovative, low-cost technologies, such as the application of priming agents;
- Research and use of priming agents should be prioritized, particularly considering the adverse stress conditions experienced due to climate change. It is also important to understand and apply the potential benefit from the postharvest use of priming agents towards enhanced cold chain management from field to consumer;

- The utilization of priming agents offers a promising strategy to improve raspberry plant health, enhance productivity, and elevate fruit quality amid changing global climatic conditions. By countering the detrimental effects of biotic and abiotic stresses, priming agents empower raspberry plants to thrive in challenging environments;
- As ongoing research continues to unravel the specific mechanisms behind these priming agents' effects on raspberry physiology and fruit quality, the potential for optimizing raspberry production and providing consumers with high-quality, nutritious berries becomes increasingly evident.

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Literature cited

- Agathokleous, E., Zhou, B., Xu, J., Ioannou, A., Feng, Z., Saitanis, C.J., Frei, M., Calabrese, E.J., and Fotopoulos, V. (2021). Exogenous application of melatonin to plants, algae, and harvested products to sustain agricultural productivity and enhance nutritional and nutraceutical value: a meta-analysis. *Environ Res* 200, 111746 <https://doi.org/10.1016/j.envres.2021.111746>. PubMed
- Aghdam, M.S., and Bodbodak, S. (2014). Postharvest heat treatment for mitigation of chilling injury in fruits and vegetables. *Food Bioprocess Technol.* 7 (1), 37–53 <https://doi.org/10.1007/s11947-013-1207-4>.
- Alcázar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P., and Tiburcio, A.F. (2010). Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* 231 (6), 1237–1249 <https://doi.org/10.1007/s00425-010-1130-0>. PubMed
- Alcázar, R., Bueno, M., and Tiburcio, A.F. (2020). Polyamines: small amines with large effects on plant abiotic stress tolerance. *Cells* 9 (11), 2373 <https://doi.org/10.3390/cells9112373>. PubMed
- Arnao, M.B., and Hernández-Ruiz, J. (2020). Melatonin in flowering, fruit set and fruit ripening. *Plant Reprod* 33 (2), 77–87 <https://doi.org/10.1007/s00497-020-00388-8>. PubMed
- Chen, D., Shao, Q., Yin, L., Younis, A., and Zheng, B. (2019). Polyamine function in plants: metabolism, regulation on development, and roles in abiotic stress responses. *Front Plant Sci* 9, 1945 <https://doi.org/10.3389/fpls.2018.01945>. PubMed
- Di Vittori, L., Mazzoni, L., Battino, M., and Mezzetti, B. (2018). Pre-harvest factors influencing the quality of berries. *Sci. Hortic. (Amsterdam)* 233, 310–322 <https://doi.org/10.1016/j.scienta.2018.01.058>.
- Dragišić Maksimović, J.J., Milivojević, J.M., Poledica, M.M., Nikolić, M.D., and Maksimović, V.M. (2013). Profiling antioxidant activity of two primocane fruiting red raspberry cultivars (Autumn Bliss and Polka). *J. Food Compos. Anal.* 31 (2), 173–179 <https://doi.org/10.1016/j.jfca.2013.05.008>.
- Dragišić Maksimović, J.J., Poledica, M.M., Radivojević, D.D., and Milivojević, J.M. (2017). Enzymatic profile of 'Willamette raspberry leaf and fruit affected by prohexadione-Ca and young canes removal treatments. *J Agric Food Chem* 65 (24), 5034–5040 <https://doi.org/10.1021/acs.jafc.7b00638>. PubMed
- Food and Agriculture Organization STAT. (2023) <https://www.fao.org/faostat/en/#home>.
- Fortes, A.M., and Agudelo-Romero, P. (2018). Polyamine metabolism in climacteric and non-climacteric fruit ripening. In *Polyamines. Methods in Molecular Biology*, Vol. 1694, R. Alcázar, and A. Tiburcio, eds. (New York, NY: Humana Press), https://doi.org/10.1007/978-1-4939-7398-9_36.
- Fotirić Akšić, M., Dabić Zagorac, D., Sredojević, M., Milivojević, J., Gašić, U., Meland, M., and Natić, M. (2019). Chemometric characterization of strawberries and blueberries according to their phenolic profile: combined effect of cultivar and cultivation system. *Molecules* 24 (23), 4310 <https://doi.org/10.3390/molecules24234310>. PubMed
- Gao, F., Mei, X., Li, Y., Guo, J., and Shen, Y. (2021). Update on the roles of polyamines in fleshy fruit ripening, senescence, and quality. *Front Plant Sci* 12, 610313 <https://doi.org/10.3389/fpls.2021.610313>. PubMed
- Hummer, K.E. (2010). *Rubus* pharmacology: antiquity to the present. *HortScience* 45 (11), 1587–1591 <https://doi.org/10.21273/HORTSCI.45.11.1587>.
- Ioannou, A., Gohari, G., Papaphilippou, P., Panahirad, S., Akbari, A., Dadpour, M.R., Krasia-Christoforou, T., and Fotopoulos, V. (2020). Advanced nanomaterials in agriculture under a changing climate: the way to the future?

- Environ. Exp. Bot. 176, 104048 <https://doi.org/10.1016/j.envexpbot.2020.104048>.
- Karim, A., Rehman, A., Feng, J., Noreen, A., Assadpour, E., Kharazmi, M.S., Lianfu, Z., and Jafari, S.M. (2022). Alginate-based nanocarriers for the delivery and controlled-release of bioactive compounds. *Adv Colloid Interface Sci* 307, 102744 <https://doi.org/10.1016/j.cis.2022.102744>. PubMed
- Kazakov, I.V., Evdokimenko, S.N., and Kulagina, V.L. (2009). Selective potential to create the everbearing raspberry varieties for machine harvesting. *Agric. Biol.* 1, 28–33.
- Khan, A.S., and Singh, Z. (2010). Pre-harvest application of putrescine influences Japanese plum fruit ripening and quality. *Food Sci Technol Int* 16 (1), 53–64 <https://doi.org/10.1177/1082013209353242>. PubMed
- Madebo, M.P., Zheng, Y., and Jin, P. (2022). Melatonin-mediated postharvest quality and antioxidant properties of fresh fruits: a comprehensive meta-analysis. *Compr Rev Food Sci Food Saf* 21 (4), 3205–3226 <https://doi.org/10.1111/1541-4337.12961>. PubMed
- Manganaris, G.A., Goulas, V., Vicente, A.R., and Terry, L.A. (2014). Berry antioxidants: small fruits providing large benefits. *J Sci Food Agric* 94 (5), 825–833 <https://doi.org/10.1002/jsfa.6432>. PubMed
- Milivojević, J., Maksimović, V., Nikolić, M., Bogdanović, J., Maletić, R., and Milatović, D. (2011). Chemical and antioxidant properties of cultivated and wild *Fragaria* and *Rubus* berries. *J. Food Qual.* 34 (1), 1–9 <https://doi.org/10.1111/j.1745-4557.2010.00360.x>.
- Milivojević, J., Radivojevic, D., Dragišić Maksimović, J., Veberic, R., and Mikulic-Petkovsek, M. (2017). Does plant growth and yield affected by Prohexadione Ca cause changes in chemical fruit composition of 'Loch Ness' and 'Triple Crown' blackberries? *Eur. J. Hortic. Sci.* 82 (4), 190–197 <https://doi.org/10.17660/eJHS.2017/82.4.4>.
- Milosavljević, D.M., Mutavdžić, D.R., Radotić, K., Milivojević, J.M., Maksimović, V.M., and Dragišić Maksimović, J.J. (2020). Phenolic profiling of 12 strawberry cultivars using different spectroscopic methods. *J Agric Food Chem* 68 (15), 4346–4354 <https://doi.org/10.1021/acs.jafc.9b07331>. PubMed
- Minocha, R., Majumdar, R., and Minocha, S.C. (2014). Polyamines and abiotic stress in plants: a complex relationship. *Front Plant Sci* 5, 175 <https://doi.org/10.3389/fpls.2014.00175>. PubMed
- Mirdehghan, S.H., and Rahimi, S. (2016). Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera* L.) quality during postharvest period. *Food Chem* 196, 1040–1047 <https://doi.org/10.1016/j.foodchem.2015.10.038>. PubMed
- Nair, M.S., Tomar, M., Punia, S., Kukula-Koch, W., and Kumar, M. (2020). Enhancing the functionality of chitosan- and alginate-based active edible coatings/films for the preservation of fruits and vegetables: a review. *Int J Biol Macromol* 164, 304–320 <https://doi.org/10.1016/j.ijbiomac.2020.07.083>. PubMed
- Nawaz, M.A., Huang, Y., Bie, Z., Ahmed, W., Reiter, R.J., Niu, M., and Hameed, S. (2016). Melatonin: current status and future perspectives in plant science. *Front Plant Sci* 6, 1230 <https://doi.org/10.3389/fpls.2015.01230>. PubMed
- Nikolić, M., and Tanović, B. (2012). *Rubus* and *Ribes* industry in Serbia: a production model for developing countries. *Acta Hortic.* 946, 405–412 <https://doi.org/10.17660/ActaHortic.2012.946.67>.
- Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., and Diamantidis, G. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid content in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem.* 102 (3), 777–783 <https://doi.org/10.1016/j.foodchem.2006.06.021>.
- Savvides, A., Ali, S., Tester, M., and Fotopoulos, V. (2016). Chemical priming of plants against multiple abiotic stresses: mission possible? *Trends Plant Sci* 21 (4), 329–340 <https://doi.org/10.1016/j.tplants.2015.11.003>. PubMed
- Shanbehpour, F., Rastegar, S., and Ghasemi, M. (2020). Effect of preharvest application of calcium chloride, putrescine, and salicylic acid on antioxidant system and biochemical changes of two Indian jujube genotypes. *J Food Biochem* 44 (11), e13474 <https://doi.org/10.1111/jfbc.13474>. PubMed
- Sherin, G., Aswathi, K.R., and Puthur, J.T. (2022). Photosynthetic functions in plants subjected to stresses are positively influenced by priming. *Plant Stress* 4, 100079 <https://doi.org/10.1016/j.stress.2022.100079>.
- Shit, S.C., and Shah, P.M. (2014). Edible polymers: challenges and opportunities. *J. Polym.* 2014, 1–13 <https://doi.org/10.1155/2014/427259>.
- Singh, V., Jawandha, S.K., and Gill, P.P.S. (2021). Effect of exogenous putrescine treatment on internal browning and colour retention of pear fruit. *J. Food Meas. Charact.* 15 (1), 905–913 <https://doi.org/10.1007/s11694-020-00696-7>.
- Singh, V., Jawandha, S.K., Gill, P.P.S., and Singh, D. (2022). Preharvest putrescine application extends the shelf life and maintains the pear fruit quality. *Int. J. Fruit Sci.* 22 (1), 514–524 <https://doi.org/10.1080/15538362.2022.2068733>.
- Torrigiani, P., Bregoli, A.M., Ziosi, V., Scaramagli, S., Ciriaci, T., Rasori, A., Biondi, S., and Costa, G. (2004). Pre-harvest

polyamine and aminoethoxyvinylglycine (AVG) applications modulate fruit ripening in Stark Red Gold nectarines (*Prunus persica* L. Batsch). *Postharvest Biol. Technol.* 33 (3), 293–308 <https://doi.org/10.1016/j.postharvbio.2004.03.008>.

United Department of Agriculture, National Agricultural Statistics Service. (2022). Noncitrus Fruits and Nuts 2021 Summary. https://www.nass.usda.gov/Publications/Todays_Reports/reports/ncit0522.pdf.

Valero, D., Martínez-Romero, D., and Serrano, M. (2002). The role of polyamines in the improvement of the shelf life of fruit. *Trends Food Sci. Technol.* 13 (6-7), 228–234 [https://doi.org/10.1016/S0924-2244\(02\)00134-6](https://doi.org/10.1016/S0924-2244(02)00134-6).

Xu, T., Chen, Y., and Kang, H. (2019). Melatonin is a potential target for improving post-harvest preservation of fruits and vegetables. *Front Plant Sci* 10, 1388 <https://doi.org/10.3389/fpls.2019.01388>. PubMed

Yerramathi, B.B., Kola, M., Muniraj, B.A., Aluru, R., Thirumanyam, M., and Zyryanov, G.V. (2021). Structural studies and bioactivity of sodium alginate edible films fabricated through ferulic acid crosslinking mechanism. *J. Food Eng.* 301, 110566 <https://doi.org/10.1016/j.jfoodeng.2021.110566>.

Ze, Y., Gao, H., Li, T., Yang, B., and Jiang, Y. (2021). Insights into the roles of melatonin in maintaining quality and extending shelf life of postharvest fruits. *Trends Food Sci. Technol.* 109, 569–578 <https://doi.org/10.1016/j.tifs.2021.01.051>.

Zeng, W., Mostafa, S., Lu, Z., and Jin, B. (2022). Melatonin-mediated abiotic stress tolerance in plants. *Front Plant Sci* 13, 847175 <https://doi.org/10.3389/fpls.2022.847175>. PubMed

Zhang, W., Cao, J., Fan, X., and Jiang, W. (2020). Applications of nitric oxide and melatonin in improving postharvest fruit quality and the separate and crosstalk biochemical mechanisms. *Trends Food Sci. Technol.* 99, 531–541 <https://doi.org/10.1016/j.tifs.2020.03.024>.

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Are priming agents a step forward to enhance soft fruit yield efficiency?

» The development of non-toxic synthetic and natural priming agents (PAs) towards sustainably-sourced and environmentally sound products towards a resource-efficient circular economy is an R&D activity that recently has received considerable attention. However, the effort of the Lead Market Initiative (LMI) Advisory Group to trigger a prospective market for innovative products still needs to be implemented. PRIME-SOFT is a European-funded project (www.prime-soft.eu), and its overarching objective is to explore innovations in applying PAs in soft fruits from a range of perspectives to take advantage of the EU-driven effort for product innovation, development, and marketization.

Dr. George Manganaris | Associate Professor at Cyprus University of Technology
Director of the CUT Fruit Sciences Group



Defining the term 'priming agent'

The process of priming involves prior exposure to abiotic stress factors, rendering a plant more resistant/tolerant to future exposure. There is a wide range of compounds that are considered to have a priming effect and can be classified into the following categories: (i) chemicals (i.e., hormones, Reactive Oxygen Nitrogen and Sulphur Species (RONSS), and small organic molecules), (ii) microorganisms (i.e., arbuscular

and (iii) nanomaterials (i.e., organic and inorganic nanoparticles, as well as polymers). Recent research on chemical priming has provided further knowledge of the complex mode of action of specific signalling molecules involved in the process and enhanced plant tolerance against individual abiotic stresses. PAs additionally offer an attractive alternative to commonly employed methodologies for enhancing tolerance to stresses, as some are particularly time-consuming (i.e. conventional breeding) and others are

The concept and the novelty

Soft fruits, also referred to as small fruits or berries, represent a wide and very diverse group of crops with high nutritional value but are very perishable with limited shelf-life potential. These crops are also greatly affected by stress conditions. The concept of the application of priming agents (PAs) to enhance yield performance and quality attributes of soft fruit crops is entirely novel. The presentation will provide information regarding the prospects



to improve stress tolerance, giving special reference to strawberry cultivation. To our knowledge, existing technologies representing major competition are limited to relatively few formulations/biostimulants based on silicon nutrition/supplementation and which do not always provide cross-protection against multiple abiotic stress factors, such as drought, salinity, and heat. The novelty of our scientific strategy lays in the fact that it encompasses (1) the exploration of both a naturally derived priming agent (PA) in the form of melatonin as well as a synthetic PA recently co-patented by

the HO (use of NOSH/NOSH-A in plants; WO/2015/123273) and (2) the employment of both advanced nanomaterial engineering and encapsulation techniques through electro-hydrodynamic processes to enhance PA's efficiency towards increment of yield, enhancement of health-promoting properties and additionally ameliorate plant damage under climate change-related abiotic stress conditions in added-value soft fruit crops, namely strawberry and raspberry.

Conclusion

Priming agents (PAs) have gathered unprecedented attention, with many

companies investing in R&D initiatives for their development, as Europe's new bioeconomy strategy and action plan aspires to drive towards an increased and sustainable use of renewable resources. We aim to bridge the gap between chemical and nanomaterial priming research and agricultural practice in order to bring the inventions (i.e., beneficial effects of advanced nanomaterials conjugated with natural metabolite-based PAs) closer to application and commercialization when it comes to agricultural practice and more broadly to sustainable or "green" technologies. ●