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Orchard Management Under Climate Change

2nd Edition

Edited by
Sergio Ruffo Roberto, Simone Rodrigues da Silva and Sarita Leonel

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Orchard Management Under Climate Change: 2nd Edition

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Guest Editors

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About the Editors

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Preface

Climate change has become one of the most pressing challenges confronting global fruit production, demanding innovative approaches to ensure the sustainability, productivity, and resilience of orchard systems. The reprint *Orchard Management Under Climate Change: 2nd Edition* brings together a collection of peer-reviewed research and review articles that address the scientific and technological advances needed to adapt fruit production to increasingly variable environmental conditions. The volume covers a broad range of topics, including irrigation and water-use efficiency, crop phenology, physiological responses to abiotic stress, nutritional management, precision horticulture, rootstock and cultivar selection, mechanization, and the expansion of fruit production into emerging growing regions. Collectively, these studies highlight practical and science-based strategies for improving orchard performance under current and future climate scenarios.

The primary aim of this reprint is to provide a comprehensive and accessible reference that consolidates recent advances in climate-smart orchard management. By bringing together contributions from researchers working across diverse climatic regions and fruit species, this volume facilitates the exchange of knowledge and promotes interdisciplinary collaboration. The motivation for publishing this collection stems from the growing need to support the horticultural sector with innovative management practices capable of mitigating climate-related risks while maintaining fruit quality, resource-use efficiency, and economic viability.

This reprint is intended for researchers, university faculty, graduate and undergraduate students, extension specialists, crop consultants, growers, and policymakers interested in sustainable fruit production. It also serves as a valuable resource for professionals seeking to understand how scientific advances in orchard management can contribute to resilient production systems and support global food security in the face of ongoing climate change. We hope this collection stimulates further research, innovation, and international cooperation toward the development of more sustainable and climate-resilient orchard systems.

Sergio Ruffo Roberto, Simone Rodrigues da Silva, and Sarita Leonel

Guest Editors

Orchard Management Under Climate Change: 2nd Edition

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1. Introduction

Climate change continues to be one of the most significant challenges facing global horticultural production. Increasing temperatures, changes in rainfall distribution, more frequent drought events, heat waves, late frosts, and extreme weather phenomena are affecting orchard productivity, fruit quality, resource use efficiency, and the long-term sustainability of fruit production systems worldwide [1–4]. Recent studies have demonstrated that climate change influences crop phenology, flowering dynamics, fruit development, water requirements, and pest and disease incidence, thereby requiring substantial adjustments in orchard management practices [1,2]. Furthermore, the increasing frequency of extreme weather events poses additional risks to fruit production systems, threatening both yield stability and economic viability [3].

To address these challenges, researchers, growers, and industry stakeholders have intensified efforts to develop innovative orchard management strategies that improve resilience and adaptation under changing environmental conditions. These strategies include improved irrigation management, precision agriculture technologies, optimized canopy architecture, climate-adapted cultivars and rootstocks, and advanced monitoring systems for environmental and crop responses [2,4].

The Special Issue “Orchard Management Under Climate Change: 2nd Edition” was organized to provide an international forum for presenting recent advances related to climate adaptation in fruit production systems. The published papers address a wide range of topics, including irrigation management, physiological responses to abiotic stress, nutritional diagnosis, crop phenology, rootstock selection, precision horticulture, and the expansion of fruit crops into new production regions.

The contributions collected in this Special Issue provide valuable scientific knowledge that supports the development of sustainable orchard systems capable of maintaining productivity and fruit quality under future climatic scenarios.

2. Overview of Published Articles

The Special Issue comprises original research articles and review papers covering several fruit crops and production systems across different climatic regions.

Water availability and drought adaptation were major themes among the published studies. Research involving ‘Hass’ avocado conducted by Kanedo et al. (Contribution 1) demonstrated the importance of optimized irrigation management under increasingly frequent drought conditions. The authors described that water deficits can negatively

affect fruit growth and yield, highlighting the need for improved irrigation scheduling and water-use efficiency strategies.

In another paper, Jitariu et al. (Contribution 2) provided a comprehensive assessment of potential changes in the growing season of plum orchards in Romania under different climate change scenarios. By integrating temperature-based phenological modeling, the authors identified a consistent trend toward an earlier onset and an extended duration of the growing season across all analyzed regions. Their findings suggest that ongoing climate change may substantially alter plum phenology, with important implications for orchard management, production practices, and the long-term sustainability of plum cultivation in Romania.

Precision horticulture and climate-based crop management were addressed in a review article by Fotouo Makouate et al. (Contribution 3), which discussed the advances in growing degree day models, environmental sensing technologies, remote sensing applications, and digital agriculture tools. The review highlighted the growing importance of integrating climate information and precision management technologies into orchard decision-making processes.

Moving towards another interesting cultivation, Brawner et al. (Contribution 4) investigated if a modified harvester height was suitable for cider apple harvest in the US. The study included 16 cider apples cultivars to assess the modified Oxbo-Korvan 930 over-the-row harvester. The authors concluded that the equipment is suitable for cider apple production and requires 40-fold less time than hand harvesting, making it significantly more labor-efficient.

Nutritional management under changing climatic conditions was addressed through a study focused on the development of updated nutritional diagnostic standards for avocado by Almeida de Oliveira Junior et al. (Contribution 5). Using DRIS and CND methodologies, the researchers proposed new nutritional reference values that may contribute to more efficient fertilization programs and improved nutrient management in commercial orchards.

Several articles explored the interaction between orchard management practices and plant physiological performance. Studies evaluating grapevine rootstocks and training systems conducted by Domingues Neto et al. (Contribution 6), demonstrated that canopy architecture influences photosynthetic activity, biochemical responses, vegetative growth, and yield components. These findings provide valuable information for adapting viticultural practices to subtropical environments. Climate adaptation through rootstock and cultivar selection was another important topic. Research on 'BRS Núbia' table grapes conducted by Monteiro et al. (Contribution 7) evaluated phenological development and thermal requirements under subtropical conditions, providing information that may assist growers in improving production scheduling and vineyard management.

The diversification of fruit production systems under changing climates was represented by studies on hop cultivation in subtropical Brazil conducted by Sabino et al. (Contribution 8). These investigations demonstrated that high-quality hop cones can be successfully produced under non-traditional growing conditions, creating opportunities for crop diversification and the development of new production regions.

Another publication conducted by Roussos et al. (Contribution 9) investigated the use of mineral particle films in olive orchards exposed to Mediterranean climatic conditions. The application of kaolin-, talc-, and attapulgite-based products reduced heat stress effects and contributed to maintaining fruit productivity and oil quality under challenging environmental conditions.

Finally, the impacts of climate variability on phenology and frost occurrence were investigated in temperate fruit species by Choi et al. (Contribution 10). The study, involving

apple and pear orchards, highlighted the challenges associated with changing flowering periods and frost risks, emphasizing the importance of regional adaptation strategies and climate monitoring tools.

Overall, the articles published in this Special Issue illustrate the multidisciplinary nature of climate adaptation research and demonstrate the importance of combining physiological, agronomic, environmental, and technological approaches to improve orchard resilience.

3. Conclusions

The studies included in this Special Issue provide valuable insights into the challenges and opportunities associated with orchard management under climate change. Collectively, the contributions demonstrate that successful adaptation requires integrated strategies involving efficient water and nutrient management, improved cultivar and rootstock selection, optimized canopy architecture, precision agriculture technologies, and climate-informed decision-making.

As climate variability continues to increase, the adoption of innovative and sustainable management practices will become increasingly important for maintaining orchard productivity, fruit quality, and economic viability. The findings presented in this collection contribute to the scientific foundation needed to support the transition toward more resilient fruit production systems worldwide.

The Guest Editors sincerely thank all authors for their valuable contributions, the reviewers for their constructive evaluations and dedication, and the editorial staff of *Horticulturae* for their support throughout the publication process. We hope that this Special Issue stimulates further research and collaboration aimed at developing sustainable orchard systems capable of meeting future climatic challenges.

Conflicts of Interest: The authors declare no conflicts of interest.

List of Contributions

1. Kaneko, T.; Gould, N.; Campbell, D.; Clearwater, M.J. Irrigation, Water Deficit and Crop Load Effects on ‘Hass’ Avocado Fruit Size Under New Zealand Growing Conditions. *Horticulturae* **2026**, *12*, 230. <https://doi.org/10.3390/horticulturae12020230>.
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9. Roussos, P.A.; Karyda, A.-G.; Mavromanolakis, G.-I.; Gkliatis, D.; Zoti, M. The Effects of Different Mineral Clay Particles on Olive Yield and Olive Oil Quality of Two Cultivars Under Rainfed or Irrigated Conditions. *Horticulturae* **2025**, *11*, 341. <https://doi.org/10.3390/horticulturae11040341>.
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Review

Advances in Growing Degree Days Models for Flowering to Harvest: Optimizing Crop Management with Methods of Precision Horticulture—A Review

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Abstract

Temperature plays a vital role in plant metabolism, and effective crop temperature appears to be influenced by variables related to climate change. While extreme weather events are widely discussed, the effects of moderate temperature changes pose consistent yet underexplored challenges for farmers. The “growing degree days” (*GDD*) also termed “heat unit”, is the most widely used approach in agricultural and ecological studies to quantify the relationship between temperature and plant development. This review provides a comprehensive examination of *GDD* methodology as applied to horticultural crop production, specifically from initial fruit development to fruit maturity, and postharvest. It is the first integrated synthesis of the conceptual evolution, methodological refinement, and broad application of *GDD*, thereby highlighting the need to optimize *GDD* approaches in light of emerging technological tools. While the *GDD* model is valuable for predicting crop development based on heat accumulation, it has limitations in capturing the effects of other environmental factors. Additionally, air temperature may not provide precise data on each plant organ. Recent advances in remote sensing, such as the integration of thermal imaging, RGB cameras, and lidar have enabled the measurement of spatially resolved temperature distribution within crop canopies, including fruit surface temperature. Recent advances, highlighted in the literature, suggest that integrating sensor innovations with machine learning approaches holds high potential for improving the precision of modeling temperature-dependent growth responses and their interactions with other environmental variables. By addressing these challenges and expanding its applications, *GDD* can continue to serve as an essential tool in promoting sustainable horticultural practices and adapting to global warming.

Keywords: biofix; fruit quality; fruit temperature; heat unit; high temperature stress; machine learning; sensors; temperature monitoring

1. Introduction

Climate change is one of the biggest challenges that global agriculture is facing in the 21st century. Under projected climate scenarios, the air temperature is expected to rise, potentially shifting growing seasons—either shortening, extending, or altering their onset and end [1,2]. Global radiation, temperature, and precipitation are the main climatic factors that affect plant phenology; and temperature is considered the most important element, influencing the growth and development of crops [3,4]. Climate change is directly or indirectly

linked to other indicators such as soil temperature, photoperiod, and apparent solar radiation [5]. Changes in ambient temperature, whether moderate or extreme, affect virtually every aspect of plant physiology simultaneously, creating a complex web of interconnected responses, through a cascade of cellular, biochemical, and molecular mechanisms, including enzyme kinetics, hormone synthesis [6], and membrane fluidity [7]. A moderate increase in temperature can enhance enzymatic reaction rates by increasing molecular collisions and lowering activation energy barriers. This acceleration benefits various metabolic processes such as photosynthesis, respiration, and nitrogen assimilation [8]. For example, under moderate temperature increases, Calvin cycle enzymes, particularly ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the primary carbon-fixing enzyme, exhibit heightened catalytic activity, leading to improved carbon fixation rates [9,10]. Respiratory enzymes also show considerable temperature dependencies that influence plant energy metabolism and growth rates. The citric acid cycle enzymes, electron transport chain components, and ATP synthase all exhibit distinct temperature response curves that collectively determine respiratory capacity across different thermal conditions [11,12]. The temperature sensitivity of respiratory processes often exceeds that of photosynthesis, leading to changes in the photosynthesis-to-respiration ratio with temperature that can affect plant carbon balance and growth [13]. Moreover, membrane fluidity is highly temperature-dependent. It becomes more rigid under cold conditions and more fluid at elevated temperature, which in turn modulates the activity of embedded proteins and signaling complexes [14,15]. These temperature-mediated alterations in membrane dynamics and transport efficiency influence hormone distribution, sugar loading, and nutrient uptake, all of which being crucial for flower initiation, fruit development, and quality [16].

While extreme temperature events often receive primary attention in climate change discussions, moderate temperature increase, typically within 0.5–4 °C of historical averages, create equally important, but more subtle impacts throughout the horticultural production process [17,18]. These moderate increases represent the most common temperature scenario under current climate projections and warrant detailed examination of their effects on plant growth and product quality. For the purpose of this review, the term high temperature or temperature stress will only be limited to moderate increase in temperature.

One of the key metrics for understanding temperature-plant interactions is the concept of Growing Degree Days (*GDD*), a fundamental tool that quantifies thermal energy accumulation for predicting the timing of various plant development stages [19,20]. The *GDD* or approach was for the first time introduced by the French scientist Rene A. F. de Réaumur as “degré de chaleur moyenne” [21]. He suggested that the duration of a particular growth stage is directly related to temperature and that this duration for a particular species could be predicted using the sum of mean daily air temperature, $\bar{T}_{air}$ [22,23]. Various terminologies are related or used interchangeably with the term *GDD* in the literature; such as “heat unit”, “degree day”, “heat requirement” [24,25], “thermal time” [26,27], “heat accumulation” [19,28], and “heat summation” [29]. Although the terminology may differ, the concept is grounded in the observation that temperature can be used to monitor or predict the development of an organism at each growth stage in its life cycle [30,31]. The *GDD* is generally calculated by summing the daily $\bar{T}_{air}$ above a base threshold, representing the minimum temperature needed for plant growth. The *GDD* methodology extends across multiple agricultural domains, enabling optimization of planting schedules [32], precise timing of harvest operations [33,34], monitoring of pest life cycles [35], and strategic scheduling of fertilizer and pesticide applications [36,37]. It provides valuable insights into how temperature variations affect the physiological growth of plants, aiding in the prediction of crop yield potential and the timing of important agricultural tasks, hence, potentially optimizing economic returns and minimizing environmental impacts [38,39].

However, there has been no recent critical analysis or review of the existing knowledge regarding this valuable tool for agricultural production and management.

The relationship between *GDD*, temperature, and plant growth is fundamental for vice versa understanding how temperature affects horticultural crops. As temperatures rise and *GDD* values increase, plants experience accelerated development. This may not always result in increased productivity or optimum quality of produce. If temperatures surpass optimal growth ranges, the benefits of higher *GDD* can be offset by high temperature stress. The latter disrupts optimum plant development and potentially causes irreversible damage in the field [40] or even affects storability of fresh produce [41]. High-temperature stress, moderate temperature, and cold are essential information used in crop management and, consistently, to frame *GDD*. However, the number of high temperature events are expected to increase under future climate scenarios, potentially causing growing seasons to shift, shorten or even be extended [1,2] depending on species, cultivar-specific traits, and region. Therefore, development of adaptive strategies based on field or even fruit data are vital for maintaining horticultural productivity and ensuring food security.

During the flowering to fruit maturation phase, the accumulation of *GDD* plays a pivotal role in determining synchronization of flowering with optimal pollination conditions as well as the length of time required for fruit ripening [42,43]. Changes in temperature can advance or delay flowering and/or fruiting by speeding or slowing heat accumulation [44]. Likewise, a rise in temperature may increase fruit growth rate and shorten the duration of fruit development [45,46]. However, rapid developmental phases may compromise final fruit quality attributes, as reduced developmental time limits dry matter accumulation, and biochemical compound synthesis and thus affecting sensory and nutritional quality attributes [46,47]. Understanding this dynamic allows farmers to make more informed decisions about crop management practices in the field and anticipate high temperature stress events that may disrupt plant growth. The knowledge is also relevant in decision-making, choosing the most suitable varieties for their region, adjusting planting times, and net or sprinkler installations.

Estimating *GDD* from flower bud to fruit maturity is not without challenges. Traditional *GDD* estimation faces inherent limitations including uncertainties in base temperature selection, optimal starting date determination, and temperature measurement accuracy—all factors that considerably influence prediction reliability [48]. Furthermore, the assumption of linear temperature-development relationships often oversimplifies complex biological responses, while environmental interactions involving soil moisture, quality and intensity of light, and humidity patterns further complicate predictive accuracy [49]. Recent studies confirm that nonlinear heat accumulation methods can substantially reduce prediction errors [50]. Standard *GDD*-based models often exhibit limited generalizability across climates and cultivars; for instance, multi-site evaluations of apple phenology revealed considerable shortcomings in model transferability between contrasting environmental settings [41]. Despite these challenges, recent advancements in technology, including precision temperature monitoring, remote sensing, the Internet of Things (IoT), and machine learning are transforming the information gaining on how agricultural systems respond to environmental stressors like increase in air temperature. With the ability to collect high amounts of data in real time and processing with these technologies, the accuracy and reliability of *GDD* models can be improved. This allows their adaptation to diverse geographic regions and growing conditions [51,52]. Additionally, it could allow for early detection or prediction of high temperature stress by identifying subtle changes in plant physiology before they are visible to the human eye and thus call for possible remedial action by farmers.

This study aims to summarize previous research on the relationship between temperature increase and *GDD*, highlighting the use of *GDD* method as a tool to monitor or predict the influence of increased temperature on flowering, fruit development, harvest maturity, and product quality. Furthermore, various modifications and extensions of the *GDD* model that have been proposed to enhance its precision and applicability across different geographic regions and plant species are discussed. Ultimately, this review lays a theoretical foundation for future work on how *GDD* can be optimized, particularly in light of emerging technological tools, to support predictions that are more accurate in fruit crop production.

2. Methodology

A literature search was conducted to identify relevant studies on the concept and applications of growing degree days (*GDD*). The search was performed across major scientific databases, including the Web of Science Core Collection, Science Direct, Scopus, PubMed, and the state library Berlin (Germany); and include literature from 1949 to 2025. A variety of keywords were employed, such as “growing degree days”, and its various variations “thermal unit”, “heat accumulation,” and “thermal time”, as well as application-specific terms like “phenology,” “temperature and flowering” “and “temperature, fruit development and quality”. The results were further refined by restricting the search to the fields of agricultural, plant, and environmental sciences. Thus, studies that focused on degree day concepts unrelated to growing degree days were systematically excluded from the review to ensure relevance and specificity in the literature selection process. Studies with a main focus on heat stress were also excluded. Duplicates were checked manually by the authors and excluded. The results indicate an increasing interest in understanding the impact of temperature on plant behavior (Figure 1), a trend likely driven by concerns over the continuous rise in global temperatures due to climate change.

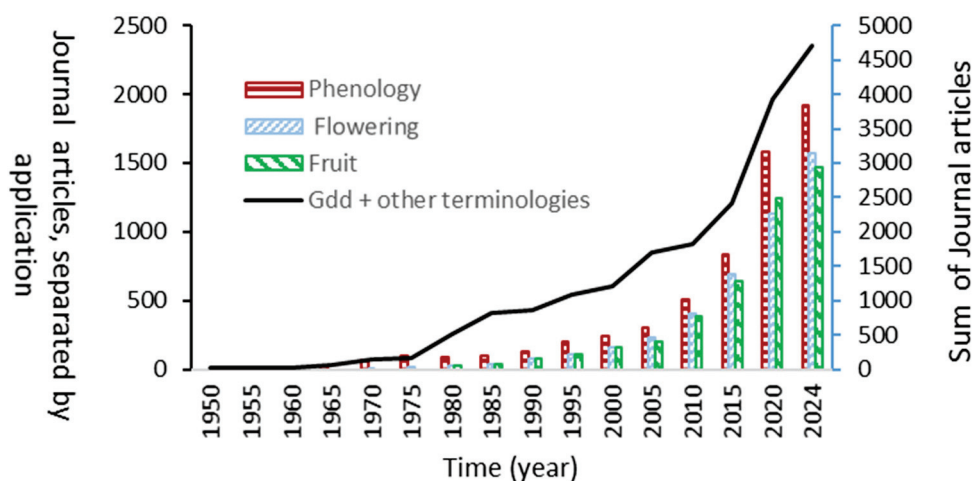


Figure 1. Number of published journal articles using the term “thermal unit”, and “heat accumulation” as well as “flowering or blooming” and “fruit”, “temperature and flowering” and “temperature, fruit development and quality”.

3. Growing Degree Days Model

3.1. Concept

The use of calendar days to predict plant development for management decision is an old practice. However, calendar days can be misleading due to local weather conditions and particularly in time of increased climate variability and unpredictability. Furthermore, the change in temperature can alter plant physiology and phenology and those modifications, e.g., plants grow faster during warm weather than in cold weather, cannot be captured by

calendar days. Thus, measuring the temperature accumulated over time provides a more accurate approach [53]. The unit of *GDD* is mostly reported as °C. Some authors refer to degree days (°d), while Kelvin (K) would be the unit for temperature differences according to SI. The quantity reported by the three units is the same; therefore, this review follows the majority of authors and reports in °C. According to the *GDD* concept, if *GDD* = 1000 °C required for maturity, the plant should reach maturity by the number of days on which 1000 °C has been accumulated [22], which is directly accessible by the farmer. The original heat unit concept uses the simple daily average [54]. When the T_{air} is below a threshold, the development rates are assumed insignificant [55]. For example, according to the *GDD* concept, if a plant has a base temperature (T^b) of 1 °C and the mean temperature ($\bar{T}$) of this day is 10 °C, the difference for that day (in this example, 9 °C) is phrased “degree days” or “heat units”. The *GDD* is calculated as

$$GDD = \sum_{d=1}^D \frac{(\max T + \min T)}{2} - T^b \quad (1)$$

d: day

D: end of growing stage

where the maximum ($\max T$) and minimum ($\min T$) (typically air) temperature and base temperature (T^b) are considered.

The model has evolved since its inception (Table 1), with advancements enhancing its accuracy and applicability across various domains. The modifications include: the use of different base temperatures [48,56]; more precise integrations of the upper and lower threshold temperature [19,57]; the inclusion of a day length factor [56,58]; and the use of a temperature index as opposed to time as the divisor in growth analysis functions [23]. The simple average [54], sine wave [19], the triangulation methods [59] and their variants [35,60,61] are classified as linear models. While nonlinear developmental rate models are common, linear-based degree day models have been shown to provide a better predictive capability in the field for some cases [50,62].

Table 1. Methods of base temperature determination (adapted from [55,63]).

Method	Variants of Base Temperature, T^b in °C	Criteria, Minimized to Select T^b for GDD Calculation
Least standard deviation in GDD	$T_{SD_{GDD}}^b = \frac{(\sum_{i=1}^N \bar{T}_i d_i \sum_{i=1}^N d_i - N \sum_{i=1}^N d_i^2 \bar{T}_i)}{(\sum_{i=1}^N d_i)^2 - N \sum_{i=1}^N d_i^2} \quad (2)$ N: number of plantings per year	$SD_{GDD} = \sqrt{\frac{\sum_{i=1}^N (GDD_i - \overline{GDD})^2}{N-1}} \quad (3)$ GDD_i: GDD of the observed timing of the phenological stage for i plantings $\overline{GDD}$: overall mean GDD of the phenological event across all plantings N: number of plantings/years. SD_{GDD} is given in °C
Least standard deviation in days	$T_{SD_d}^b = \bar{T} - \frac{(\sum_{i=1}^N \bar{T}_i d_i)^2 - N \sum_{i=1}^N \bar{T}_i^2 d_i^2}{N \sum_{i=1}^N d_i^2 \bar{T}_i - \sum_{i=1}^N d_i \bar{T}_i \sum_{i=1}^N d_i} \quad (4),$ where $\bar{T}_i = \bar{T} - \bar{T}$ $\bar{T}_i$: overall mean of temperature in all plantings $\bar{T}$: mean temperature of ith planting/year	$SD_d = \frac{SD_{GDD}}{(\max T - T_{SD_d}^b)} \quad (5)$ SD_{GDD}: standard deviation in GDD $\bar{T}$: overall mean temperature of the developmental period of all years or all plantings SD_d is given in days
Coefficient of variation in days	$T_{CV}^b = \frac{\sum_{i=1}^N \bar{T}_i d_i^2 \sum_{i=1}^N \bar{T}_i d_i - \sum_{i=1}^N d_i \sum_{i=1}^N \bar{T}_i^2 d_i^2}{\sum_{i=1}^N d_i^2 \sum_{i=1}^N \bar{T}_i d_i - \sum_{i=1}^N n_i \sum_{i=1}^N \bar{T}_i d_i^2} \quad (6)$	$CV_d = \frac{SD_d}{\bar{d}} \times 100 \quad (7)$ Mean $\bar{d}$ is the number of days observed to reach the phenological stage across all plantings CV_d is given as percentage
Linear regression	$T_r^b = \frac{\sum_{i=1}^N \bar{T}_i \sum_{i=1}^N \bar{T}_i d_i - N \sum_{i=1}^N d_i \bar{T}_i^2}{\sum_{i=1}^N \bar{T}_i \sum_{i=1}^N \bar{T}_i d_i - N \sum_{i=1}^N d_i \bar{T}_i} \quad (8)$	$RMSE = \sqrt{\frac{\sum_{i=1}^N (n_i^p - n_i^o)^2}{N}} \quad (9)$ n_i^p: Predicted number of d in the ith year. n_i^o: Observed number of d in the ith year

3.2. Starting Date

In cultivated annual plants, phenological events are typically traced and dated back to the first visible phenophases, most commonly the initial germination, and occasionally from the date of sowing [48]. For perennial plants, the starting dates, also known as biofix, for growth and development can differ substantially among species, ecotypes, and plant age [64,65]. Identifying optimal starting dates for physiological processes in perennial plants is often challenging. In the 1900s, the 1st of January was used as the biofix date for phenological studies in temperate regions [66,67], which proved problematic, especially in northern temperate areas, where the growing season may commence in March or later [48]. The timing of flowering can vary widely based on local climate conditions, even within a single region [68]. This variability complicates the selection of an appropriate starting date, potentially leading to inaccurate predictions of subsequent developmental stages or mismanagement of agricultural practices.

Furthermore, the definition of “flowering” “further contributes to inconsistencies among studies, as it depends on both crop type and modeling objectives. Most past and recent studies refer to the “first flowering date” when discussing flower phenology [69–71]. However, there is some ambiguity regarding what represents the “first flowering date,” even across Europe, as the BBCH scale is not crop specific [72]. Phenological datasets may be recorded from the day the first flower is spotted [71,73] or later, when it is assumed that approximately 10% of the plants are in bloom, particularly in fruit trees [74,75]. Some studies initiate monitoring when 20–25% of blossoms have opened [76,77] or at 50% [78,79]. The BBCH scale suggests six secondary stages, namely first flower, 10%, 30%, and 50% flower open, flowering finishing, and visible fruit set [72,80]. Conversely, some studies consider three secondary stages: first flowering (25%), peak flowering (50%), and last flowering (75% faded) [81], or first flowering (10% open), full flower (80% open), and petal fall (90% fallen) [74]. Regardless of percentages, determination of flowering date through visual observation can be subjective, adding to variability. In some instances, early flowers may not fully develop or may drop before maturity, resulting in discrepancies between the observed “start” of flowering and the actual growth processes that affect development [82]. Furthermore, some flowers may wither or fall off, while others bloom within the same period [74]. Relying solely on the date of first flowering may overestimate shifts in peak flowering and fail to predict changes in subsequent growth stages, making it difficult to detect responses to climate change [83]. Additionally, variations in population size can lead to earlier or later first flowering dates [71].

3.3. Base Temperature

Just as the starting date is crucial, the selection of a threshold temperature and the accuracy of calculations are vital to ensure that *GDD* models are adjusted to local farming conditions. Typically, the base temperature is determined by the minimum temperature required for crop development, below which the biological processes of interest do not progress [30]. The base temperature, T^b , is not universally fixed. It can be species-specific for each phase of a crop and may depend on environmental conditions and the objectives of the study [31,84]. Environmental factors that influence the T^b include soil characteristics, day length, general air and soil temperatures, water conditions, and light quality and quantity [22,85]. In the 1800s, only heat units above 0 °C were typically summed as the threshold temperature [48]. By the 1900s, a T^b of 5–6 °C became the most commonly used standard for cold-season crops, particularly in agriculture [57,86,87]. In warm-season crops, such as corn and soybean, higher T^b (8–10 °C) were considered [30,88]. The mid-20th century marked a more systematic effort to define and calculate base temperatures. Experimental

approaches became prevalent, with greater emphasis placed on the biological status of the plant rather than solely relying on historical growth data or previous observations [19].

The value of estimated T^b may also vary considerably with methodologies used to determine thresholds [54] and GDD model [30]. The most common mathematical formula for calculating the base temperature for GDD are the least standard deviation in GDD , SD_{GDD} (Equation (2), Table 1) [89,90]; the least standard deviation in days, SD_d (Equation (4), Table 1) [54]; the coefficient of variation in days, CV_d (Equation (6), Table 1) [91]; and the regression coefficient (Equation (8), Tables 1 and 2) [92].

Table 2. Example data comparing three methods standard deviation for setting the base temperature, T^b , and resulting degree days given in parenthesis, calculated between flowering and fruit harvest maturity, for apricot [75] and cherry [55]. Terms refer to least standard deviation ($T^b_{SD_{GDD}}$), least coefficient of variation (T^b_{CV}), linear regression (T^b_r). The unit °C has been used as synonym for degree days (°d) or Kelvin.

Crop	Cultivar	$T^b_{SD_{GDD}}$ in °C	T^b_{CV} in °C	T^b_r in °C
Apricot	Harcot	6.4 (925)	3.0 (1248)	4.3 (1117)
	Bergeron	5.0 (1311)	2.6 (1578)	3.7 (1472)
	Stella	3.5 (1280)	2.0 (1417)	2.5 (1367)
Cherry	Burlat C1	−3 (887)	-	1 (650)
	Burlat	1 (649)	-	4 (475)
	Forli	2 (673)	-	3 (585)

These empirical methods seek to determine the T^b that best aligns with the growth patterns observed in a particular species by minimizing the standard deviation, coefficient of variation, and root mean square error in the prediction of plant phenological events, such as flowering or fruit ripening. The T^b is usually set across multiple years. GDD is calculated using a series of candidate base temperatures, each one resulting in a set of GDD s and standard deviations. The temperature that generates GDD s with the smallest standard deviation is selected as the base temperature [56,75]. In annual crops, it is usually selected using a series of plantings [93]. The SD_{GDD} focuses on minimizing the variability in the total GDD accumulation or heat unit for predicting a phenological event, while the SD_d approach aims to minimize variation between calendar days. The $CV_{GDD/d}$ aims to calculate the relative magnitudes of the variation in days, as this is relevant for practical application [56]. Another method that uses Equation (1) for T^b determination is the iteration method [55]. The iteration model minimizes the root mean square error ($RMSE$, Equation (9), Table 1) between the observed and predicted number of days in development period. Similar to the least standard deviation, in the iteration approach, adjustments of the base temperature are performed until it no longer results in meaningful improvements in the accuracy of the predictions. The regression coefficient model assumes that GDD is a constant for crop development and can be expressed as a function of the threshold temperature, T^b (Table 2) [56,92]. For a series of base temperatures, regression equations are computed, the resulting GDD values are plotted versus $\bar{T}$, and the correct T^b appears where the regression equation equals zero. That is until the plant no longer accumulates enough heat for meaningful growth.

The upper threshold is less well defined, while it is considered as the temperature at which the rate of growth or development begins to decrease or stop [22,94]. Determining a consistent upper threshold is difficult. Often, they are unavailable for use in phenology models and may occur at higher temperatures than the one recorded in the study site [53]. Some authors have even argued that upper thresholds do not sufficiently increase the accuracy of the prediction for growth or development [19,95]. The introduction of the upper threshold led to an increase in the coefficient of variation in GDD when determining

the heat requirement from blooming to maturing in apricot cultivars [95]. However, it is likely that the upper threshold will become more crucial in the future due to enhanced number of heat (wave) events.

3.4. Simple Average Method

A straightforward model has been used since the 1800s, and remains relevant for farmers, extension workers, and parts of the scientific community. It is computed directly by averaging the daily maximum and minimum temperatures, then subtracting a base temperature specific to the organism or the growth stage over the specified period (Equation (1), [54,96,97]). This method assumes a linear relationship between the heat units accumulated and daily extremes temperatures and does not account for upper temperature threshold [19]. Ignoring intra-day temperature patterns can result in inaccuracies during periods of rapid temperature changes, leading to underestimate/overestimate heat accumulation [29,98,99]. To overcome this challenge, *GDD* is frequently calculated with the growing degree hour (GDH) [100,101] if hourly temperatures are available. When considering hourly data, *GDD* is determined by first calculating degree-hours for each hour of the day, followed by the summation of the degree-hours over the 24 h day and dividing the total by 24 [62,94]. In a comparative study, it was found that if temperatures exceed the base temperature during part of the day, while the daily mean remains at or below the base temperature, then the accumulated growing degree using *GDH* method is meaningful [29]. In comparison, *GDD* may lead to an underestimation of heat summation. Few authors recommended using *GDH* estimates for more accurate heat summation, a conclusion supported by other researchers [3,102,103].

3.5. Sine Wave Approximation (Baskerville-Emin Method)

This mathematical approach employs a sinusoidal curve to model daily temperature and estimates *GDD* by calculating using the area above the base temperature [19,98]. The method allows for both upper and lower threshold. The *GDD* is estimated by integrating the area under the sine curve using one or more of four cases (Table 3, [19,62]). Advancement of the sine-wave method was approached by calculating degree days from half-day curves. This approach was published as double sine method [98]. The single sine method fits a sine curve through the day's minimum and the maximum temperature, while the double sine method uses separate sine curves for the maximum temperature period of the day and the minimum temperature of the following day [98]. This approach can be particularly useful in climates with extreme day–night temperature variation [35]. While some researchers prefer the double sin method, studies [35,94] indicate that it is more accurate when considering only few days. In contrast, the difference between the two methods becomes negligible over longer observation period. Similar to the sine curve method, the triangulation method utilizes a day's low and high temperatures to produce an equilateral triangle over a 24 h or 12 h period. Degree days are estimated by calculating the area between the two thresholds enclosed by either the single or the double triangle [60,94].

Table 3. Calculating growing degree days (*GDD*) by the single sine method. Accumulated degree days are shaded areas of diagrams [19,35].

	Diagram	Equation
1		$GDD = \sum \left(\frac{max_T + min_T}{2} \right) - T^b$
2		$GDD = \frac{1}{\pi} \left[\left(\frac{max_T + min_T}{2} \right) - T^b \right] (\pi/2 - \theta_1) + \alpha \cos(\theta_1)$ $\theta_1 = \sin^{-1} \left[\left(T^b - \frac{max_T + min_T}{2} \right) \div \alpha \right]$
3		$GDD = \frac{1}{\pi} \left\{ \left(\frac{max_T + min_T}{2} \right) - T^b \right\} (\theta_2 + \pi/2) +$ $\left(T^u - T^b \right) \left(\frac{\pi}{2} - \theta_2 \right) - [\alpha \cos(\theta_1)]$ $\theta_2 = \sin^{-1} \left[\left(T^u - \frac{max_T + min_T}{2} \right) \div \alpha \right]$ T^u: upper threshold
4		$GDD = \frac{1}{\pi} \left\{ \left(\frac{max_T + min_T}{2} \right) - T^b \right\} (\theta_2 - \theta_1) + \alpha$ $[\cos(\theta_1) - \cos(\theta_2)] + \left(T^u - T^b \right) \left(\frac{\pi}{2} - \theta_2 \right)$ $\theta_1 = \sin^{-1} \left[\left(T^b - \frac{max_T + min_T}{2} \right) \div \alpha \right]$ $\theta_2 = \sin^{-1} \left[\left(T^u - \frac{max_T + min_T}{2} \right) \div \alpha \right]$

Case 1: The daily minimum temperature is above the single threshold temperature. The *GDD* is calculated following the simple average method. *Case 2:* The daily minimum temperature is below a single threshold temperature. Degree days are calculated by integrating the curve to receive the area under the sine curve above the base temperature. *Case 3 and Case 4:* There are both upper and base threshold temperatures. Degree days are calculated as the difference considering the area above the base and below the upper threshold temperatures under the sine curve.

However, the use of *GDD* as a tool to monitor or predict when a crop will reach important developmental stages is not without criticism. One of the major concerns is that most models are based on the assumption of a linearity between plant developmental rate and temperature. However, this is not strictly the case, particularly at lower temperatures [66,104] and in events of high temperature spikes that occur outside of typical growth ranges [105]. Secondly, the dependence on only one of the many environmental parameters

to explain the variation in biological response [22,106]. There have been attempts to improve *GDD* models by adding other environmental factors such as the photoperiod [85,107]. It is predicted that, as a response to climatic warming, species with a high sensitivity to temperature and little sensitivity to photoperiod would be expected to undergo noticeable changes in flowering time. In contrast, less change would be observed in species strictly controlled by photoperiod [108]. Thus, a model that estimates the rate of development at this stage of growth must represent the interaction of these factors, to increase the accuracy of *GDD* [109,110]. The first model of *GDD* that integrated photoperiod was developed by Nuttonson, based on the observation that the number of degree days from emergence to maturity multiplied by the average day length was more reliable compared on degree days used alone [85]. Several researchers continue to advocate on the importance of either considering *GDD* model concurrently with (or integrate *GDD* in) other models for crop growth prediction [111–113]. In any application, some progress on using advanced technology may support improved *GDD* accuracy.

4. Using Growing Degree Days to Guide Harvest and Quality Decisions

4.1. Flowering

In annual crops, which complete their life cycle in a single season, *GDD* accumulation typically begins from planting, making predictions relatively straightforward [30,31]. In perennial fruit crops, *GDD* models must consider carry-over effects from the previous season, particularly the accumulation of chilling hours during dormancy. For species of temperate climate, the timing and duration of chilling play a crucial role in determining bud break or bud opening. A specific number of chill hours (or chilling requirements) at low temperatures is required to break endodormancy and prepare for spring growth. Apple and peach trees may require approximately 600 to 1000 chill hours, depending on the variety, before bud break can occur [114]. Several chilling accumulation models have been developed to estimate chill accumulation, including the Chilling Hours Model, the Utah Model [96], the Dynamic Model [115], and Phenoflex [116].

In temperate deciduous fruit trees, flowering is closely related to heat accumulation following dormancy release, and cultivar-specific *GDD* thresholds allow reliable prediction. Apple cultivars such as ‘Gala’ and ‘Fuji’ differ substantially in their *GDD* requirements for flowering and harvest, with early cultivars flowering at lower heat sums than late cultivars [117]. Sweet cherry (*Prunus avium*) exhibits predictable *GDD* ranges: approximately 100–200 °C for bud break, 300–500 °C for full bloom, and 1200–1800 °C from bloom to harvest depending on cultivar [118]. In Nordic climates, it was confirmed that sweet cherry flowering can be accurately modeled using *GDD* despite low spring temperatures [119], as opposed to in Mediterranean regions. It was shown that almond (5654–10,201 °C) and apple (7471–9690 °C) cultivars require distinct heat sums for bloom [120]. Subtropical crops such as mango (*Mangifera indica*) also follow *GDD*-dependent flowering patterns [121,122], with a cultivar-dependent range of 382–500 °C in India [121], and a yearly variation of 665–714 °C in Brazil [122] from bud swelling to flowering. These differences showcase the broad applicability of *GDD*-based models across climatic zone, and also the need for adaptation of the models (Table 4).

Warmer summer temperatures often accelerate the accumulation of *GDD*, speeding up physiological processes such as floral initiation and development in several species, reported for strawberry [123], grape [124], and peach [125]. Budburst, flowering, and veraison occur earlier by an average of 5–7 days, 12–14 days, and 17–19 days, respectively, in various cultivars of grape due to rising temperatures [124]. However, the relationship between temperature and flowering is complex. While earlier onset of flowering is commonly observed with temperature increases, the magnitude and direction of phenological shifts

vary between early- and late-flowering crop species or varieties. Crops that flower early in the season (e.g., apricot, plum, peaches) often exhibit stronger advancements in phenology [126,127] compared to those with later flowering periods, e.g., apple, pear [117,128]. Furthermore, increased winter temperatures could delay blooming by reducing chilling hour accumulation, while excessive temperatures may adversely affect flower development, leading to poor pistil development and reduced fruit set [129,130].

GDD modeling for flowering provides actionable guidance for frost protection, pollination scheduling, and labor planning. By using locally calibrated cultivar-specific base temperatures and thermal thresholds, growers can anticipate bloom timing, even under climate variability [131,132].

Table 4. Examples of growing degree day (GDD) requirements, and the base temperature, T^b or flowering prediction across crops and climates. The unit °C has been used as synonym for degree days (°d) or Kelvin.

Crop	Climatic Region	T^b in °C	Starting Date/Duration	GDD (°C)	Reference
Olive	Mediterranean	5.0	1 January	1434	[133]
Apple	Mediterranean	4.0	February	7471–9690 *	[120]
Sweet cherry	Temperate	2.0	1 March	254	[119]
Sweet cherry	Temperate	4.5	3 Feb–28 March	3233–4343 *	[134]
			11 Feb–14 April	5444–6988 *	
Sour cherry	Temperate	2.0	1 January	492	[135]
Sour cherry	Temperate	4.0	1 March	125	[38]
Grapevine	Temperate	10.0	1 January	1600–1850	[136]
Grapevine	Temperate	10.0	1 January	318–552	[131]
Mango	Subtropical	10.0	from floral bud swelling-floral opening	623	[122]

* Calculated with growing degree hours model.

4.2. Fruit Development and Maturity

Once flowering has occurred, GDD accumulation governs fruit development and the timing of harvest (Table 5). Temperature influences fruit development by altering the plant's metabolic activity. Generally, warmer temperatures speed up biochemical processes, resulting in faster fruit growth and ripening, although this relationship varies across development stages [137]. For every 1 °C increase in average spring temperature, the bloom-to-harvest period shortens by about 3.5 days in temperate fruit trees [138], a trend also observed in Kinnow mandarin (*Citrus nobilis* Lour $\times$ *Citrus deliciosa* Tenora), where warmer temperatures lead to an increase in GDD accumulation, accelerating fruit set, cell division, and overall maturation [139]. In peaches (*Prunus persica*), GDD is used alongside chilling requirements to forecast flowering and fruit development, critical for frost management and harvest optimization [96].

Table 5. Growing degree day (GDD) requirements, and the base temperature, T^b for fruit maturity prediction across crops and climates.

Crop	Climatic Region	T^b in °C	Starting Date/Measurement Duration	GDD (°C)	Reference
Peach	Sub-temperate	7.0	January	814–1894	[140]
Peach	Temperate	7.0	May–August	1651–1826	[141]
Grapevine	Temperate	10.0	April–September	1204–1940	[142]
Grapevine	Temperate	10.0	After bloom	556–800	[131]
Grapevine	Humid continental	10.0	1 April–31 Oct.	1070–1700	[143]
Mango	Tropical	17.9	July–November	1062.71–1309.41	[121]
Mango	Tropical	12.0	Asparagus stage of flower	1740–2185	[144]
Mango	Subtropical	10.0	Floral bud swelling to commercial maturity	2399	[122]
Papaya	Subtropical	15.0	Flowering to harvest	1293–1488	[34]
Orange	Subtropical (Humid)	12.8	9 August	4462–5090	[145]
Eggplant	Subtropical	15.6	June–November	892–1078	[146]

In peaches, *GDD* exceeding 494 °C in the first 30 days post-bloom resulted in less than 60% of peaches being classified as large, compared to years with combined *GDD* of 468 °C or less during the same timeframe [147]. Similarly, strong correlation between *GDD* and the timing of maturity stages were found in other experiments on peaches, providing a reliable index beyond simple calendar days [140,141]. Grapevines (*Vitis vinifera* L.) rely heavily on *GDD* for predicting veraison, and harvest, aiding vineyard zoning and quality forecasting [148,149]. For reported grapevine grown in temperate semi-arid conditions, development from bloom to veraison occurred after accumulating 556–800 °C with a base temperature of 10 °C [131]. In comparison, grapevines cultivated in a humid continental climate required a higher heat accumulation of 1070–1700 °C from April 1 to October 31 to reach harvest [143]. Subtropical oranges (*Citrus sinensis*) showed even greater thermal requirements, needing 4462–5090 °C from August 9 onward at a base temperature of 12.8 °C [145], illustrating how *GDD* accumulation varies substantially among species and climatic regions. Modeling the impact of air temperature and cumulated temperature on the duration of fruit developmental stages from flowering to green, white, and turning stages, has been proposed to optimize the harvest date in strawberry [150].

Similarly, in cherries, *GDH* accumulation closely tracks key developmental stages, including flowering and fruit set, allowing precise phenological predictions. This was demonstrated in sweet cherry under temperate conditions [38], and its importance was subsequently confirmed for sour cherry as well [151]. *GDD* models are also effective in tropical and subtropical crops. For instance, papaya (*Carica papaya* L.) requires approximately 293–1488 °C from anthesis to ripening, enabling optimized harvest timing [34]. The relationship between *GDD* and crop outcomes is also evident in vegetable production. Research on eggplant (*Solanum melongena*) cultivation, for example, revealed a multifaceted association [145]. The latter authors found that *GDD* reliably predict the timing of key phenological stages, from flowering to fruit maturity, which directly influences yield potential by affecting the rate of fruit set and development. Furthermore, the study showed that *GDD* affects marketable quality, finding that sufficient thermal time was necessary to achieve desirable commercial characteristics such as optimal fruit size. An earlier study found that the heat units model was more effective than calendar-day methods for determining optimal harvest time in cucumber production [152].

Beyond predicting phenology, *GDD* models can evaluate whether new cultivars will reach full maturity within the growing season, as shown in kiwifruit by Kovaleski et al. [153]. In warmer regions, model accuracy can be improved by incorporating both lower and upper temperature thresholds, as demonstrated for mango in Australia [143].

4.3. Fruit Quality

While *GDD* is primarily used to predict phenological timing, their influence extends deeply into the final quality of harvested fruit (Table 6). The *GDD* accumulated throughout a growing season directly shapes critical postharvest attributes like flavor, texture, color, and shelf-life, as these traits are intrinsically linked to fruit maturity [154,155]. Achieving an optimal *GDD* accumulation is essential; insufficient heat results in underdeveloped fruit, while excessive heat can accelerate ripening to the detriment of overall quality [156,157].

In grapevine development, cumulative *GDD* critically shapes fruit composition, particularly the sugar and acid balance that determines a wine's quality potential. Several authors [132,136,145] observed that increased *GDD* accelerates berry ripening by promoting sugar accumulation, while reducing titratable acidity. It was further emphasized that this process could be reliably predicted using cultivar-specific models, highlighting the need to tailor such predictions to different grape varieties [132]. While these compositional changes can enhance stylistic attributes like body and perceived sweetness, they also risk creating

imbalances in warmer vintages by advancing ripening too quickly and diminishing acidity below optimal levels. These findings underscore the dual role of *GDD*: it is both a key predictor of phenological progress and a potential driver of compositional trade-offs that must be managed to preserve wine quality under changing climatic conditions.

Table 6. Fruit quality monitoring using growing degree day across crops and climates. Titratable acidity (TA), soluble solids content (SSC).

Crop	Climatic Region	T^b in °C	Starting Date/Measurement Duration	<i>GDD</i> (°C)	Fruit Quality Attributes Related to <i>GDD</i>	Reference
Mango	Subtropical	12.0	From <i>asparagus</i> stage of flowering	1728–2185	Flesh color, dry matter content, SSC:TA ratio	[144]
Peach	Subtropical	7.0	From full bloom to fruit senescence/harvest	1106–1547	Fruit size and mass, mesocarp cell number	[158]
Grape	Humid continental	10.0	August–October	1330–1500	SSC, timing of ripening	[159]
Apple	Humid continental	10.0	Seasonal accumulation up to harvest (29 Aug–3 Oct. 2012, 11 Sept–26 Sept. 2013)	1444–2406	SSC, TA, starch index, firmness, internal ethylene and soft-scald susceptibility	[160]
Apple	Temperate	7.2	From anthesis to harvest	892	SSC, firmness, diameter	[161]

In peaches, a strong correlation between *GDD* thresholds and the onset of physiological maturity has been documented, allowing for more accurate estimation of harvest readiness [142]. Similarly, changes in fruit texture and sugar–acid balance, important determinants of firmness and flavor, has been found to be temporally linked to specific *GDD* accumulations [141]. Concurrent monitoring of fruit quality variables (firmness, soluble solids content (SSC), and titratable acidity) along with temperature from full bloom to harvest led to the identification of 2469 °C as the optimum harvest date for pears [162]. Generally, warmer conditions tend to decrease fruit firmness and titratable acidity (TA) while increasing SSC. This pattern has been observed in apples grown at warmer temperatures [45] as well as in peaches [163] and plums. In tomatoes, increased heat unit accumulation prior to harvest substantially enhances sugar content but also reduces fruit firmness and increases the risk of cracking [164]. Adding to this complexity, other studies have shown that warmer temperatures raise SSC and acidity, but concurrently decrease levels of lycopene and total carotenoids compounds essential for both fruit color and nutritional quality [165,166].

Collectively, these studies demonstrate that *GDD* and *GDH* models are versatile and effective tools for predicting key developmental stages such as flowering, fruit set, and harvest maturity across a wide range of horticultural species and climatic conditions. Their robustness and low cost make *GDD*-based modeling particularly valuable for practical applications, including frost risk assessment, optimizing pollination timing, irrigation scheduling, thinning, and harvest planning. The accuracy and reliability of these models depend heavily on careful calibration tailored to specific cultivars, phenological stages, and local climate conditions. For deciduous temperate crops, it is crucial to consider the interactions between chilling requirements and heat accumulation to improve model precision. Furthermore, ensuring that input data are precise and representative using accurately calibrated sensors is essential for producing reliable model predictions.

5. Remote Sensing Approaches

In most studies, meteorological data on T_{air} from the closest official weather stations are used for this purpose [167]. The contribution of those studies to scientific knowledge cannot be disputed. Aimed at more precise analysis, the importance of microclimatic temperatures for limiting deviation from actual *GDD* at the plant and even plant organ scale was highlighted since the late 20 century [62,167,168]. According to Roltsch et al. [62], the most important factor to consider when employing *GDD* models in the field is “How

well do available temperature readings reflect the microclimatic temperatures that an organism actually experiences within the field environment?”. The actual conditions depend on all climate variables, such as global radiation, relative humidity, wind, affected by within-canopy shading and airflow resistance. Sources of error of *GDD* calculations linked to data collection has been discussed: the temporal resolution of data recorded, collections of data once daily using separate thermometers and the use of $\bar{T}$ calculated using daily maximum and minimum versus the $\bar{T}$ calculated by averaging values recorded every minutes, and the position of the sensors [27]. The latter researcher emphasized the fact that even when temperature sensors are located in the field, the T_{air} at shelter height may differ from the temperature affecting the plant.

A good understanding of changes in flowering phenology requires monitoring from the beginning to the end of the growth stage. Recording flower phenology throughout the full growing seasons using real-time monitoring tools, such as remote sensing, can help mitigate these challenges [169,170]. Automatic image-based monitoring technologies for flower phenology are being progressively explored in ecological studies, yielding promising results [171–174]. For example, a precision of up to 0.92 in their flower detection model was reported when using automated method to monitor the phenology of two species in the South Greenland [173]. A national flowering map of Southern Beech in Australia was created with overall classification of 90.8% accuracy using a Sentinel-2 Imagery, as fully automated remote sensing system [174].

Consistently, it was shown that degree days calculated from local weather stations, using the single sine method failed to reflect the predicted delay in tomato development and also lead to erratic prediction of ripening rate [167]. Even if it may be reasonable in some research work to assume that $T_{air} = T_{fruit}$, a more accurate measurement would be supportive [175,176]. Furthermore, considering the different mass, absorption coefficients, and heat transfer coefficient [177] when comparing fruit and leaf, a difference between T_{air} and T_{leaf} or T_{fruit} can be reasonably assumed.

Based on weather data and thermodynamic model considering the absorption and emissivity of apple fruit, the fruit surface temperature was modeled [178]. Since the radiative fruit properties are requested in this approach, direct measurements appear as an alternative for analysis of a high number of fruit and implementation in practice: Temperature measurements at the fruit surface in the field were mostly performed by means of thermocouples [176,179] (Figure 2). For this purpose, the probes were inserted under the epidermis. The data are collected by data logger or wireless sensor network. Influencing factors are the penetration depth of the wire, wind conditions, direct exposure to the sun, as well as tissue decay due to the sensor implementation. While only low numbers of fruit can be measured with this approach, the methodology is frequently used as reference for new sensor approaches and machine learning [180].

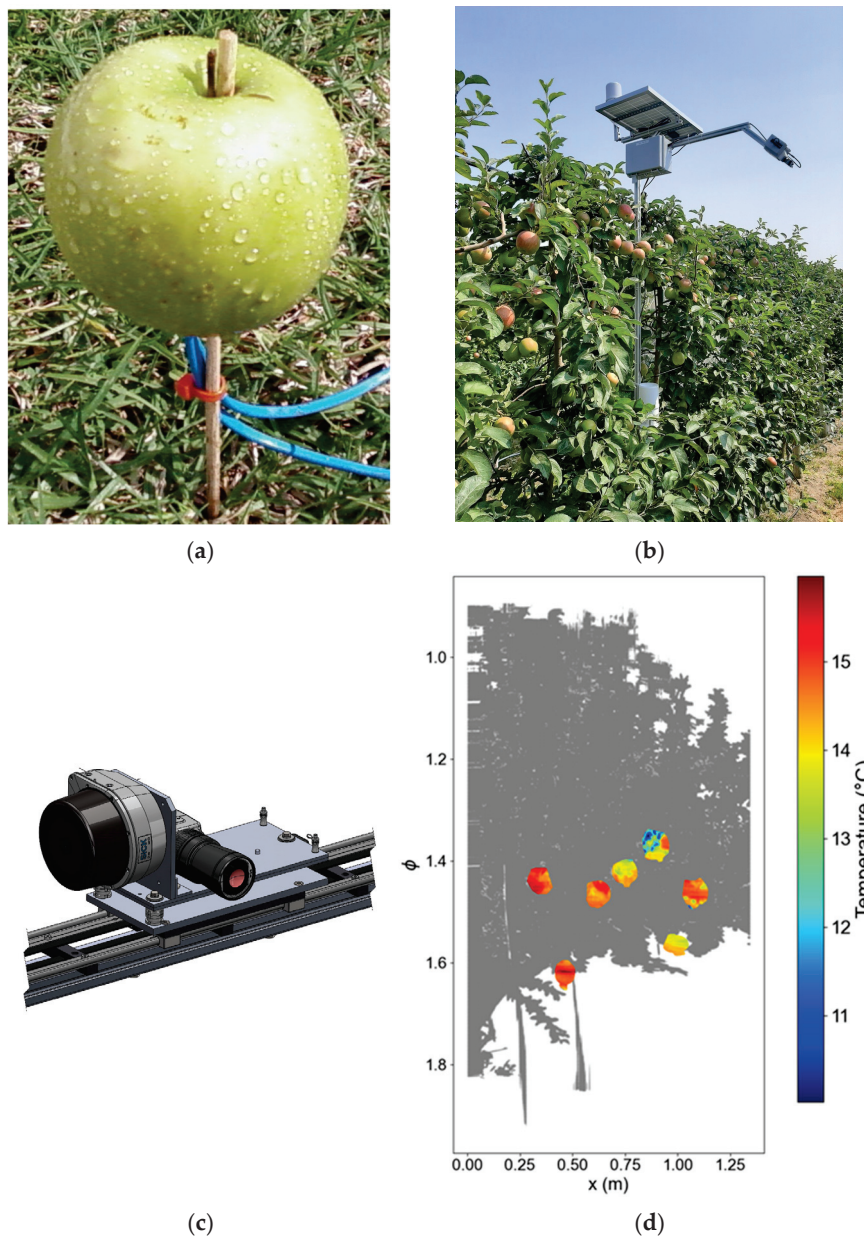


Figure 2. Temperature sensing at the fruit level employing thermocouples inserted below the epidermis of apple ((a) [181]), using weather sensors and thermal-RGB imager for analysing 2D T data of apple ((b) [182]), mobile sensor array with thermal camera and lidar sensors ((c) [183]) and, obtained by sensor array (c), measured T -annotated 3D point cloud that shows temperature of fruit surface in false color scale ((d), data derived from open access database [183]).

Thermal imaging has been applied in orchards, initially employed to detect fruit in the canopy, followed by the interest in fruit surface temperature due to global warming. Using a sensor combination of RGB and thermal cameras, T_{fruit} was estimated with a non-contact approach [182,184]. The root mean square error, $RMSE$, was documented in the range 0.50–1.08 Kelvin (Figure 2, [182]). By means of this approach, data from a higher number of fruit were acquired, compared to fruit readings with thermocouples. Recent studies applied thermal 2D information to study the susceptibility of grape to heat stress [185]. In apple, the T_{fruit} was extracted with 2D spatial resolution, considering a min, max, and mean value [186]. Such data have been applied for the prediction of free water on the fruit surface of sweet cherry [187,188]. Using this range of temperature obtained may also provide valuable input in GDD models. Thermal cameras have the advantage of

being precalibrated by the manufacturer capturing environmental variables such as relative humidity. However, the heat emission coefficients of fruit surface are different from the surrounding [177], which results in a certain error in field measurement. Under conditions of free water on the fruit surface due to precipitation, irrigation, condensation as well as dense fog, thermal imaging cannot measure the fruit surface temperature.

Furthermore, measuring spatial temperature distribution in 3D would address individual fruit to capture the large variation between fruit appearing within a canopy, resulting from the microclimate and individual fruit's radiative properties [176]. The estimation of 3D spatially resolved temperature distribution has recently gained interest in other disciplines such as in building layouts, where linear textures appear [189]. Subsequently, the concept of merging mobile terrestrial light detection and ranging (lidar) sensor and thermal imaging has been introduced in crop production. The methodology has been in an early stage of development, showing the temperature distribution of leafy structures in an apple orchard in Chile [190]. While 3D point clouds obtained by lidar provide 3D information on the geometric structure of tree canopies, the merging with thermal data results in in situ data on the 3D distribution of temperature in the canopy, as shown with high resolution at the fruit level (Figure 2). Temperature information is provided in the x , y , z coordinate system as points, either in local or global coordinate system. The approach of analyzing position and temperature of individual fruit was enabled in a data pipeline. The first step captures the data fusion of lidar and thermal imaging, resulting in T annotated point clouds [191]. As a second step, segmentation of T annotated fruit point clouds from the canopy point cloud is necessary [192]. The entire data pipeline enabled the analysis of T_{fruit} spatially within the canopy (Figure 2) with a documented RMSE of 1.9% [193]. The spatial monitoring of variation in fruit surface temperature, affected by environmental changes, can provide specific information for crop management. Physiological changes observed on fruit could be related to tree architecture and climate change with high density of fruit data. In a study on Gala–Brookfield apples, seasonal and diel courses of T_{fruit} were described [193]. In previous studies, it was proposed that a time gap between air and fruit temperature exists throughout the diel course, which is diminished when working with daily mean temperatures [176]. In diurnal courses measured with the 3D approach, the gap between the course of T_{air} and T_{fruit} was described in the Gala–Brookfield apples [193], which provides new opportunities for 3D GDD modeling. As an addition, this approach allows the assessment of position, fruit size, and distribution of T_{fruit} in canopies, potentially capturing a high number of fruit also for these variables [194]. A low-cost solution, using RGB-D sensors instead of lidar and low-cost thermal cameras, was already approached [195] and tested in apple and grape. However, the methodology of analyzing spatial temperature distribution has been introduced only recently by few workgroups and more research is requested on the implementation of the data as a tool for GDD applications.

Concurrently, there have been recent advancements in fruit maturity identification leveraging radiometric information gained from lidar's return signal strength intensity [196]. Computer vision and deep learning have greatly enhanced the precision and scalability of automated fruit ripeness assessment. Convolutional neural networks (CNNs) have been pivotal in automating feature extraction from fruit images, enabling accurate classification of maturity stages without manual intervention [197,198]. Notably, the YOLO family, including models like the YOLO-BLBE (combines innovative image enhancement (I-MSRCR) and attention mechanisms) developed for blueberries addressing challenges such as occlusion, variable lighting, and dense fruit clusters [199]. This illustrates the transformative potential of interdisciplinary research, integrating close-range remote sensing,

machine learning, and phenological studies potentially in combined models, capturing GDD and maturity analysis for optimized crop management.

6. Conclusions

Growing degree days (*GDD*) have emerged as a pivotal tool in understanding plant growth and development, offering a quantitative approach to measure the accumulation of heat necessary for crops to progress through their life cycles. It is a simple yet effective approach to understanding temperature-driven biological processes relevant in horticultural production. As the effects of global warming intensify, understanding and adapting to the relationship between *GDD* and flowering, fruit development, and postharvest quality will be critical for maintaining the standards of fruit production and ensuring sustainability in the face of changing environmental conditions.

Despite its widespread utility, the application of *GDD* is not without challenges. Considerable effort has been made over years to improve *GDD* calculation, but there are still limitations in determining appropriate base temperatures, which vary across crops and phenological development. Additionally, *GDD* models often assume a linear relationship between temperature and development, disregarding the complexities of plant responses to extreme weather, particularly events of heat stress and other environmental factors. Variability in local climate conditions, data availability at the fruit level, and the influence of factors like patterns of rainfall, soil–water potential, carbon dioxide partial pressure, and photoperiod also complicate the accuracy and reliability of *GDD* models.

Advancements and initial use of satellite data analysis and close-range remote sensing, such as thermal imaging, combining RGB and thermal imaging, or lidar and thermal imaging can enhance the spatially-resolved analysis of temperature distribution in crop canopies at the fruit level. These technologies, along with machine learning algorithms, provide high-density data of potentially all fruit at the tree. In addition, the consideration of dynamic factors has been enabled, e.g., diurnal course of fruit surface temperature (T_{fruit}) and the gap between air and fruit temperatures. The new approaches are seen as a new driving force for analyzing the impact of fruit temperature by means of the widely accepted *GDD* concept. By capturing detailed, 3D temperature data across different spatial scales, these innovations are also predicted to support nonlinear growth responses and interactions with other environmental factors. However, these are research challenges for the future.

In summary, *GDD* provides an essential framework for understanding and managing plant growth in relation to temperature. While challenges considering the availability of easy-to-use tools remain, advancements in close-range remote sensing technology offer opportunities to enhance the precision and spatial analysis of *GDD* models. Therefore, *GDD* can continue to serve as a vital tool in supporting sustainable agricultural practices and adapting to climate change.

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Article

Irrigation, Water Deficit and Crop Load Effects on ‘Hass’ Avocado Fruit Size Under New Zealand Growing Conditions

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Abstract

The potential for ‘Hass’ avocado production is predicted to increase with climate warming in New Zealand, a country where avocado orchards often lack irrigation because of a cooler and wetter climate compared to most other major growing regions. However, intermittent summer droughts are also predicted to increase in frequency and intensity. This study assessed the effects of summer soil water deficits on fruit growth of ‘Hass’ avocado in the Bay of Plenty, New Zealand, by comparing irrigated and non-irrigated treatments. Rainfall was variable over the three years of the study (2016–17, 2017–18, and 2018–19), but each summer there was a dry period without any rainfall for 2–3 weeks that decreased soil water content in the non-irrigated treatment. Fruit number and final yields were highly variable between trees and years, an effect of variable fruit set during the spring flowering period, and were not affected by the irrigation treatments because soil water deficits did not occur until later, during the summer. Increasing tree crop load caused decreasing individual fruit weight and dry matter content at harvest. However, in the year with the highest average crop load a dry period occurred during early fruit development, and mean fruit weight at harvest was decreased by 26.4 g (10%) in the non-irrigated treatment, an effect that was only apparent after accounting for the effects of variable crop load. The trees responded to dry conditions by reducing stomatal conductance (g_s) by 20%, preventing midday leaf water potential (Ψ_{leaf}) from decreasing below -0.25 MPa. Irrigation of avocado under the conditions at this site is therefore recommended when soil tension decreases below -30 kPa at 30 cm depth, and adverse effects on fruit growth are likely when tension decreases below -50 kPa. Irregular bearing of avocado under New Zealand growing conditions causes highly variable crop loads that obscure economically significant effects of mild to moderate water deficits on fruit growth. However, irrigation is still an important consideration for avocado production under current growing conditions and is likely to become more important under future climate scenarios as the risk of summer droughts increases.

Keywords: avocado; Hass; water; irrigation; fruit; yield; stress; deficit; soil; drought; climate change

1. Introduction

Careful management of plant water status is critical for production of many fleshy fruit crops, particularly during early fruit development when water deficits can have large impacts on final fruit size and quality [1,2]. However, climate change is altering

evaporation and rainfall patterns globally, and water resources for irrigation in many regions where these crops are grown [3]. Avocado (*Persea americana* Mill.) originates from subtropical and tropical regions that have a wet-summer and dry-winter climate, but as a crop it is now widespread in drier climate zones such as in parts of California, Chile, Israel, Peru and Australia, where frequent summer irrigation is vital for successful production. Avocado has also become an important horticultural crop in New Zealand, with a production area of approximately 5000 ha in 2025, producing 2% of global supply as the 9th largest exporter international exporter of avocado. In contrast to many other producing regions, the current climate of New Zealand is considered one of the coolest in the world for commercial avocado production [4], where many avocado orchards lack irrigation because of milder temperatures, lower evaporative demand and year-round rainfall [5]. However, climate change is causing more frequent short- and medium-term summer droughts in New Zealand [6], a trend that is predicted to continue, particularly in the more northern and eastern regions where avocado is currently grown [7]. These changes pose a challenge for the continued viability of the horticultural industry, because of the significant impacts of short-term water deficits on fruit growth across many crops, including avocado, and limitations on fresh water supplies for irrigation [8].

Avocado is known to be highly sensitive to water status, and reductions in water supply below well-watered crop demand can decrease fruit number, size and yield [9–11]. However, identifying when and how short periods of water deficit become limiting for fruit growth can be difficult because crop responses vary with weather conditions, soil type, physiological status, crop load, and the timing or degree of water scarcity [12]. There are several plant-based indicators that can be used to estimate the level of water stress in perennial horticultural crops. These include stem water potential (Ψ_{stem}) and leaf water potential (Ψ_{leaf}) [13,14], stomatal conductance (g_s) [15,16], changes in trunk diameter [17,18], leaf temperatures [19,20], and sap flow [21,22]. Significant water stress has been reported for avocado when Ψ_{stem} falls below -0.8 under Mediterranean climate conditions [23]. However, avocado is known to exhibit isohydric stomatal responses to soil water deficits, with plant water status regulated through stomatal closure [24]. Reductions in photosynthesis in avocado, caused by stomatal closure in response to water stress, have been observed when Ψ_{stem} decreased to -0.9 MPa in South Africa [25] and -1.2 MPa in California [26]. Fruit require water and carbohydrates to grow, and carbohydrates are derived from photosynthesis [27]. In an isohydric species like avocado, mild or moderate levels of stress may not be detectable as further decreases in Ψ_{stem} as the soil dries, but both water and carbohydrate supply to the fruit may be affected [28]. Fruit size and oil content in fruit (correlated with dry matter content—the ratio of dry weight to fresh weight) are both important fruit quality parameters for avocado. Periods of inadequate water supply can have a large negative impact on both.

Understanding how water stress influences fruit quality and yield in avocado is complicated by the variable nature of tree growth and reproduction from year to year. Avocado has unique characteristics that include protogynous flowering [24], a tendency for biennial bearing [29,30], and exceptionally high levels of flower and immature fruit abscission [31]. The temperate maritime climate of New Zealand, with relatively cool and variable temperatures in spring, directly influences these characteristics, causing frequent failure of the flowering opening process, irregular bearing from year to year, and a prolonged fruit growth period [4,32]. The resulting high levels of tree-to-tree variation in crop load, fruit size and flowering patterns, often observed within individual orchards, make it more difficult to detect and ameliorate the effects of moderate levels of water stress on fruit development. In the main avocado growing region of the Bay of Plenty with allophanic soils, soil water deficit is rare during the flowering period in spring but becomes

more likely during the period of rapid fruit growth in summer [28,33]. However, guidelines for irrigation management have only recently been developed for avocado production in New Zealand [33], and if irrigation is available, the strategies for its use remain variable from grower to grower.

The aim of this research was to quantify the effects of short-term water deficits on avocado fruit growth, yield, and quality under New Zealand conditions, and to provide supporting information for irrigation recommendations as producers adapt to a more variable climate. Irrigated and non-irrigated ‘Hass’ avocado trees were compared on an orchard in the Bay of Plenty, New Zealand, over three seasons. ‘Hass’ avocado is the prevailing avocado cultivar grown internationally, and currently accounts for 95% of plantings in New Zealand. It was hypothesized that a decrease in soil water content during the early fruit development stage from November to February, associated with intermittent rainfall, would reduce fruit size at harvest. However, it was also hypothesized that the naturally high and uncontrolled levels of variation in crop load between trees would interact with the effect of water stress on fruit size and dry matter content, making it more difficult to detect the effect of short-term water deficits on fruit size [24]. Potential reductions in fruit dry matter content were also considered, because of avocado’s tendency for stomatal closure and decreased photosynthesis in response to decreased soil water content.

2. Materials and Methods

2.1. Study Site

This research was conducted in a commercial avocado orchard, Omokoroa, Bay of Plenty, New Zealand ($37^{\circ}39'17.964''$ S, $176^{\circ}1'12.936''$ E). The region is characterized by a mild climate with rainfall throughout the year. In Tauranga (25 km from the study site), the 30-years (1981–2010) normal mean monthly maximum and minimum air temperatures were 24 and 15 °C in January, and 14 and 6 °C in July respectively, and normal annual rainfall was 1189 mm, with mean monthly total rainfalls of 74, 95, 78, 86, and 97 mm in November, December, January, February, and March, respectively (Figure 1) [34]. The soil type of the study site was sandy loam (Typic Orthic Allophanic soil) [35]. Management of the orchard, including fertilizer applications, pruning, pest control and irrigation followed recommended practices for commercial orchards in this region [36].

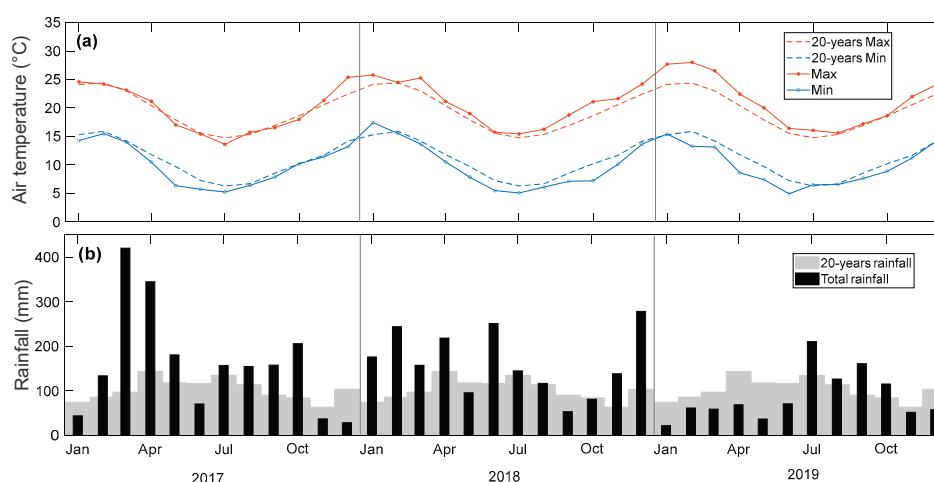


Figure 1. Mean monthly daily maximum and minimum air temperatures compared to normal maximum and minimum air temperatures (1981 to 2010) (a), and monthly total rainfall with normal rainfall (1981 to 2010) (b), at the orchard in the Bay of Plenty from January 2017 to December 2019.

2.2. Plant Materials and Experimental Design

Ten year old *Persea americana* ‘Hass’ avocado trees were growing on ‘Zutano’ seedling rootstocks, with 18 trees per row, 7.5 m between trees, and 9 m between rows. Within three adjacent rows, 24 healthy and mature trees with a height of 7 m and a canopy diameter of 7.5 m were selected for this study.

A complete block design with two treatments (irrigated and non-irrigated) and three rows as blocks was established (Supplementary Figure S1). There were four replicate trees per row, providing 12 trees in total per treatment. Trees of the same treatment were paired so that trees adjacent to non-irrigated pairs of trees could also be non-irrigated, to provide a buffer between treatments within rows.

Micro-sprinklers with a convex rotor (Rondo 75 lph, Rivulis, Migdal HaEmek, Israel) had been previously installed by the orchard owner below the canopy 2.5 m above the ground. For this experiment irrigation was applied for 6–8 h when soil water content of the 0–30 cm soil layer, as measured under the trees using soil moisture probes, dropped below 0.4 m³ m^{−3}. This threshold corresponded approximately to soil water tensions reaching 30 kPa at 30 cm depth (see Section 3), the recommended irrigation trigger point for commercial orchards in this region [36]. The volume of water recommended for irrigated trees was calculated as:

I = ET_oK_c − R (1)

where *I* is the depth of irrigation (mm), *ET_o* is the FAO-56 Penman-Montieth reference evapotranspiration (mm), *K_c* is a crop coefficient from 0.45 in December to 0.70 in March [33], and *R* is total rainfall (mm), calculated weekly. Groundwater does not contribute to the soil water balance of trees at this site [33]. Actual irrigation was controlled by the orchard owner, and tended to be less than the recommended amount before and during the experiment, because of restrictions on water availability and requirements for other parts of the orchard (see Section 3).

Sprinklers were disabled for the non-irrigated treatment from January to April in Year 0 (2016–17), and from November to April in Year 1 (2017–18) and Year 2 (2018–19), with the non-irrigated trees receiving only precipitation during the three summers of the study period. Trees in the irrigated treatment continued to receive irrigation as described above.

2.3. Experiment Timeline

Under New Zealand’s conditions, the flowering season for avocado is from early-October to early-November, and harvest starts in September the following year. The potential irrigation period, including at the study site, is generally from November to March (Table 1). From April to September rainfall usually exceeds evapotranspiration, soil water content at the study site did not fall below 0.4 m³ m^{−3}, and irrigation was not used [33].

Table 1. A typical timeline for flowering, rapid and slow fruit growth, harvest, and an irrigation period under New Zealand conditions.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Flowering												
Rapid fruit growth												
Slow fruit growth												
Harvest												
Irrigation period												

The experiment was conducted for three cropping years; ‘Year 0’ from October 2016 to September 2017, ‘Year 1’ from October 2017 to September 2018, and ‘Year 2’ from October 2018 to September 2019. Overall, there was no difference in soil water content between the

two treatments in Year 0, because the non-irrigated treatments were established during that summer, after the dry period that occurred in that year had begun (Table 2). Differences in soil moisture appeared more clearly in Years 1 and Year 2 after irrigation treatments were applied from the beginning of the summer period. Plant water potential was measured periodically during the summer of all three years. Fruit growth and fruit abscission were monitored throughout the fruit growth period of Year 1. Fruit yield, size and dry matter content were measured at harvest in Year 0 and Year 1. Fruit set was extremely low in Year 2 despite sufficient flower numbers, resulting in a lack of fruit over the entire orchard. However, monitoring of the impact of irrigation treatments on tree water status was continued for that year, and measurement of the effects of treatments on stomatal conductance were added.

Table 2. Summary of the three experimental years, including the timing of periods of dry conditions, tree cropping status, and the years the irrigation treatment, weather and soil moisture monitoring, water potential and stomatal conductance measurements, fruit monitoring and fruit analysis were conducted. Irrigation treatment is described as partial in Year 0 because the dry period had already begun when the monitoring of soil moisture and irrigation treatments were established, and ended with heavy rainfall before a full treatment effect on soil moisture could be established. The three years of the study are included when reporting the results because they illustrate the variability of summer rainfall and avocado yields and fruit size in New Zealand (see Section 3).

Experiment Conditions & Variables Measured	Year 0 2016–17	Year 1 2017–18	Year 2 2018–19
Dry period	mid-January to early-February	mid-November to late-December	mid-February to late-March
Cropping status of alternate bearing cycle	Off	On	Off
Irrigation treatment	Partial	Yes	Yes
Weather monitoring	Yes	Yes	Yes
Soil moisture monitoring	Yes	Yes	Yes
Water potential measurement	Predawn Stem Leaf	Predawn Stem Leaf	Stem Leaf
Stomatal conductance measurement	No	No	Yes
Fruit growth and fruit abscission monitoring	No	Yes	-
Harvest and fruit analysis	Yes	Yes	-

2.4. Weather

A meteorological station was installed in an open area within the orchard to record rainfall (TR-525I, tipping bucket rain gauge, Texas Electronics Inc., Dallas, TX, USA), solar radiation (LI200 pyranometer, LI-COR Inc., Lincoln, NE, USA), air temperature and humidity (HMP50 temperature and relative humidity probe, Campbell Scientific, Logan, UT, USA), and wind speed (Vector A101M anemometer, Vector Instruments, Denbighshire, UK) from January 2016 to November 2019. Sensors were connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT, USA), meteorological data were recorded every 15 min, and daily potential evapotranspiration (ET_0) for a short vegetated surface was calculated by the data logger using the FAO-56 Penman-Monteith equation [37]. To fill a gap caused by equipment failure, meteorological data for the nearby city of Tauranga were obtained for the period October to December 2016 [34].

2.5. Soil Water Content

To monitor volumetric soil water content, soil moisture probes (CS616, and CS650, Campbell Scientific Inc., Logan, UT, USA) were installed in January 2017. The soil moisture probes were calibrated in the laboratory before installation using soil samples taken from the orchard at the depths where the probes were buried. Dry soil was moistened to a known water content and packed to the same bulk density as measured in the orchard to obtain the relationship between the output values of the probes and actual volumetric soil water content.

The soil moisture probes were installed vertically halfway between the trunk and the edge of the canopy at the three depth ranges (0–30, 31–60, and 61–90 cm) on the eastern side of six irrigated trees and two non-irrigated trees. More irrigated trees were monitored because these trees were part of a separate trial monitoring tree water use at the same site [33]. These probes were connected to a data logger (CR1000, Campbell Scientific, Inc., Logan, UT, USA), and soil water content was recorded hourly. Tensiometers are commonly used by avocado growers in New Zealand (30 and 60 cm, SR Irrrometer, Irrrometer Company Inc., Riverside, CA, USA) and therefore were also installed next to a set of the electronic soil moisture probes to obtain a cross-reference between volumetric soil moisture and manual tensiometer measurements. A soil moisture release curve was prepared from periodic tensiometer readings and the output recorded from soil moisture probes at the same times.

2.6. Plant Water Potential

Predawn leaf water potential (Ψ_{pd}) was measured using a pressure chamber [13] (PMS Instrument Co., Ltd., Corvallis, OR, USA) on 17 and 29 January, and 4 March in 2017 (Year 0), and 12, 21 and 31 December 2017 (Year 1). At each measurement, a leaf was placed in a plastic bag before removing from the tree using a razor blade.

Leaf water potential (Ψ_{leaf}) and stem water potential (Ψ_{stem}) were measured at midday on 17 and 28 January, and 12 February 2017 (Year 0), and 30 December 2017 (Year 1), and 12 February and 6 April 2019 (Year 2), when the soil water content of non-irrigated trees decreased below $0.35 \text{ m}^3 \text{ m}^{-3}$. At each time, for the Ψ_{leaf} measurement, a fully sun-exposed leaf was selected, placed in a plastic bag before removing from the tree using a razor blade, and then measured. For the Ψ_{stem} measurements, leaves were covered with aluminium foil for two to three hours before measurements.

2.7. Fruit Size, Dry Matter and Yield

At harvest on 2 October 2017 (Year 0) and 15 September 2018 (Year 1) fruit yield was obtained for each experimental tree by weighing all fruit, then a 50 fruit sample was selected randomly and weighed as a measure of average fruit weight. Three to five fruit per tree were placed in a plastic bag and carried to the laboratory for analysis.

In the laboratory, a 10 mm corer was used to take a longitudinal core from the stem end to the stylar end. The mesocarp was separated from the skin and seed, weighed and dried at 60°C in an oven for three days. Dry matter content was calculated as the ratio of dry weight to fresh weight of the mesocarp sample.

2.8. Fruit Growth and Abscission

Non-destructive fruit growth measurements were conducted from December 2017 until harvest in September 2018 (Year 1). For each tree, 20 fruit were labelled, and one length and two diameters of labelled fruit were measured using digital callipers every two to three weeks.

Fruit weight was estimated using the equation [38]:

$$W_{\text{fruit}} = 0.4644L_1D_1D_2 + 8.7219 \quad (2)$$

where W_{fruit} is fruit weight (g), L_1 (cm) is fruit length, and D_1 (cm) and D_2 (cm) are fruit diameters.

Fruit abscission was monitored from December 2017 until harvest (Year 1). To monitor 50 fruit per tree, 5–7 fruiting branches were selected and labelled, and the remaining fruit on these branches counted every 2–3 weeks.

2.9. Stomatal Conductance

Stomatal conductance (g_s) was measured on six dates during the dry period of Year 2 with a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). Measurements were conducted five times a day from 11:00 to 16:00 h on six irrigated and six non-irrigated trees. At each time, five mature and fully sun-exposed leaves per tree were selected.

2.10. Statistical Analysis

All statistical analyses were carried out using R v.4.4.2 [39]. Water potential and stomatal conductance were compared by analysis of variance (ANOVA) for each date of measurement, and Tukey's HSD test was performed for post-hoc comparison. Non-destructive fruit growth measurements were compared by ANOVA for the first and last dates of measurement only (to minimize pseudo-replication caused by repeated measurements on the same fruit over time), with fruit nested within tree. Analysis of covariance (ANCOVA) was used to test for the effect of irrigation on fruit weight and dry matter content, with yield per tree (crop load) as a covariate and row as a blocking factor. A p -value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Weather

Over the three summers, January was consistently the warmest month with a monthly mean daily maximum air temperature of 29.9 °C in Year 0, 28.6 °C in Year 1, and 31 °C in Year 2 (Figure 2a–c). An unusually cool spring was observed in October of Year 2, with 15 days of minimum temperatures below 8 °C (Figures 1a and 2c). An extended dry period occurred in all three summers, although its timing and length was variable: January in Year 0, December in Year 1, and February in Year 2, with a total rainfall of 43.6, 28.2, and 61.1 mm for each of these months, respectively (Figure 2d–f).

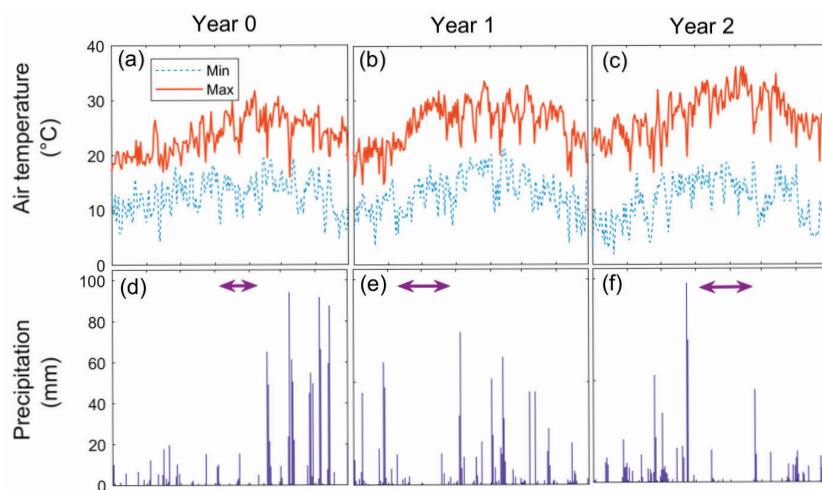


Figure 2. Cont.

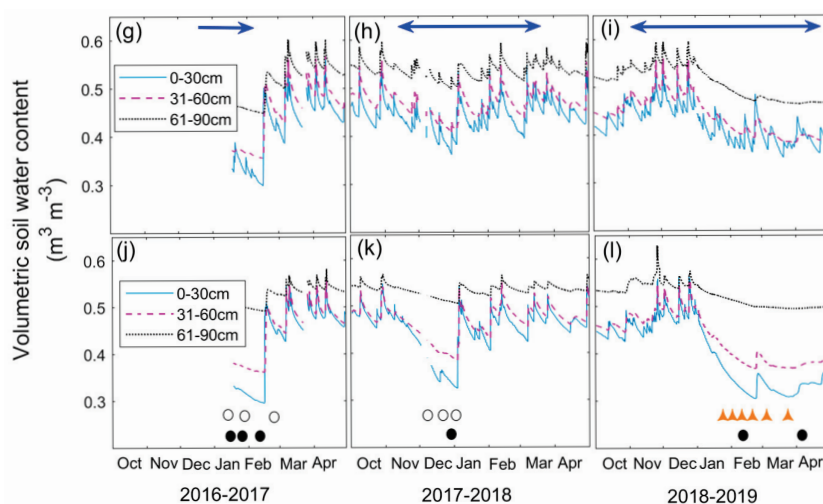


Figure 2. Daily maximum and minimum temperatures ($^{\circ}\text{C}$) (a–c) and daily total rainfall (d–f), soil water content for irrigated trees (g–i) and non-irrigated trees (j–l) at three depths (0–30, 31–60, and 61–90 cm) for the summer period from October to April in three years; Year 0 (2016–17), Year 1 (2017–18) and Year 2 (2018–19). The purple horizontal arrows indicate low rainfall periods, the blue arrows the irrigation seasons, white circles ($\circ$) indicate dates of Ψ_{pd} measurement (17 and 29 January, and 4 March in 2017, Year 0, and 12, 21 and 31 December 2017, Year 1), black circles ($\bullet$) date of Ψ_{stem} and Ψ_{leaf} measurements (17 and 28 January, and 12 February 2017, Year 0, and 30 December 2017, Year 1, and 12 February and 6 April 2019, Year 2, and orange triangles ($\blacktriangle$) dates of g_s measurements.

3.2. Soil Water Content

In Year 0, the irrigation treatments did not start until partway through the summer, therefore, soil water content was similar between the two treatments for the remainder of the summer period. In this year, soil water in the top layer (0–30 cm) decreased to $0.3 \text{ m}^3 \text{ m}^{-3}$ until a significant rain event occurred on 16 February (Figure 2g,j). In Year 1 and Year 2 the soil water content of the top layer remained above $0.35 \text{ m}^3 \text{ m}^{-3}$ for the irrigated trees (Figure 2h,i). In contrast, the soil water content for the non-irrigated trees declined below this level during the dry periods (Figure 2k,l). The driest measurement of the top layer was $0.32 \text{ m}^3 \text{ m}^{-3}$ (−80 kPa) on 3 January in Year 1, and $0.30 \text{ m}^3 \text{ m}^{-3}$ (−110 kPa) on 22 February in Year 2 (Figure 2k,l). Figure 3 provides the relationship between volumetric soil water content and soil water tension measured using soil tensiometers at two soil depths.

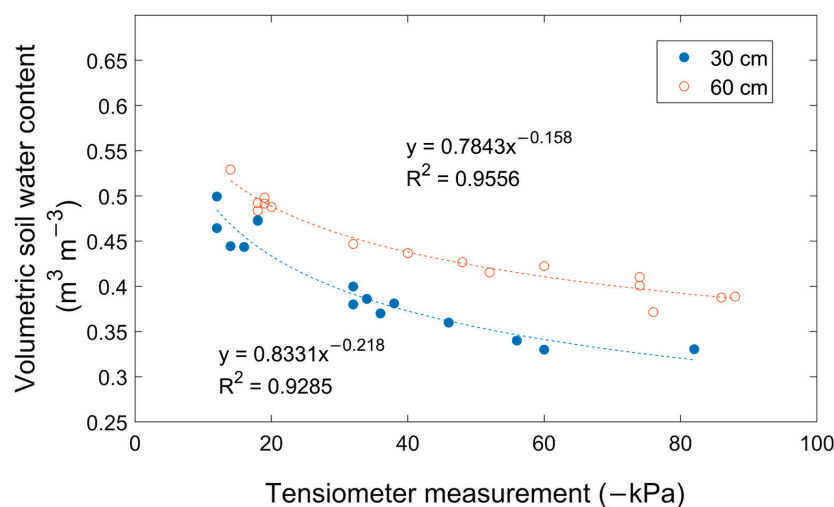


Figure 3. Soil moisture release curves for depths of 30 and 60 cm at the orchard in the Bay of Plenty. The values were obtained from tensiometer readings and output values of soil moisture probes recorded during the experiment at the same time.

3.3. Plant Water Potential

Ψ_{pd} was measured in Year 0 and Year 1 (Figure 4a,b). Both treatments were above -0.06 MPa, and no difference was detected between the two treatments in either year ($p > 0.05$). Ψ_{stem} and Ψ_{leaf} were also measured during the dry periods in Year 0 and Year 1 (Figure 4c,d). Overall, there was no difference in Ψ_{stem} and Ψ_{leaf} in Year 0. In Year 1, non-irrigated tree Ψ_{stem} was 0.1 MPa more negative than the irrigated treatment ($p < 0.05$) during the dry period, but there was no difference in Ψ_{leaf} ($p > 0.05$), suggesting a mild level of soil moisture stress for avocado.

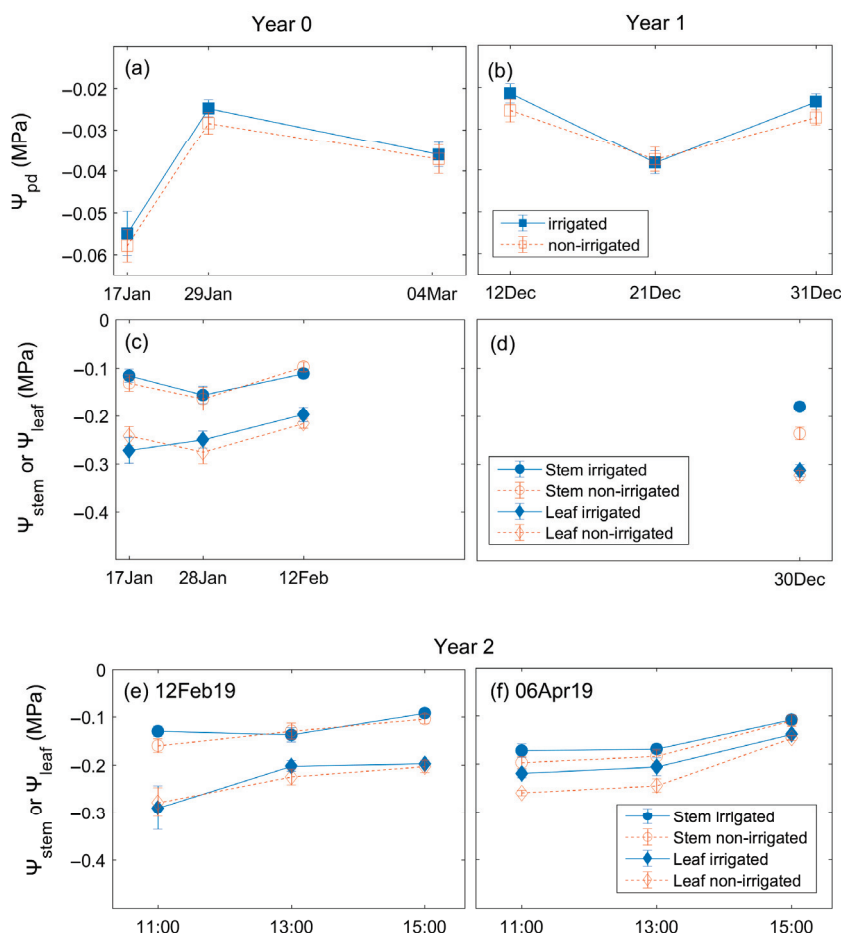


Figure 4. Predawn leaf water potential (Ψ_{pd}) in Year 0 (a) and Year 1 (b), stem water potential (Ψ_{stem}) and leaf water potential (Ψ_{leaf}) in Year 0 (c) and Year 1 (d), and a diurnal pattern of Ψ_{stem} and Ψ_{leaf} in Year 2 on 12 February (e) and 6 April 2019 (f) for irrigated and non-irrigated treatments. The measurements were taken during low rainfall periods when the continuous soil water content measurements (Figure 2) indicated significant differences between irrigation treatments. Values are means $\pm$ SE ($n = 12$).

Diurnal variation in Ψ_{stem} and Ψ_{leaf} was observed during the dry period in Year 2 (Figure 4e,f). Ψ_{stem} and Ψ_{leaf} was lowest in the morning and increased in the afternoon. On 12 February, the two treatments had similar values of Ψ_{stem} and Ψ_{leaf} ($p > 0.05$). However, by 6 April, at 1100 h, the non-irrigated treatment had lower Ψ_{stem} and Ψ_{leaf} values, relative to the irrigated treatment, and the difference in Ψ_{leaf} between treatments was significant ($p < 0.05$), suggesting that a mild level of water stress also developed during this dry period.

3.4. Stomatal Conductance

Stomatal conductance varied diurnally from late-January to late-March during the dry period in Year 2, usually reaching a maximum around the middle of the day, be-

fore declining (Figure 5). Stomatal conductance of the irrigated trees was usually above $0.3 \text{ mol m}^{-2} \text{ s}^{-1}$, whereas for non-irrigated trees g_s was slightly lower, around $0.25 \text{ mol m}^{-2} \text{ s}^{-1}$, and the difference between the two treatments became more significant when the vapour pressure deficit (VPD) increased above 2.0 kPa ($p < 0.05$). Variation in the diurnal patterns of g_s and VPD were caused by variable weather and cloud cover at the site, however mean g_s was consistently lower for the non-irrigated trees during this period when soil water content was different between treatments (Figure 2).

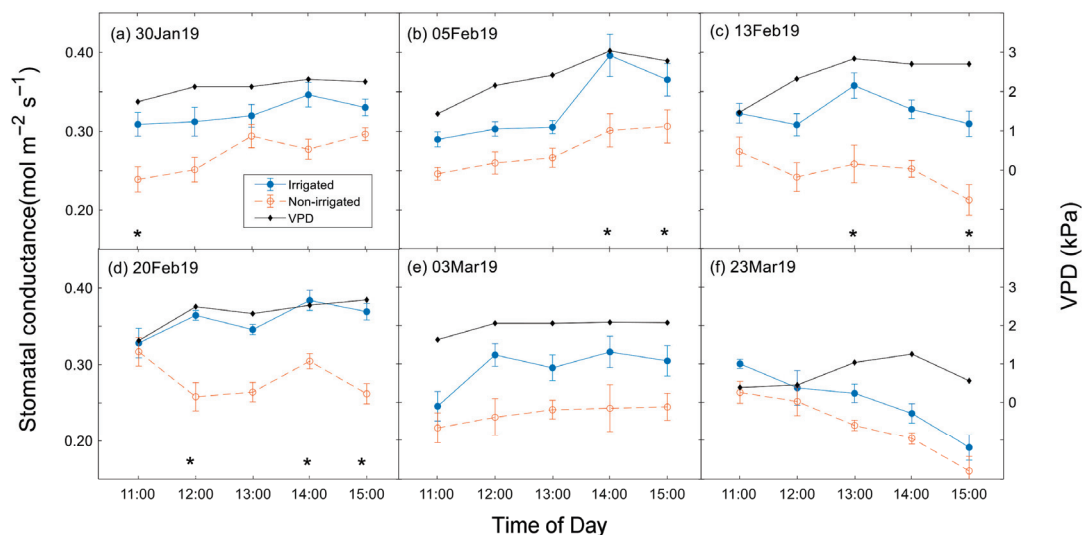


Figure 5. Diurnal stomatal conductance (g_s) (± 1 SE) of the irrigated and non-irrigated ‘Hass’ avocado trees, and VPD obtained from meteorological measurement at the study site. The measurements were made during the dry period between late-January and late-March, 2019 (Year 2) on (a) 30 January, (b) 5 February, (c) 13 February, (d) 20 February, (e) 3 March and (f) 23 March (▲ in Figure 2). (* below a time point indicates significant differences between g_s of irrigated and non-irrigated trees for that time point, $p < 0.05$) ($n = 6$).

3.5. Fruit Growth and Fruit Abscission

Fruit grew rapidly until early-March in Year 1, then more slowly until harvest in September (Figure 6a). Overall, from February to September, fruit increased in size from $66 \pm 1.82 \text{ g}$ and $61 \pm 1.50 \text{ g}$ ($p < 0.05$) in the irrigated and non-irrigated treatments, respectively, to $246 \pm 8.63 \text{ g}$ and $234 \pm 3.90 \text{ g}$ ($p > 0.05$) on the final date of measurement. The fruit abscission rate was highest from January to early-March, and both the irrigated and non-irrigated trees lost more than one-third of their fruit in this period (Figure 6b). The irrigated trees had higher early fruit abscission, compared to the non-irrigated trees. However, there was a high level of variation in the proportion of fruit abscised between trees, and there were no significant differences in fruit abscission between the two treatments.

3.6. Fruit Size, Dry Matter and Yield

Fruit yield varied widely between trees and years. In both Year 0 and Year 1 the lowest yields were below 30 kg per tree, whereas the highest yields were more than 200 kg per tree. Year 0 was an off-cropping year, with only one tree producing more than 200 kg , whereas Year 1 was an on-cropping year, when more than half of all trees produced more than 200 kg . Overall, there was no significant effect of irrigation on total yield per tree, but yield was significantly higher in Year 1 (Table 3). In Year 2 very few fruit were set and no harvest was possible.

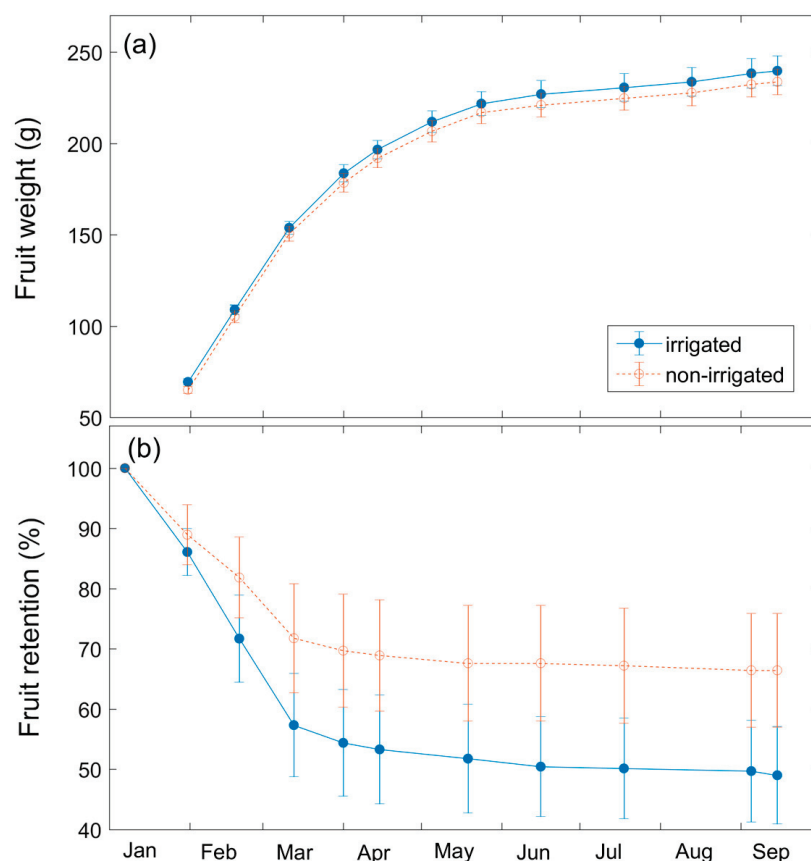


Figure 6. Non-destructive estimates of fruit weight (a), and fruit retention (%) (b) of the irrigated and non-irrigated trees, from January to harvest in September in Year 1. Values are means $\pm$ SE ($n = 12$).

Table 3. Average fruit yield (kg) of 12 irrigated and 12 non-irrigated avocado trees with S.E. over the three years of the study. Irrigation treatments were established in January 2017, during the summer that preceded the Year 0 harvest in October 2017. Yield per tree is an estimate of individual tree crop load, and was a significant covariate in the analysis of irrigation treatment effects on final fruit size (Figure 7). Variable fruit set resulted in no harvestable yield in 2019 (Year 2). Irrigation had no significant effect on yield (no irrigation effect, ANOVA, $p = 0.14$), but yield was significantly higher in 2018 (significant year effect, $p < 0.001$; no significant interaction between year and irrigation).

Calendar Year of Harvest	Irrigated	Non-Irrigated
2017 (Year 0)	91 $\pm$ 14.5	130 $\pm$ 10.5
2018 (Year 1)	204 $\pm$ 22.5	220 $\pm$ 22.0
2019 (Year 2)	-	-

Individual fruit weight decreased with increasing fruit yield per tree (-17.0 g per fruit with every 100 kg increase in yield per tree, ANCOVA, $p < 0.001$; Figure 7a,b). The effect of yield on fruit weight did not change with year or irrigation (no year or irrigation interactions with yield, $p > 0.05$; Figure 7a,b). Lack of irrigation in Year 1 decreased individual fruit weight by 26.4 g per fruit, regardless of yield per tree (significant interaction between irrigation and year, $p < 0.05$; Figure 7b). Fruit dry matter content also decreased with increasing yield per tree in both years (-1.2% for every 100 kg increase in yield, ANCOVA, $p < 0.001$; Figure 7c,d), but there were no separate effects of irrigation or year on fruit dry matter content (no irrigation, year or interaction effects $p > 0.05$; Figure 7c,d).

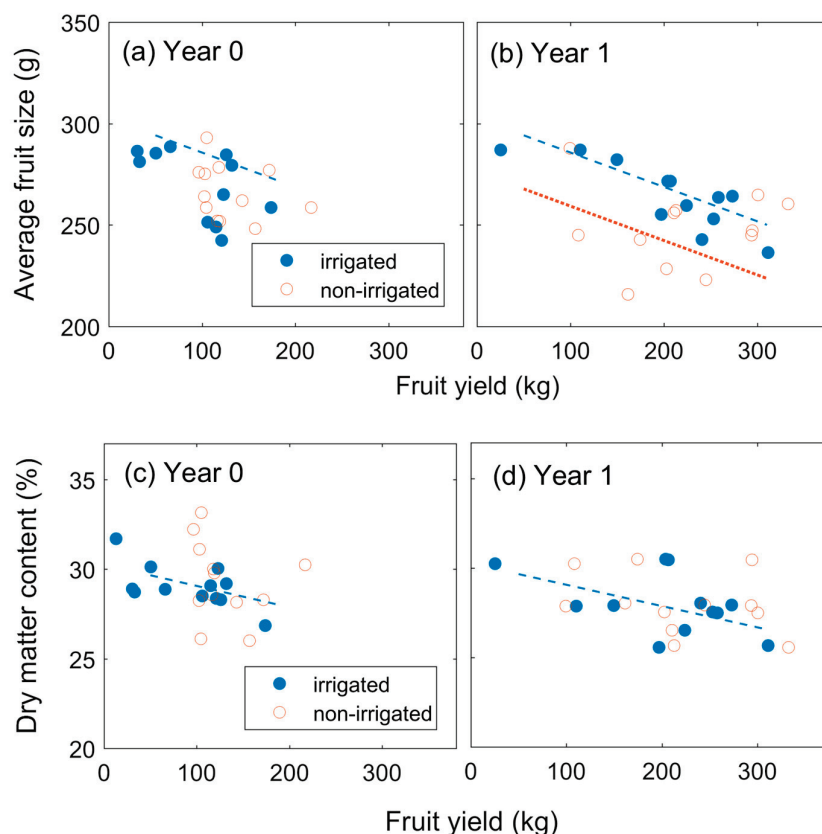


Figure 7. The relationship between fruit yield and average individual fruit size for irrigated and non-irrigated trees in 2017 (a) and 2018 (b), and the relationship between average fruit yield and dry matter content for irrigated and non-irrigated trees in 2017 (c) and 2018 (d). Fruit size and dry matter content decreased with increasing yield per tree in both years, with no significant difference in the slope of these relationships between years (ANCOVA, blue dashed lines; yield effect $p < 0.001$; year effect $p > 0.05$). However, there was a 26.4 g decrease in average fruit size in non-irrigated trees in year 1 (decreased intercept of orange dashed line; significant year and irrigation interaction, $p < 0.05$).

4. Discussion

This study demonstrates the sensitivity of avocado fruit growth to water status, even under the comparatively mild growing conditions of New Zealand, where irrigation of avocado is often considered ‘optional’. Highly variable crop loads between years and trees can mask the effects of short periods of water deficits on fruit size and total yields. However, after accounting for the effects of crop load, fruit weight was reduced in the absence of irrigation, even when the level of water stress was mild compared to the levels typically seen in avocado in other countries. Isohydric stomatal closure minimized changes in plant water status as the soil dried, contributing to the difficulty of detecting water stress, and may have contributed to impacts on fruit growth by reducing carbohydrate supply during early fruit development. The sensitivity of fruit growth to even mild water deficits, combined with an increasing probability of summer water deficits linked to climate change within current avocado growing regions of New Zealand presents a clear risk for orchard productivity and continued expansion of the industry.

4.1. Weather and Soil Water Content

Soil water deficit during the flowering period (from late-September to late-October) is rare in the Bay of Plenty region of New Zealand, indicating that there is a low probability that water stress affects the flowering process [38]. Soil water content was high at all depths in early-spring in the three years of this study and no difference was detected

between the two treatments. However, rainfall was irregular in summer (from December to March). Although the timing of the dry period was variable, each summer there was a month-long period with lower precipitation that caused the upper soil water content of the non-irrigated trees to decrease below $0.35 \text{ m}^3 \text{ m}^{-3}$ (-40 kPa). Water application to the irrigated treatment was slightly under the recommended amount because of constraints on irrigation management at the orchard, however, the dry periods still created clear differences between the two treatments.

Avocado is known to have a shallow root system, with two-thirds of the root system is located in the top 60 cm of soil [10,40,41], although the actual proportion is likely to depend on the soil type. In this study on sandy loam soil the top (0–30 cm) and middle layers (31–60 cm) dried quickly during the dry periods, and soil water content in the top 30 cm appeared to be most important. In contrast, soil water content in the deeper layer (61–90 cm) was high (above $0.45 \text{ m}^3 \text{ m}^{-3}$) throughout the year. These observations suggest that a shallow root system makes avocado highly sensitive to water scarcity.

4.2. Plant Water Status

Water potential is a good water stress indicator for a plant, and the level of water stress observed in this study was less than that recorded elsewhere. For avocado in Chile, Celedon, et al. [42] stated that Ψ_{stem} values were between -0.45 and -0.6 MPa under well-watered conditions, and dropped to -0.9 MPa during water stress with a Mediterranean marine climate, when the VPD was 3.6 kPa on sunny days. Compared to these values, the Ψ_{stem} values obtained here were higher and usually above -0.3 MPa in both treatments. Environmental conditions such as temperature and evaporative demand affect plant water potential measurements [43]. In New Zealand, the VPD rarely exceeds 3.0 kPa , possibly explaining why the Ψ_{stem} values were higher in this experiment. Overall, the water potential measurements suggest only a mild level of water stress developed in the non-irrigated treatments, with water potential differences potentially minimized by stomatal closure in response to soil drying.

Stomatal regulation is complex and involves many factors, such as plant physiological conditions, water status, nutrient availability, and weather conditions [44–46]. The description of stomatal behaviour of avocado is somewhat controversial, with some studies [28,47–49] having suggested that water stress induces stomatal closure, while others [42,50] have reported that water deficit did not influence g_s but caused a decrease in Ψ_{stem} . Measurements of stomatal conductance were added to monitoring of the trees in the final year of the present study because of the lack of strong differences in water potential observed during dry periods in the first two years. The non-irrigated trees had approximately 20% lower g_s compared to the irrigated treatment when soil water content was below $0.35 \text{ m}^3 \text{ m}^{-3}$. The effect of lack of irrigation on stomatal conductance was more pronounced than the difference in water potential. This suggests isohydric stomatal behaviour, with stomatal closure in response to water stress reducing differences in water potential, but potentially also causing a decrease in carbohydrate production by restricting photosynthetic assimilation.

Carbohydrate supply is important for fruit growth. In many crop species, such as plum [51], apple [52], citrus [53] and avocado [54], a higher photosynthetic rate has been observed during fruit development. This is because fruit are a strong sink for carbohydrates, and high demand for carbohydrate for fruit growth lowers carbohydrate concentration in leaves, leading to increased photosynthetic rates [54]. However, in isohydric species such as avocado, photosynthetic activity decreases when soil water availability decreases, limiting carbohydrate accumulation in fruit. This limitation on fruit growth should be more significant for trees with a heavy crop.

4.3. Fruit Growth, Fruit Abscission and Irregular Bearing

In year 1 the dry period occurred early, before the first period of very high rates of fruit abscission had subsided and non-destructive fruit growth measurements could begin [38]. Mean fruit size was already lower in the non-irrigated treatment at the start of the monitoring period, a possible effect of the early water deficits on initial fruit growth. From the first measurement onwards fruit growth rate did not differ, and fruit size remained lower in the non-irrigated treatment for the rest of the season, although this difference gradually became less statistically significant over time. However, a larger destructive sample of fruit at harvest confirmed that the non-irrigated treatment had smaller fruit, after the effect of variable crop load between trees was accounted for (see below). Tree-to-tree variation in crop load, and the early timing of the dry period, made it difficult to detect treatment effects on fruit growth rate using non-destructive fruit monitoring alone.

Fruit abscission caused by soil water deficit was not detected in this experiment. Some studies suggested that immature fruit abscission is increased by heat stress [55,56] or carbohydrate stress [57,58], rather than water stress [31]. Severe water stress might lead to carbohydrate stress if a photosynthetic activity is reduced for an extended period, resulting in excessive fruit abscission [56]. However, rainfall patterns meant that the level of water stress imposed on the non-irrigated trees was moderate in this experiment, the stress period occurred before monitoring of the later stages of fruit abscission began, and the final yields for year 1 did not indicate that there were significant differences in mean crop load between treatments.

Crop load in avocado is strongly influenced by highly variable flower numbers and flower and fruitlet abscission during the flowering period in spring, when soil water deficits are rare in the Bay of Plenty growing region of New Zealand. Regardless of the climate, alternate bearing by avocado is typically observed as alternating on-cropping and off-cropping years [30]. In the present experiment fruit production was low in Year 0 and Year 2, and high in Year 1 across the entire orchard. However, it appears that the exceptionally low fruit yield in Year 2 was caused by weather conditions during flowering for that year, rather than the more regular alternate bearing effects on flower production. This orchard experienced an unusually cold spring in Year 2. The October monthly average daily minimum air temperature in Year 2 was 3 °C lower than that of Year 1. The reproductive process of avocado is known to be highly sensitive to temperature [59], and unusually low temperature can negatively affect the synchrony of male and female flower development [60,61], ovule viability [62], and pollen tube growth [63]. This is an example of how New Zealand's current climate creates a more irregular pattern of avocado production compared to other countries [32].

4.4. Effect of Water Stress on Harvested Fruit Yield and Fruit Quality

Water stress in the absence of irrigation decreased avocado fruit weight at harvest by approximately 10% or 26.4 g in the second year of the study, when a period of low rainfall occurred during early fruit development. This effect was detected against a background of very high levels of variation in individual fruit weight between trees in response to crop load, with mean fruit weight decreasing as total fruit weight per tree increased. As discussed above, irregular variation in crop load is a common feature of avocado production in New Zealand, driven primarily by variation in fruit set in spring. Regardless of the cause of variable crop load, a negative correlation between crop load and fruit size is commonly observed in avocado and many other fruit crops [64]. In our study the effect of variation in crop load on fruit size was of a similar or larger magnitude compared to that of the effect of irrigation, potentially obscuring the negative effects of mild water stress that may occur at any time during summer under New Zealand growing conditions if irrigation is not

used. Irrigation did not have a detectable effect on total yield per tree, because yield was determined primarily by fruit number rather than fruit size, and varied from 13 to 330 kg per tree across the two years of harvestable yields. Both total yields and mean fruit size are important determinants of the commercial viability of avocado production.

Water stress reduced fruit size, but did not influence fruit dry matter content, an indicator of fruit oil content, maturity and eating quality. Holzapfel, et al. [65] and Moreno-Ortega, et al. [66] also reported that water stress reduced avocado fruit size without affecting fruit dry matter content, but Zuazo, et al. [11] did observe reductions in fruit size and increases in fruit lipid content with deficit irrigation under semi-arid conditions. Fruit size in avocado may be determined by variation in the rate of cell division in the mesocarp [67]. Cell division continues throughout avocado fruit growth, but is most active during the first six weeks after fruit set [68]. This is the period from November to December under New Zealand conditions, and water stress during this period may directly affect fruit growth by reducing the rate of early cell division. The lack of reduction in dry matter content may be because avocado fruit have a long growing period, and oil accumulates throughout fruit growth, in particular during later fruit developmental stages in winter [69]. Therefore, water stress that occurs only in early- or mid-summer is less likely to affect oil accumulation.

4.5. Irrigation and Climate Change

Avocado is frequently cited as an exemplar of a crop that will increase in land-use suitability and potential spatial extent in New Zealand with climate warming [70,71]. These recommendations are based on reductions in the negative impacts of frost and cool spring temperatures on flowering and fruit set, but do not consider irrigation requirements. Current water use for avocado production in New Zealand is comparatively low compared to production in other countries, where avocado is often grown in water-scarce regions, and is therefore considered a water intensive crop [5]. However, over the 10 years prior to the current study the average daily temperatures in the Bay of Plenty region increased by 0.5 °C [34], and the three summers included in this study were the warmest of the preceding 20 years [34]. Drought is predicted to occur more frequently and more intensely in the northern and eastern regions of New Zealand where avocado is currently grown, likely affecting both the demand for and availability of water resources for agricultural use [3,7]. This study demonstrates that even relatively brief and mild summer water deficits can impact avocado productivity under New Zealand growing conditions, suggesting that access to irrigation is a potential limiting factor for continued expansion of this crop with climate warming.

Sustainable and effective irrigation of a horticultural crop requires accurate identification of the threshold level of water stress required for negative impacts to occur. In this experiment, the amount of irrigation water applied was calculated based on ET_o and K_c . Irrigation was usually applied when soil water content dropped below 0.40 m³ m⁻³ (−30 kPa) at a depth of 30 cm, half-way between the trunk and the edge of the tree canopy. This threshold corresponded to the point on the soil moisture release curve where soil water potential measurements became increasingly negative, resulting in subtle but significant effects on harvest fruits size, and marked the onset of reductions in g_s as soil water content decreased below 0.35 m³ m⁻³ (−50 kPa).

Deficit irrigation, the application of water at rates below crop demand, can improve the efficiency of irrigation water use for fruit crops such as olive [72] and grape [73]. A recent study of sustained deficit irrigation of avocado under semi-arid conditions demonstrated that a 25% reduction in irrigation below crop demand reduced overall yield by only 8%, and improved fruit quality through increases in fruit lipid concentrations [11]. A similar

strategy could be beneficial even under the more humid and temperate conditions of New Zealand. Crop load also has an important role in irrigation management of avocado, as trees with a light crop have a significantly lower water demand [33,74]. Larger reductions in irrigation may be acceptable for light or non-cropping trees without affecting longer-term fruit yields, provided that the physiological response to water stress throughout the cropping cycle is well understood.

5. Conclusions

This study assessed the impact of water stress on avocado fruit size and yield by comparing between the irrigated and non-irrigated treatments in the Bay of Plenty, New Zealand. The results demonstrated that a lack of irrigation reduced avocado fruit size when there was a period of low precipitation during early fruit development, even though drought conditions were not prolonged and there were no changes in leaf water potential. However, the benefits of irrigation can be hidden by high levels of variation in fruit size caused by tree-to-tree and inter-annual variation in crop load. The results highlight the importance of irrigation for avocado under current and future New Zealand growing conditions, and provide an indication of the threshold level of soil and plant water stress that may cause reductions in yield. Additional research is required to compare the irrigation requirement of avocado between on- and off-cropping years.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12020230/s1>, Figure S1: The experimental plot design with 12 irrigated and 12 non-irrigated avocado trees within three orchard rows (blocks). Non-irrigated trees were paired within rows to allow for separation from the adjacent irrigated trees by non-irrigated ‘buffer’ trees.

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Article

Projected Shifts in the Growing Season for Plum Orchards in Romania Under Future Climate Change

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Abstract

Climate change strongly influences the phenology of temperate fruit species, yet its long-term effects on Romanian plum orchards (*Prunus domestica* L.) remain insufficiently quantified. This study analyzes projected changes in the start (SGS), end (EGS), and duration (GSL) of the growing season under two emission scenarios (RCP 4.5 and RCP 8.5) throughout the 21st century. Using temperature-based phenological thresholds, SGS and EGS were modeled for six orchard clusters representing distinct regional and altitudinal conditions across Romania. Results reveal a consistent advancement of SGS and a marked extension of GSL, particularly under RCP 8.5, where the growing season may lengthen by up to 60 days compared with early-century conditions. Under RCP 4.5, changes are more moderate but directionally similar, indicating a robust climatic signal across all clusters. These findings highlight that earlier and longer vegetation periods may enhance fruit development potential but also increase risks associated with late spring frosts, heat stress, and pollination mismatches. Despite inherent model uncertainties, the convergence of trends suggests reliable projections that can support adaptive orchard management and long-term strategies for sustainable fruit production under a changing climate.

Keywords: phenological simulation; plum orchards; Romania; RCP scenario; growing season shift

1. Introduction

Fruit cultivation represents a key component of agricultural production, where yield and quality depend on continuous interaction between environmental factors and farm management practices [1,2]. Beyond their nutritional value, fruits increasingly contribute to public health and wellbeing [3–5], and pomology supports both agricultural production and related economic sectors [6,7]. As global population increases, fruit demand is also expected to rise; however, long-term supply may be constrained by environmental limitations [8].

Climate change is among the most widely investigated environmental issues due to its multiple implications for agriculture and food security [9]. Numerous studies reports that rising temperatures reduce winter chill accumulation and alter the phenological development of fruit trees [10,11]. Earlier onset of the growing season increases frost exposure risk during sensitive flowering stages, as illustrated by the severe event of 2017, which affected apple orchards across Central and Eastern Europe [12]. Fraga et al. [13] demonstrated, under RCP 4.5 and RCP 8.5 scenarios, accelerated heat accumulation and significant phenological shifts in olive and vineyard systems, particularly in Mediterranean regions. Subsequent research extended these findings to apple, plum, orange and pear [14],

highlighting that warm winters may delay flowering and reduce yields. Similar trends were detected in Spain, where apple, almond and plum orchards are projected to experience stronger climatic stress than cherry [15], and in France, where milder winters and earlier flowering in peach sometimes led to bloom failure [16].

Romania is the leading plum producer in Europe, with traditional cultivars such as Tuleu Gras, Grase Românești and Vinete Românești, alongside commercial varieties like Reine Victoria grown for export. Annual production typically ranges between 400,000–500,000 tons, but exceptional yields of 830,000 and 693,000 tons were recorded in 2018 and 2019, respectively [17].

Several Romanian studies have explored climate–fruit interactions. Jitariu et al. [18] evaluated apple orchards in the northeast, while [19,20] examined grapevine suitability. Gitea et al. [21] assessed climate impacts on apple, plum and almond orchards in the west, and Cosmulescu [22] analyzed apricot yields in drought-prone southern regions. FAO's Climate Change Strategy [23] acknowledges similar concerns, emphasizing the need to enhance resilience and reduce vulnerability in agri-food systems in alignment with the Paris Agreement and the 2030 Agenda.

Understanding the relationship between climatic variability and fruit species is essential for identifying zones with different suitability levels and those at higher risk of extreme events [24]. Orchard establishment involves long-term investment and low short-term reversibility compared to annual crops, making climate-sensitive planning crucial. Although effects of climate change on major fruit crops are increasingly documented across Western and Central Europe, information remains limited for plum phenology in Romania, particularly regarding projected shifts in the timing and duration of the growing season under future emission pathways.

Despite Romania being one of the leading plum producers in Europe, the response of plum phenology to future climate trajectories remains insufficiently investigated. Existing studies mainly address yield trends or climatic stress factors, but few quantify how the timing and duration of the growing season may shift across different regions under future warming. Therefore, this study intends to fill this research gap by analyzing projected changes in the thermal growing season across Romania's main plum-producing zones, comparing spatial patterns under RCP4.5 and RCP8.5. By identifying areas with potential season extension, increased phenological vulnerability, or heightened frost risk, our results aim to provide a scientific basis for adaptation strategies, orchard planning, and long-term management. This work thus contributes new regional-scale evidence to a topic that is currently underrepresented in literature, offering a foundation for future phenological research, cultivar-specific assessment, and practical decision-making in Romanian fruit production.

2. Materials and Methods

2.1. Study Area

Romania is a medium-sized country (approx. 239,000 km²), which is located in the southeastern region of the European continent (Figure 1). The Romanian relief is defined by the central presence of the Carpathian arc, which encircles the Transylvanian Depression, and by its progressive transition toward lower landforms on the periphery, including the Subcarpathians, Western Hills, Moldavian Plateau, Romanian Plain, and Western Plain [25].

Romania is characterized by a temperate-continental climate, resulting from its position at the intersection of the main European atmospheric circulation pathways and from the pronounced contrasts between major landform units. According to the Köppen–Geiger classification, three main climate types can be identified across the country: Cfa (temperate, without dry season, hot summer), Cfb (temperate, without dry season, warm summer), and Dfb (cold-continental, without dry season, warm summer) [26,27]. The country lies at the

confluence of Atlantic, Continental, and Scandinavian high-pressure systems, and under the influence of Mediterranean and Atlantic cyclone tracks, which explains the alternation of air mass advections and the frequent variability of weather types [28]. The Carpathian Mountains play a key role in shaping the climatic individuality of Romania, generating strong contrasts between the intra- and extra-Carpathian regions through their effects on air circulation, temperature, and precipitation patterns [28].

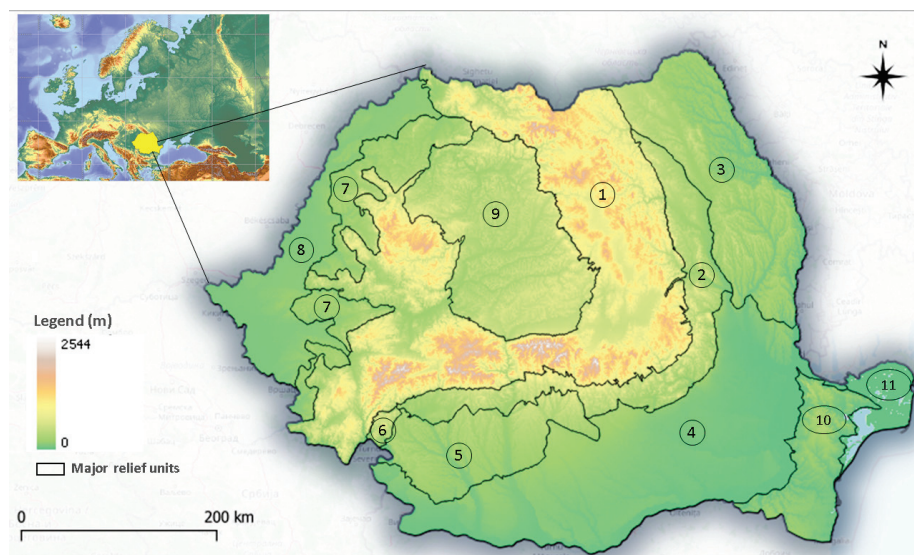


Figure 1. Major relief units of the study area (1—Carpathians; 2—Subcarpathians; 3—Moldavian Plateau; 4—Romanian Plain; 5—Getic Plateau; 6—Mehedinti Plateau; 7—Western Hills; 8—Western Plain; 9—Transilvanian Depression; 10—Dobrogea Plateau; 11—Danube Delta).

The Romanian climate is temperate continental, with mean annual temperatures ranging from values below 0 °C at elevations above 2000 m to more than 11 °C in the southern and south-eastern regions. A significant warming trend has been observed over recent decades, with the national average increasing from 8.81 °C (1961–1990) to 9.52 °C (1991–2013). July is typically the hottest month, with mean values around 21 °C and extremes between 2.5 °C and 26 °C, while January is the coldest, averaging −2 °C, with a range from −12 °C to 3 °C. Mean annual precipitation for the 1990–2019 period is approximately 700 mm, though it varies markedly across the country, from less than 400 mm in the Danube Delta to more than 1200 mm in high mountain areas. June is generally the wettest month (28–216 mm), whereas March tends to be the driest, with a mean of 41 mm and values ranging between 20 mm and 128 mm [25,29].

In addition, the westerly atmospheric circulation exerts a dominant influence on the country's climate, particularly during the cold season, controlling the regional thermal response to external forcings such as solar variability [30]. The climatic regionalization of Romania reflects the complex interplay among relief, general atmospheric circulation, and solar-radiative forcing, positioning the country within a transitional climatic zone between the oceanic climate of Western Europe and the continental climate of Eastern Europe.

Given its relatively modest climatic requirements, plum cultivation is well suited to a wide range of Romanian regions, from lowland plains to hilly areas. According to the guide for fruit growing issued by the Ministry of Agriculture and Rural Development, the species tolerates a broad thermal spectrum, with optimal hourly growth conditions between 18 and 33 °C, and growth ceasing only outside absolute limits of approximately 8–36 °C. It also shows good resistance to winter frost, enduring temperatures down to −35 °C, while the chilling requirement during dormancy (0–7 °C) typically ranges between 800 and 900 h depending on the cultivar [31]. These characteristics explain why plum orchards occupy

a significant share of the national fruit-growing area (over 67,000 ha), being distributed across diverse landscapes where both traditional varieties and commercial cultivars are well adapted to Romania's temperate continental climate.

2.2. Data

2.2.1. Spatial Distribution of Plum Orchards

In order to carry out this study, regarding potential shifts in the plum growing season in the context of future climate scenarios, the spatialization of current plum orchard locations was taken into account, and these data were obtained from the Agency for Payments and Interventions in Agriculture (www.apia.ro). Although plum orchards are widespread across Romania, their distribution is highly fragmented, which complicates the identification of regional patterns and the assessment of their relationship with environmental factors. To address this limitation, we applied a spatial clustering procedure using the K-means algorithm [32,33]. This method aggregated the individual orchard polygons into 6 clusters (C1, C2, C3, C4, C5, C6) based on their geographical position, reducing spatial dispersion and creating more coherent analytical units. The resulting clusters correspond to major fruit-growing regions such as the Subcarpathians (C1, C4, C6), the Moldavian Plateau (3) [17,34]. K-means clustering was performed on all orchard locations using latitude, longitude, elevation as input variables, with the final number of six clusters selected based on geographic interpretability. These units provide a robust spatial framework for subsequent analyses, facilitating the evaluation of climate change impacts, phenological shifts, and potential adaptation strategies at the regional level (Figure 2).

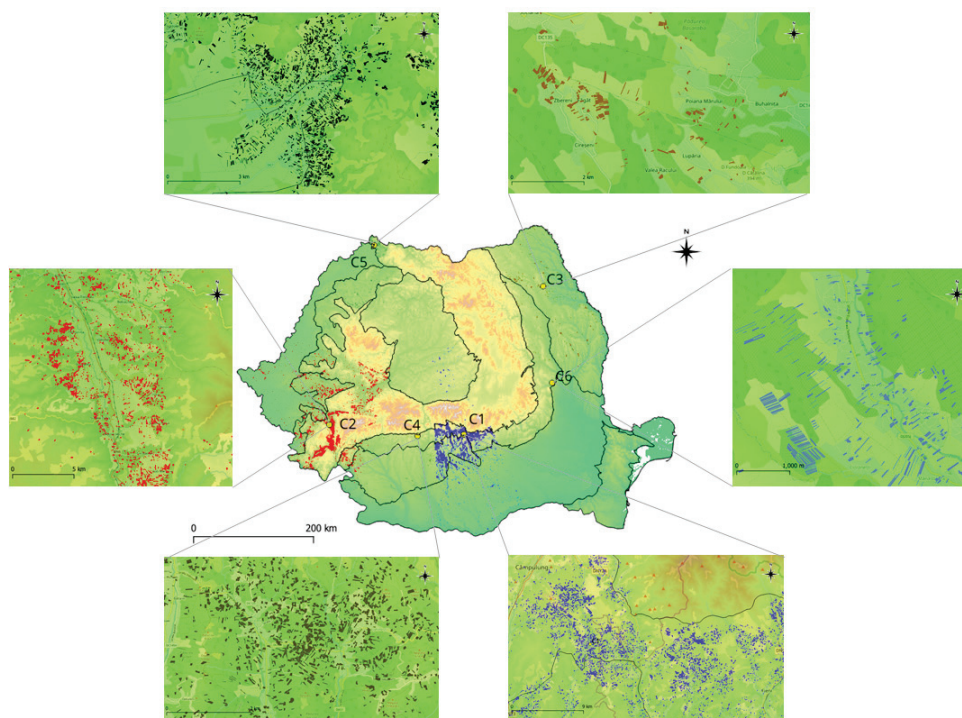


Figure 2. Orchard clusters.

2.2.2. Climatic Data

Climatic data were obtained from the CHELSA project, which provides high-resolution (30 arc-second) downscaled estimates of temperature and precipitation [35]. For future climate projections, we used the CHELSA CMIP5 timeseries dataset, which includes four global circulation models (GCMs) covering the period 2006–2100 at a spatial resolution of 0.049° (~5 km at the equator). The analysis was based on monthly averages of maximum,

minimum, and mean air temperatures derived from the MIROC5 (Model for Interdisciplinary Research on Climate), developed at the University of Tokyo. This model was selected because it provides more realistic estimates of air temperature under RCP2.6, RCP4.5, and RCP8.5 scenarios [36]. Kawamiya et al. [37] have highlighted the robustness and broad applicability of the MIROC5 model in a comprehensive review. Beyond its theoretical framework, the model has been extensively applied in diverse fields, including soil erosion studies under climate change conditions [38,39], yield estimation for cotton production in Pakistan [40], assessments of crop suitability [41–43], as well as analyses of extreme climatic events affecting agricultural systems in China [44–46]. Downscaled MIROC5 climate data were extracted at point level for all meteorological stations in Romania (157 stations), after which the start (SGS), end (EGS), and length (GSL) of the growing season were calculated individually for each station, and the resulting values were subsequently interpolated spatially. For this study were used RCP 4.5 and RCP 8.5 in order to capture a moderate stabilization trajectory and a high-emission future, respectively. RCP 4.5 reflects a scenario in which radiative forcing stabilizes at 4.5 W/m^2 through the implementation of mitigation measures, whereas RCP 8.5 represents a “business-as-usual” pathway characterized by continuously increasing emissions and radiative forcing reaching 8.5 W/m^2 by the end of the century [47].

2.2.3. Growing Season Calculation

The previously described climatic data were used to determine the start and end dates of the growing season, defined as the period during which the mean daily air temperature exceeds the biological threshold of 6°C . This threshold-based approach has been widely applied in previous studies [48,49]. To identify the start and end of the growing season, we employed the mathematical equations proposed by Gumiński [50]. The method assumes that the mean monthly temperature corresponds to the 15th day of each month, that each month contains 30 days, and that temperature changes linearly between consecutive months. Based on these assumptions, the start of the growing season (SGS) and end of the growing season (EGS) were calculated using the following equations:

$$\text{SGS} = 30 (tt - t_1) / (t_2 - t_1) \quad (1)$$

$$\text{EGS} = 30 (t_1 - tt) / (t_1 - t_2) \quad (2)$$

where tt represents the temperature threshold (6°C base temperature used for plum—[51]), t_1 is the mean temperature of the month preceding the threshold crossing, t_2 is the mean temperature of the following month, and SGS/EGS denotes the number of days between the threshold day and the 15th day of the previous month.

The number of days calculated using the above equations was added to the 15th day of the month preceding the threshold temperature. When the resulting value exceeded 15, the actual number of days in that month was considered to ensure temporal accuracy. The obtained date thus represents either the start or the end of the growing season and it is expressed in the number of the day of the year (DOY). This method is commonly applied in determining the length of the growing season as well as other thermal seasons of the year [52,53]. Pluta et al. [54] demonstrated a strong agreement between the average start dates of the growing season estimated using Gumiński’s [50] method and those derived from satellite-based remote sensing data for the 2001–2010 period. The growing season length (GSL) was calculated as the difference between the day of the year marking its end and that marking its beginning. The purpose of using the Gumiński approach was not to obtain highly precise phenological dates, but to provide a standardized and comparable climatic indicator that can be applied across large spatial domains and long time periods.

The temporal analysis was conducted for successive time intervals of 15 and 11 years, respectively: 2030–2044, 2045–2059, 2060–2074, 2075–2089, and 2090–2100. The reason why these intervals were chosen is related to the operating duration of a super-intensive plum orchard (approximately 15 years), which could make this study a good benchmark to take into account when talking about establishing such a type of orchard. To map the obtained results (SGS, EGS and GSL), the co-kriging method was used, taking into account the altitude as an auxiliary variable [55–57]. Based on the methodology described previously, a synthetic work scheme was created, intended to illustrate the sequence of analysis stages and the relationships between the components used (Figure 3).

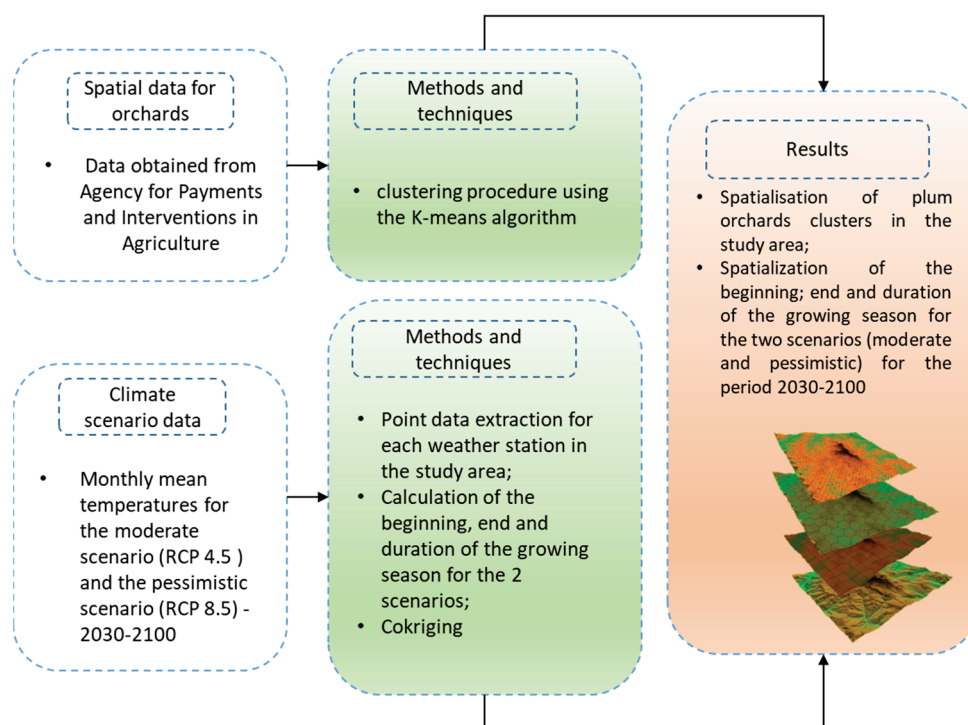


Figure 3. Workflow diagram summarizing the methodological steps.

3. Results

3.1. Spatial Distribution and Temporal Evolution of the Start of the Growing Season (SGS) in Romania Under the RCP4.5 Scenario for the 2030–2100 Time Horizon

In response to anticipated temperature increases under the RCP 4.5 scenario, the start of the growing season (SGS) has gradually advanced over the century, especially after 2060. In the early future period (2030–2059), the start of vegetation occurs mainly between late February and mid-March, with limited regional differences. From 2060 onward, however, the onset becomes markedly earlier by 10–20 days compared to the 2030–2044 baseline, especially across the southern and eastern lowlands, where the mean DOY decreases below 60 (early March). In contrast, the Subcarpathian clusters maintain later starts (mid- to late March), reflecting the persistence of lower temperatures at higher altitudes. By the end of the century (2090–2100), most orchards exhibit an average GS onset around $\text{DOY } 65 \pm 5$, while upland areas remain above $\text{DOY } 70$, indicating an overall north–south and altitudinal gradient in phenological response.

Overall, the RCP45 scenario highlights the following fact: mean start dates for the studied clusters advance from $\text{DOY } 63\text{--}67$ (~early March) in 2030–2044 to $\text{DOY } 50\text{--}55$ (~late February) during 2060–2089, with a slight stabilization towards the end of the century (Figure 4).

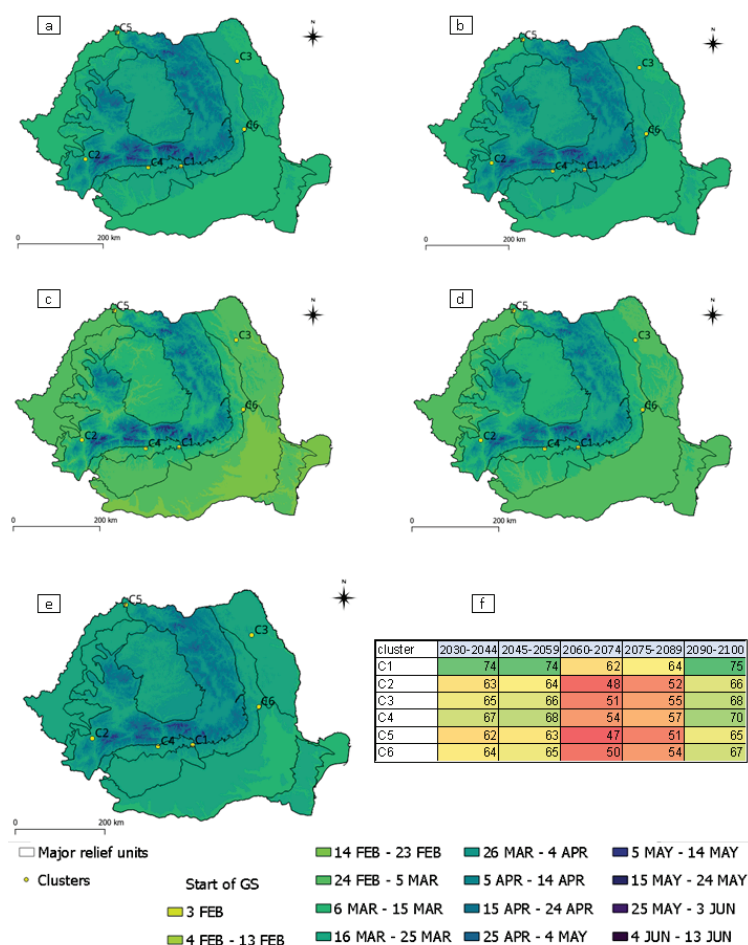


Figure 4. Spatial distribution and temporal evolution of the start of the GS in Romania under the RCP4.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the mean day of year (DOY) corresponding to the beginning of the vegetative season for the six orchard clusters (C1–C6).

3.2. Spatial Distribution and Temporal Evolution of the End of the Growing Season (EGS) in Romania Under the RCP4.5 Scenario for the 2030–2100 Time Horizon

The results regarding the EGS indicate a consistent delay of the end of the growing season throughout the 21st century, reflecting the gradual warming of autumn months under the RCP 4.5 scenario. In the near future (2030–2059), the EGS typically occurs between early and mid-November, while by 2060–2074 it extends to late November or even early December across most of the lowlands and hilly regions. This delay corresponds to a 15–25-day extension of the vegetation period compared to the early-century baseline.

The high-altitude and Subcarpathian zones retain an earlier end of season, usually between late September and mid-October, due to lower air temperatures and faster cooling rates in autumn. In contrast, extensive lowland regions in the south, east, and west experience a much later EGS, with modeled values surpassing DOY 315–320 (late November to early December) in the final projection intervals (2075–2100) (Figure 5).

According to the mean values summarized in Figure 5f, the end of the growing season (EGS) exhibits a clear temporal delay across all orchard clusters, consistent with the general warming trend projected under the RCP 4.5 scenario. During the first two studied intervals (2030–2044 and 2045–2059), most clusters show relatively stable mean values between DOY 297–309, corresponding to late October–early November. From 2060 onward, all clusters indicate a distinct shift towards later autumn termination, with mean EGS dates ranging from DOY 312 to 318 (approximately early to mid-November), suggesting an

extension of the vegetation period by nearly two to three weeks compared to the early projection horizon.

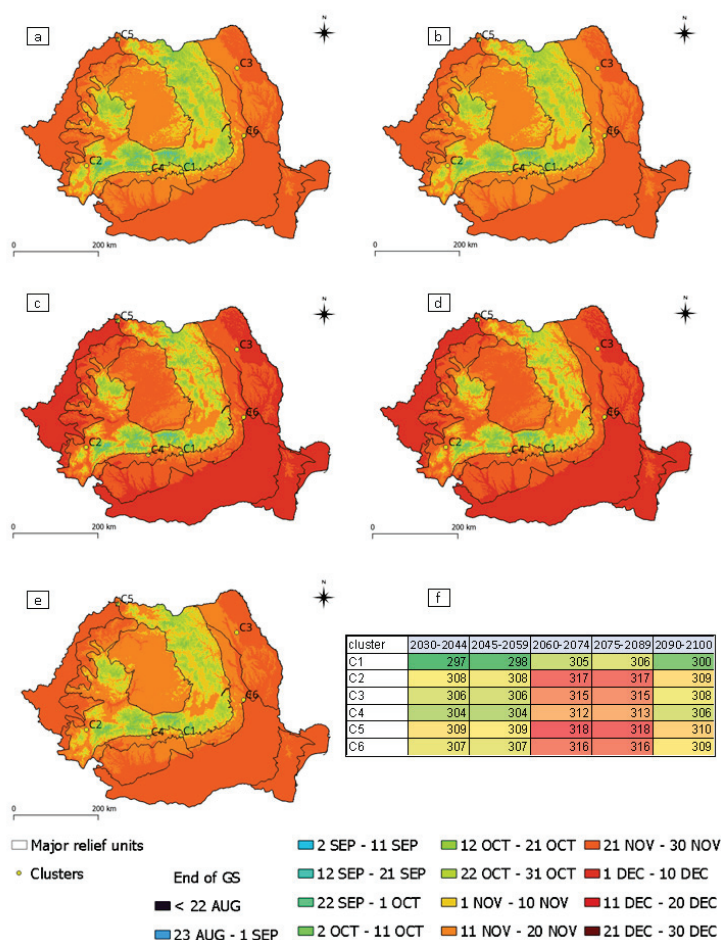


Figure 5. Spatial distribution and temporal evolution of the end of the GS in Romania under the RCP4.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the mean day of year (DOY) corresponding to the end of the vegetative season for the six orchard clusters (C1–C6).

The maximum delay is recorded in the 2075–2089 interval, when most of the studied clusters (C2, C3, C6) reach mean values above DOY 315, highlighting an autumn phenological limit around late November in lowland and hilly regions. By the end of the century (2090–2100), the EGS slightly retracts toward earlier dates (around DOY 306–310), indicating a possible stabilization of autumn conditions and reduced interdecadal variability after 2090.

3.3. Spatial Distribution and Temporal Evolution of the Growing Season Length (GSL) in Romania Under the RCP 4.5 Scenario for the 2030–2100 Period

Under RCP 4.5, the model results show an overall lengthening of the growing season length throughout the majority of Romania in the twenty-first century. The average length of the growing season in the lowlands and hilly regions ranges between 220 and 250 days over the early projection periods (2030–2044 and 2045–2059), while shorter seasons (less than 200 days) continue in the intra-Carpathian depressions and mountainous regions.

In the southern, eastern, and western regions of the country, the vegetation period lasts more than 260–270 days, starting with the 2060–2074 interval. The maximum duration locally reaches 280–300 days by the end of the century (2090–2100), indicating a 20–40 day increase over the early-century baseline. The maps also reveal that the lowland and sub-

Carpathian regions experience the most noticeable increases, with both delayed autumn senescence and earlier spring start contributing to the total expansion.

Despite this general trend, a slight stabilization or minor reduction in GSL is noticeable in some regions after 2080, likely reflecting the balancing effect of mid-century warming stabilization in RCP 4.5 (Figure 6).

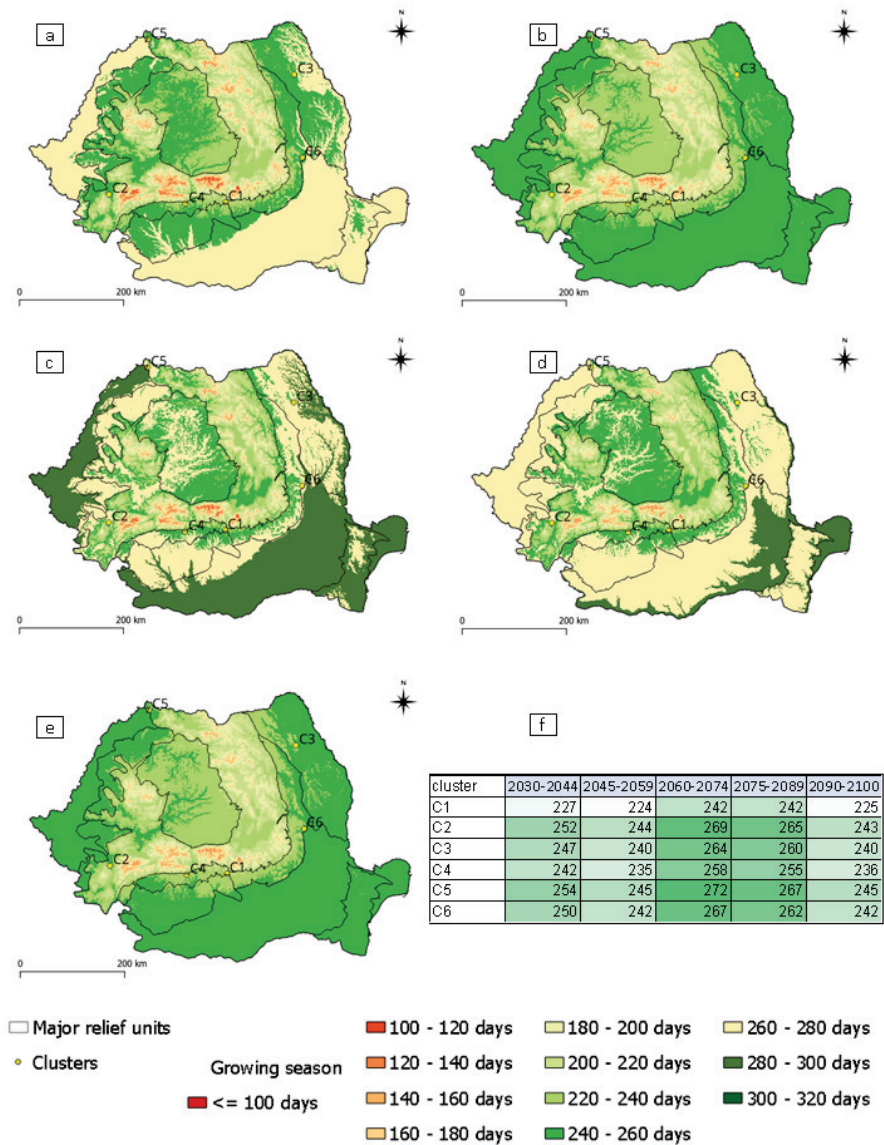


Figure 6. Spatial distribution and temporal evolution of the GSL in Romania under the RCP4.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the GSL corresponding for the six orchard clusters (C1–C6).

According to the mean values summarized in Figure 6f, the growing season length (GSL) exhibits a heterogeneous but consistent increase across all orchard clusters, reflecting both regional climatic differences and altitudinal gradients. In the early projection periods (2030–2044 and 2045–2059), clusters located in central-southern and eastern Romania (C2, C4, C6) display longer vegetation durations (around 245–255 days), while those situated in central and northwestern highlands (C1, C3, C5) maintain shorter seasons (approximately 225–245 days).

From the 2060–2074 horizon onward, the growing season extends markedly in all clusters, reaching values of 258–272 days, which implies an extension of 20–30 days compared with the early-century baseline. The most substantial increases occur in the southern

and western lowlands (C2 and C5), where both earlier spring onset and delayed autumn senescence contribute to vegetation periods approaching nine months per year.

During 2075–2089, the GSL remains similarly elevated, confirming a persistent lengthening trend, particularly in lowland orchards where DOY based estimates indicate values exceeding 265 days. By the end of the century (2090–2100), a modest decline is observed in most clusters, especially in C1, C3, and C4, with mean values returning to approximately 240–245 days, suggesting a stabilization of the phenological response under the moderate RCP 4.5 warming trajectory.

3.4. Spatial Distribution and Temporal Evolution of the Start of the Growing Season (GS) in Romania Under the RCP8.5 Scenario for the 2030–2100 Time Horizon

Under the high-emission RCP 8.5 scenario, the onset of the growing season exhibits a faster and more spatially homogeneous advancement than under the moderate RCP 4.5 pathway. In the early projection period (2030–2044), the simulated start of vegetation remains within the last decade of February to early March, broadly comparable with present conditions. By mid-century (2045–2059), the advancement becomes increasingly evident: large parts of the southern, western, and eastern plains reach DOY values near 50–55, meaning that the vegetative season can begin as early as mid-February, while mountainous regions still show onsets in early March. After 2060, the signal intensifies considerably. The 2060–2074 and 2075–2089 intervals reveal a rapid shift toward earlier activation of vegetation, with extended areas of the country recording SGS around DOY 40–45—equivalent to the first half of February. By the end of the century (2090–2100), most lowland and plateau zones show an advance exceeding three weeks relative to the 2030–2044 horizon, and in several warm southern and eastern regions the models suggest vegetation resumption possibly even in late January.

The spatial contrasts gradually diminish, indicating a tendency toward climatic homogenization of the spring phenophase as winter temperatures rise. The earlier initiation of vegetation could enhance annual biomass accumulation and fruit yield potential, yet it simultaneously amplifies vulnerability to late frosts and early-spring cold surges, especially in regions where temperature variability remains high (Figure 7).

The values presented in Figure 7f reveal a pronounced temporal advancement of the start of the growing season (SGS) across all orchard clusters under the RCP 8.5 scenario, with clear acceleration after the mid-21st century. During the early projection periods (2030–2044 and 2045–2059), most clusters register mean SGS values between DOY 60–70, corresponding roughly to late February–early March, comparable with present-day or RCP 4.5 conditions. From 2060 onward, however, the onset of vegetation advances sharply, with all clusters showing reductions of 10–15 days per period. By 2060–2074, average SGS values range between DOY 51–64, and by 2075–2089 they drop further to DOY 42–55, indicating that the start of vegetative activity occurs predominantly in early to mid-February. By the end of the century (2090–2100), most clusters record the earliest onset ever simulated, between DOY 32–47, equivalent to late January to early February, confirming a total advancement of about 25–30 days compared to the 2030–2044 interval.

The lowest values (earliest SGS) are recorded in the clusters located within southern and eastern Romania (C2, C4, C6), which reach mean dates as early as DOY 32–36 in 2090–2100, while northern and central clusters (C1, C3, C5) maintain relatively later onsets, between DOY 39–47, reflecting the residual influence of altitude and cooler regional climates. This strong spatial gradient, however, tends to weaken toward the end of the century, suggesting a progressive climatic homogenization under intense warming.

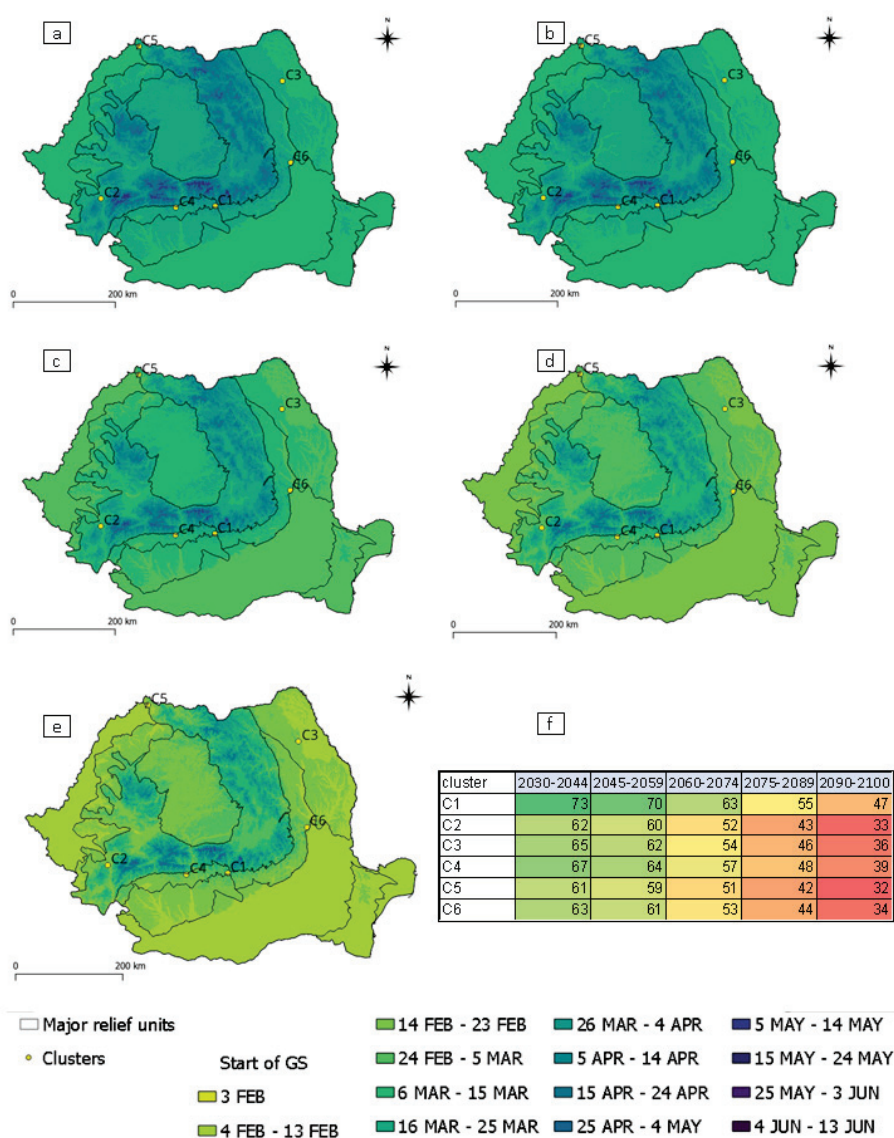


Figure 7. Spatial distribution and temporal evolution of the start of the GS in Romania under the RCP8.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the mean day of year (DOY) corresponding to the beginning of the vegetative season for the six orchard clusters (C1–C6).

3.5. Spatial Distribution and Temporal Evolution of the End of the Growing Season (EGS) in Romania Under the RCP8.5 Scenario for the 2030–2100 Time Horizon

The projections under the RCP 8.5 scenario reveal a marked and continuous delay of the end of the growing season throughout the 21st century, with the most substantial changes occurring after mid-century. During the first projection interval (2030–2044), the EGS largely coincides with present-day conditions, ending between late October and mid-November across most regions. By 2045–2059, however, the end of vegetation progressively shifts towards late November, especially within the southern and eastern lowlands, suggesting the onset of a prolonged autumn growing phase.

From 2060 onwards, the simulated extension becomes more accentuated and spatially coherent. Between 2060–2074, large parts of Romania exhibit EGS dates surpassing DOY 310–320 (late November–early December), while some lowland and hilly areas approach DOY 325–330, corresponding to the second decade of December. By the final part of the century (2090–2100), almost the entire territory—excluding high-altitude regions—

shows the end of the growing season shifted well into mid or even late December, indicating a phenological delay of nearly one month compared to the early projection horizon.

Regarding the orchard clusters (Figure 8f), the results indicate a pronounced and nearly continuous delay of the end of the growing season (EGS) in all orchard clusters under the RCP 8.5 scenario, with particularly rapid changes after the mid-21st century. During the early projection horizons (2030–2044), EGS values range between DOY 295–305, meaning that vegetation typically ends between late October and early November. These dates are close to current climatic conditions, suggesting limited alterations in the near future.

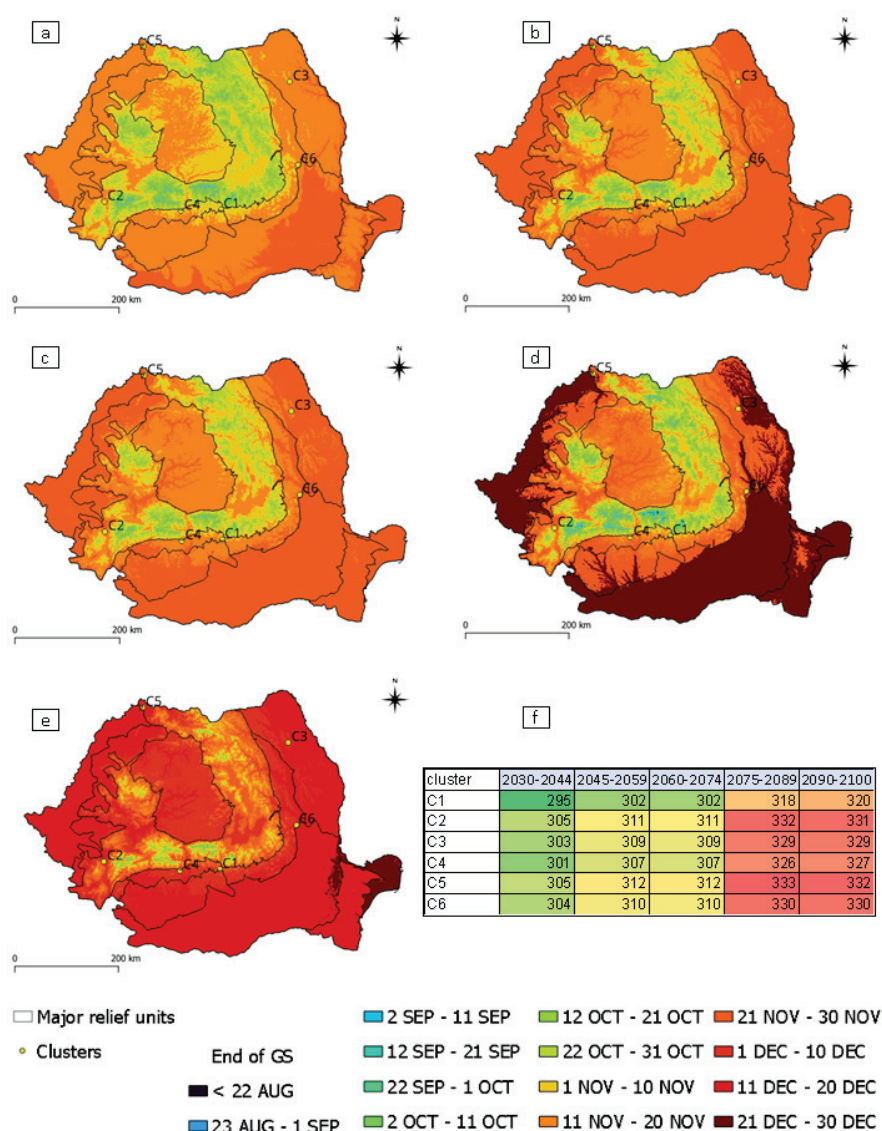


Figure 8. Spatial distribution and temporal evolution of the end of the GS in Romania under the RCP8.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the mean day of year (DOY) corresponding to the end of the vegetative season for the six orchard clusters (C1–C6).

However, by 2045–2059, all clusters register increases of 5–10 days, with mean values around DOY 307–312, pointing to a transition of the vegetative limit towards mid-November. This trend continues through 2060–2074, when most clusters stabilize temporarily at similar values, reflecting an intermediate stage before the accelerated extension seen in the late-century projections.

From 2075 onward, a sharp shift toward later autumn termination becomes evident. Clusters C2, C5, and C6 reach the highest mean values, between DOY 330–333, equivalent to mid or even late December. Meanwhile, C1, C3, and C4 record slightly earlier EGS values around DOY 320–327, but still indicate a delay of almost one month compared to early-century conditions.

By the end of the century (2090–2100), most clusters maintain this late-season plateau, with differences of only a few days among them, suggesting a quasi-uniform extension of the vegetation period across Romania. The cumulative lengthening relative to 2030–2044 reaches approximately 25–30 days for the lowlands and 20–25 days in the upland clusters.

3.6. Spatial Distribution and Temporal Evolution of the Growing Season Length (GSL) in Romania Under the RCP 8.5 Scenario for the 2030–2100 Period

The simulations depict a progressive and strong lengthening of the growing season, with the most dynamic changes occurring from mid-century onward. In the near-term (2030–2044), GSL values are broadly comparable to present-day conditions, typically spanning ~220–245 days over large areas. By 2045–2059, the distribution shifts upward, with extensive regions entering the 240–260-day class, signaling the early imprint of warmer springs and milder autumns.

A visible change emerges after 2060. During 2060–2074, much of the country crosses the 260-day threshold, and in favored lowland and plateau zones the season frequently extends toward 270–280 days. The late-century intervals (2075–2089 and 2090–2100) exhibit the most expansive increases, with broad swaths reaching 280–300 days and localized maxima approaching ~300+ days. This pattern reflects the compound effect of earlier spring onset and delayed autumn termination documented in the SGS and EGS maps.

A tendency toward thermal homogenization under prolonged warming is indicated by the diminishing of spatial disparities that were noticeable in the early horizons (longer seasons in lowlands versus shorter seasons at higher elevations) approaching 2100.

The results (Figure 9f) show a consistent and substantial increase in the duration of the growing season (GSL) across all orchard clusters under the RCP 8.5 scenario, reflecting in the early projection horizons (2030–2044), mean GSL values range from 222 to 244 days, depending on altitude and regional climate. C2, C5 and C6 clusters already exhibit slightly longer growing seasons (240–244 days), while C1, C3, and C4 maintain shorter intervals (around 222–238 days). By mid-century (2045–2059), a uniform increase of roughly 8–10 days is observed across the country, marking the transition toward a climate regime favorable to prolonged vegetative activity. From 2060–2074, the rate of extension accelerates further, with mean GSL values ranging between 251 and 261 days, indicating a clear shift to nearly nine-month vegetation periods in several lowland and sub-Carpathian clusters.

The most substantial lengthening occurs after 2075, when all clusters surpass 270 days, and lowland areas reach 290–300 days by the end of the century. The northwestern and southwestern clusters (C5 and C2) record the highest values—up to 298–300 days in 2090–2100 representing an increase of nearly 50–60 days relative to the early-century baseline. Even in the cooler and higher elevation clusters, GSL extends to 273–288 days, underscoring the warming trend.

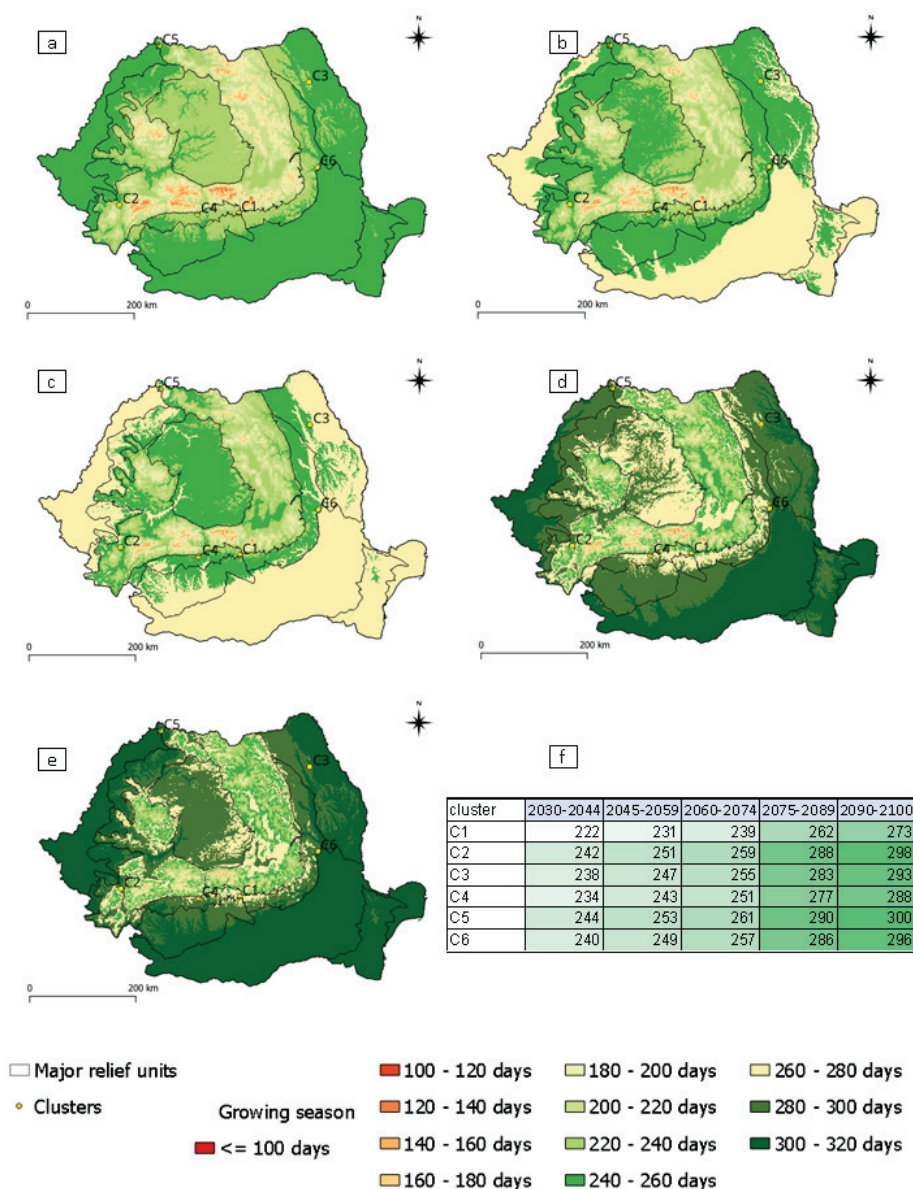


Figure 9. Spatial distribution and temporal evolution of the GSL in Romania under the RCP8.5 scenario; (a) 2030–2044, (b) 2045–2059, (c) 2060–2074, (d) 2075–2089, and (e) 2090–2100. Panel (f) summarizes the GSL corresponding for the six orchard clusters (C1–C6).

4. Discussion

In the specialized literature, the duration of the growing season for plum (*Prunus domestica*) in Romania is reported to range between 235 and 250 days, varying among regions according to local climatic characteristics [58]. For instance, in clusters C1, C4, and C6, the growing season length (GSL) averages around 235 days, while in C2 and C5, it can extend to 240–250 days. In southern Romania, the onset of the growing season for plum orchards generally occurs in late March or early April, a pattern also observed in the north-eastern region of the country [59,60]. The end of the vegetative period typically takes place between the second half of October and mid-November, depending on local temperature conditions and altitude.

The temporal evolution of the growing season across Romania displays a clear advancing and lengthening trend throughout the 21st century, with distinct rates of change under the two emission scenarios. Under RCP 4.5, both the onset (SGS) and termination (EGS) of the growing season show a gradual yet statistically significant shift. The SGS advances

by approximately 10–15 days, and the EGS is delayed by about 15–20 days, resulting in an overall extension of the growing season by three to four weeks compared to the early projection horizon (2030–2044).

In contrast, the RCP 8.5 scenario produces a markedly stronger and non-linear response. After 2060, the phenological indicators accelerate sharply: the SGS advances by 25–30 days, frequently occurring in late January or early February, while the EGS extends into late November or December, leading to a growing season 40–60 days longer than at the beginning of the century. This temporal expansion intensifies between 2060 and 2089, after which most variables stabilize or plateau, indicating a potential climatic equilibrium toward the end of the century. Such behavior highlights the sensitivity of perennial orchard systems to cumulative thermal forcing, with the strongest adjustments concentrated in the second half of the century (Figure 10).

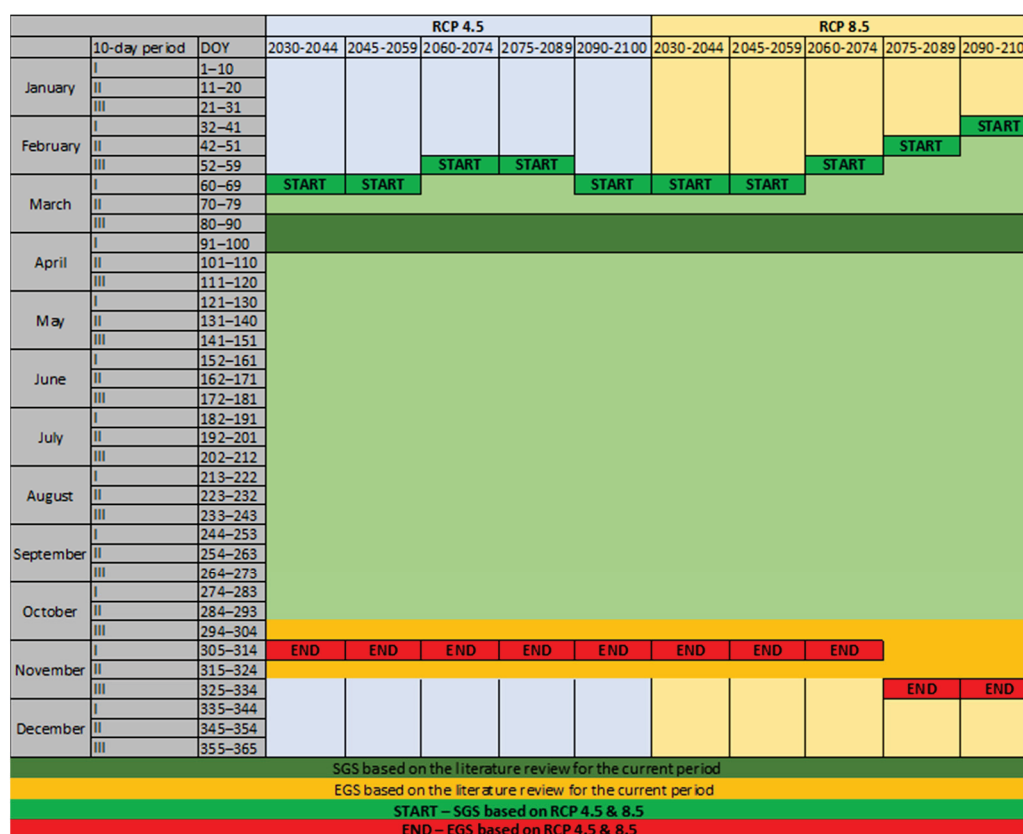


Figure 10. Calendar representation of the growing season timing (SGS, EGS, GSL) for current and projected climatic conditions. The SGS and the EGS represent the mean value for the 6 clusters.

Following the evolution of the SGS and EGS indicators by cluster, C1 shows the lowest variations compared to the standards mentioned in the literature, followed in each case by C4. This indicates the importance of taking into account the vertical gradient which, in the context of RCP 4.5, is presented in a clearer manner, the two clusters mentioned above (C1 and C4) being located in areas with higher hills with a subcarpathian character. This clear difference seems to fade in the context of the RCP 8.5 scenario, when although the two clusters maintain their order, the differences compared to the other clusters are much smaller. Irímia et al. [19], in a study targeting the relationship between climate change and the suitability for wine production, note that from a climatic perspective, an altitudinal shift is also observed, with areas suitable for wine production moving to higher altitudes in the context of recent climate change.

Although the scale of Romania is too small to draw such conclusions, it should be noted that for RCP 8.5 there is an increase in homogeneity in the territory. We associate this with a visible decrease in the differences between clusters for SGS and EGS. The decrease in heterogeneity in the context of future climate scenarios was also observed by [61] (Figure 11).

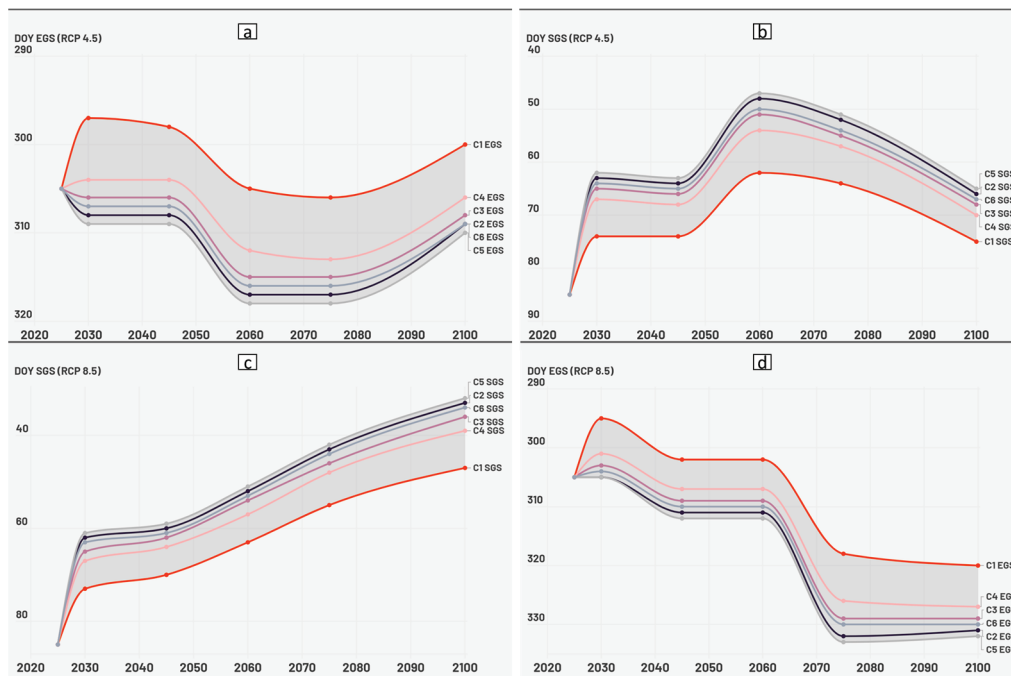


Figure 11. Projected changes in: (a) SGS under RCP 4.5; (b) EGS under RCP 4.5; (c) SGS under RCP 8.5; (d) EGS under RCP 8.5 for the 2030–2100 period.

Regarding the start of the growing season (SGS), Xia et al. [62] reported a marked advancement across northern latitudes. For the late-century period (2080–2099), the mean shift relative to 1985–2004 is projected to reach approximately -18.6 days under RCP 4.5 and -37.5 days under RCP 8.5, indicating a substantial early onset of vegetation under intensified warming conditions. Similarly, a notable extension of the growing season has been documented for various regions of Finland based on multiple climate model simulations. According to Ruosteenoja et al. [63], thermal winter is shrinking much faster than thermal summer is lengthening, primarily because winter temperatures are expected to increase more than twice as rapidly as summer temperatures. As a result, areas that are currently characterized by long and cold winters, such as Lapland or the Carpathian uplands are projected to develop thermal regimes comparable to those of present-day lowlands or even Central Europe by the end of the 21st century.

Based on the obtained results, it can be observed that the variability is more pronounced in the changes affecting the start of the growing season (SGS) than in those related to its end (EGS) under both climate scenarios analyzed. This indicates that spring phenophases are more sensitive to temperature increases and interannual thermal fluctuations than autumn ones, as the onset of vegetation responds directly to early-season warming. Overall, the analysis highlights a non-linear but accelerated shift in spring phenology under RCP 8.5, where each successive period brings an earlier SGS by approximately one week compared with the previous decade. Such advancement is projected to lengthen the overall growing season but also heightens the frost risk window, particularly for early-flowering with implication over the fruit production, respectively, over the economic sector [12,47]. Climate change can affect multiple components of agricultural ecosystems,

including the activity and efficiency of pollinating insects. Temperature extremes or insufficient chilling may disrupt flowering–pollinator synchrony, reducing fertilization and ultimately fruit set. The optimal temperature range for pollination and fertilization in temperate fruit species such as plum, apple, or cherry is between 20–25 °C, while prolonged periods of cold, rain, or heat stress can severely limit pollination success [64]. Under projected climatic conditions in Romania, phenological mismatches between flowering periods and pollinator activity may therefore become an additional factor influencing the productivity and stability of orchard ecosystems.

In the context of a future warmer climate, the reduction in winter chill is expected to have some of the most severe implications for fruit production in temperate regions. Insufficient chilling can delay or even inhibit flowering, produce uneven or prolonged bloom, and generate abnormal vegetative growth, ultimately affecting fruit quality and yield [11,65]. In the context of the Romanian orchard regions, this process could intensify under warmer winters projected by both RCP 4.5 and RCP 8.5 scenarios. The observed advancement of the (SGS), combined with weaker chilling accumulation, may disrupt the physiological balance between dormancy and flowering, posing a critical challenge for the long-term adaptation and productivity of plum orchards. Although the extended growing period may enhance productivity potential, it also increases the exposure window to frost, heat [66,67], and drought extremes [68,69], redefining the risk calendar for Romanian orchards. For plum orchards, in the context of future climate change, Ref. [70] mentions that the projected temperature increases in the Bydgoszcz region are expected to raise the water needs of plum orchards (the annual optimal precipitation requirement for plum is estimated to rise from 712 mm to 807 mm, representing a 95 mm increase. In Great Britain, Ref. [71] revealed emerging geographic mismatches between suitable orchard areas and suitable pollinator habitats, suggesting a potential decline in pollination services unless production shifts towards more climatically favorable north-western regions. A recent study [72] highlighted that under 2050 conditions, the median bloom date shift in apples, pears and European plum ranged between −12 days to −19 days. According to [73], adapting agriculture to ongoing climate change requires coordinated and integrated measures across the production sector. In fruit growing, this process is particularly challenging and costly due to the perennial nature of orchards, which cannot be rapidly replaced or relocated. The most vulnerable crops are also the most widely cultivated in Bosnia and Herzegovina, including plum, apple, pear, cherry and sour cherry, making their adaptation especially urgent.

Although the results obtained depend on the assumptions and resolution of the climate models used, they provide a coherent picture of the direction and magnitude of phenological changes at the regional level. This study relies on a single GCM (MIROC5), which, although appropriate for the methodological structure adopted here, does not capture the full range of climate model uncertainty; therefore, future research should incorporate multi-model ensembles to enhance robustness and quantify variability among projections. The main sources of uncertainty, which can also be considered limitations, come from the differences between global and regional climate models [74], but also from the sensitivity of phenological thresholds to altitude, exposure and microclimate [75,76]. However, the convergence of trends between clusters and scenarios (RCP 4.5 and RCP 8.5) suggests a general robustness of the climate signal, which allows the use of these results in comparative assessments and fruit-growing adaptation plans. In the future, the integration of observational phenological series, monitoring data in orchards and the incorporation of varietal stratification to better reflect phenological variability within orchards, together with multi-model and multi-scenario analyses, can reduce uncertainty and refine local estimates of SGS and EGS periods. Although the projections presented here reveal coherent spatial and temporal patterns, several sources of uncertainty may influence the direction and mag-

nitude of the estimated changes. Climate model bias is an inherent limitation, as different GCMs may produce warmer or cooler seasonal trajectories, potentially shifting the timing of SGS and EGS by several days. Likewise, the selection of the 6 °C phenological threshold may affect the absolute values of start and end dates, especially in transitional years, although the relative trends across time periods are expected to remain robust. Even so, the consistent advancement of SGS and extension of GSL across scenarios and clusters suggests that the underlying climatic signal is strong and resilient to methodological uncertainty.

5. Conclusions

This research provides a comprehensive assessment of possible changes in the growing season of plum (*Prunus domestica* L.) orchards in Romania, under moderate (RCP 4.5) and severe (RCP 8.5) climate scenarios during the 21st century. By integrating temperature-based phenology modeling with a regional cluster approach, the results highlight a clear trend towards advancing the start and lengthening the duration of the growing season in all regions analyzed.

Under the RCP 4.5 scenario, the start of the growing season (SGS) is projected to occur about 10–15 days earlier and the end of the growing season (EGS) 15–20 days later, implying a moderate extension of the growing season by about three to four weeks compared to the first-time interval analyzed in this paper. These trends reflect a gradual but steady warming, compatible with moderate adaptation needs in fruit systems. This moderate extension of the season may call for revised orchard management calendars, including earlier pruning, earlier pest and disease monitoring, and improved preparedness for late spring frost events.

In contrast, under the RCP 8.5 scenario, both the magnitude and the pace of phenological changes increase significantly after mid-century. In this case, the beginning of the growing season is expected to occur 25–30 days earlier and the end 25–35 days later, which determines a total extension of up to 60 days in some clusters. The most pronounced changes are anticipated between 2060 and 2089, an interval characterized by an intensification of the increase in temperatures. From a spatial point of view, the differences between the plain and the hill or mountain areas tend to reduce, indicating a thermal homogenization of the phenological behavior across the territory of Romania.

In the context of projected advances of SGS and extensions of the growing season, adaptation in plum orchards will require more targeted strategies. Selecting cultivars with later flowering dates or improved frost tolerance could help minimize exposure to early-season frost risk. In addition, assessing future winter chill availability will be essential for guiding cultivar suitability and orchard renewal decisions. Water management will also become increasingly important under longer growing seasons, particularly in regions projected to experience reduced precipitation and higher evaporative demand.

Although the results may be influenced by uncertainties specific to the climate scenarios (the limitations of the models used or local microclimatic particularities), the general direction of the trends is clear and consistent across regions and scenarios. This provides confidence that the obtained projections reflect a real and significant climate signal, which can serve as a solid basis for anticipating and managing the planning of future plum orchards in Romania.

In conclusion, the progressive advancement and extension of the plum growing season in Romania, especially under the high emissions scenario, indicates a fundamental phenological shift that will redefine the biological calendar of fruit ecosystems. The results obtained emphasize the need for adaptive strategies and continuous monitoring of phenological responses, to ensure the sustainability and resilience of fruit production in the context of current climate change.

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Abbreviations

The following abbreviations are used in this manuscript:

SGS	Start of the growing season
EGS	End of the growing season
GSL	Growing season length
DOY	Day of the year
GS	Growing season

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Article

Over-the-Row Mechanical Harvest of Cider Apples (*Malus domestica* Borkh.)

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Abstract

The single greatest annual production cost for an established cider apple (*Malus domestica* Borkh.) orchard is the labor required to hand harvest. Reducing harvest labor time may increase the appeal and profitability of growing cider apples. Over-the-row mechanical harvest of cider apples using a modified Oxbo-Korvan 930 was evaluated in northwestern Washington, USA, in 2021, 2022, and 2023 in a fully mature cider apple orchard that was planted in 2014–2016. Sixteen cider apple cultivars grafted on ‘Geneva 935’ rootstock were summer hedged between 7 and 20 July each year of this study. Plant growth regulators were applied before harvest to equalize the timing of harvest among cultivars. There were no differences among cultivars for the percent of apples captured by the Oxbo-Korvan 930 harvester for the 3 years of this study. Across all years and cultivars studied, 82% of fruit were captured by the harvester. There also were no differences among cultivars for the percentage of fruit left on the tree by the harvester (9% of fruit on average), nor in the percentage of fruit dropped on the ground during harvest (9% of fruit on average). The overall mean number of branches broken during mechanical harvest across all cultivars was 1.4 per tree, and there were no differences among cultivars. ‘Sweet Alford’ had high spur removal (26 removed per tree), but excluding this outlier, only 6 spurs on average were removed per tree for all other cultivars. Laceration to fruit during mechanical harvest were positively correlated with mean fruit weight and mean fruit diameter. The overall average time required to mechanically harvest one tree in this orchard (1.8 m in-row spacing, 1495 trees·ha^{−1}) was 5.3 s, averaging 2.9 s per row-meter traveled. The average time required to manually harvest one tree was 229 s (3.8 min). The juice quality of the mechanically harvested apples that were kept in cold storage and pressed within 42 d of harvest did not differ largely or consistently from juice quality of apples that were pressed within 3 d of harvest, except that sugars (measured through °Brix and specific gravity) increased with storage time, as expected. Mechanical harvest using the modified Oxbo-Korvan 930 appears to be a labor-efficient and effective method of harvesting cider apples, and testing is needed in commercial orchards to evaluate its viability compared to other harvest technologies.

Keywords: labor efficiency; mechanization; orchard management

1. Introduction

The U.S. cider industry has expanded in the past two decades, increasing the demand for apple (*Malus domestica* Borkh.) cultivars with cider-making attributes such as high polyphenol content, acidity and sugars [1]. Due to the high cost of these specialty cider apples (USD 0.19 and USD 0.21 per kg in the Pacific Northwest in 2018 and 2019, respectively), many cidermakers purchase cull dessert apples or apple juice (USD 0.02 to USD 0.11 per kg) to make their ciders [2–6]. The high cost of cider apple fruit is largely due to their limited supply and high production costs.

Following establishment, the single greatest annual orchard production cost for cider apples is the labor required to hand harvest, which represents ~46% of annual variable costs [3,7]. Cider apples tend to be smaller than standard dessert fruit, thus can take up to four times longer to pick by hand [8]. Additionally, a large, capable, and consistent labor force can be difficult to recruit and house only for the brief harvest period [9], especially in areas where tree fruit are not abundantly grown such as western Washington, or when harvest dates for other crops conflict. Cider apples are suitable for mechanical harvest as they are pressed into juice soon after harvest; thus, cosmetic damage is not a critical quality concern. Further, bruising and damage of the fruit during mechanical harvest has minimal impact on juice quality [10].

In European cider apple orchards, shake-and-sweep mechanical harvest systems are the dominant harvest method. These systems utilize a hydraulic arm that grasps the tree trunk to “shake” apples onto the ground, then a machine comes along to “sweep” the apples off the ground. In this process, all apples that are on the orchard floor are picked up, including those of poor quality. In New York, mechanical harvest of cider apples using shake-and-sweep machinery was found to be more economical than hand harvest regardless of orchard scale [11]. However, shake-and-sweep harvest systems are not suitable for high density planting systems that utilize dwarfing rootstocks. Trunk shakers require a significant amount of time to use in high density orchards, and they can break smaller trees. The time required to shake trees in a 1-ha orchard of 2500 trees would be at least 41.5 h (~1 min per tree) [11]. Further, fruit needs to be collected from the ground, usually with a harvester that can cost USD 2000 to USD 200,000 depending on model and scale, and includes a mix of poor quality fruit [11]. Another consideration in the U.S. is that food safety requirements result in the restricted use of apples that have come in contact with the ground [12]. Smaller processors and/or cider producers may be willing to complete disclosure documentation and proper sanitation associated with processing ground-fallen fruit. However, larger processing facilities that mostly process handpicked apples may be less likely to accept ground-fallen fruit due to the sanitation required. A shake-and-catch mechanical harvester that prevents apple-to-ground contact would be more favorable in the U.S. This is especially important in Washington State, USA, where processing facilities are well-established for handpicked apples.

Over-the-row harvesters that remove and catch fruit have been utilized in olives, citrus, and processed berry and stone fruit production for many years. In 2014 and 2015, an over-the-row berry harvester (model OR0012; Littau Harvester, Lynden, WA, USA) was tested on low-trellised ‘Brown Snout’ cider apples in Washington State, USA, capturing 74% of apples on average with insignificant effects on juice quality [10,13]. The goal of the current study was to determine if an over-the-row modified Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA) was suitable for harvesting cider apples in an orchard that was pruned through summer hedging to a fruiting wall system [14]. A further goal was to assess if mechanically harvested fruit could be cold-stored post-harvest without loss due to rot or decline in juice quality. The modified Oxbo-Korvan 930 harvester has the same drum, rod and conveyer belt system as the Littau harvester used in other

studies; this study was to determine if the modified harvester height was suitable for cider apple harvest. Apple cultivars exhibit variation in stem length, stem abscission, fruit size and other factors that affect mechanical harvest efficiency [15]; thus, this study included 16 cider apples cultivars to assess the modified Oxbo-Korvan 930 over-the-row harvester.

2. Materials and Methods

2.1. Research Orchard

This experiment was carried out in a cider apple research orchard at Washington State University (WSU) Northwestern Washington Research and Extension Center (NWREC) (48°26'14.4" N, 122°23'40.7" W), located near Mount Vernon, WA, USA. The 0.35-ha cider apple research orchard was planted in 2014 and included two replicates of 65 cultivars in a randomized complete block design with three trees per plot. Trees were spaced 1.8 m apart in-row, with 3.7 m between rows (605 trees·acre⁻¹). Trees were grafted to ‘Geneva 935’ (*Malus hybrid*) rootstock and were trellised and trained to a 3.0 m tall vertical axis, with a central leader. All trees had reached full maturity at the start of the experiment.

2.2. Mechanical Harvester

This experiment utilized an over-the-row Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA;) modified only by increasing the tunnel height from 2.1 m to 3.7 m (Kathryn Vanweerdhuizen, personal communication, December 2022). This harvester was designed to harvest *Jatropha* (*Jatropha curcas*) and was purchased in 2016 (cost USD 71,000) and modified by a grower (Supplemental information). The modified Oxbo-Korvan 930 includes heavy duty Dynarotor head and rods (48 cm length), which rotate at variable speeds that is based on contact resistance. A diesel tractor was used to pull the harvester (Figure 1), with the PTO operating at 540 rpm and the tractor moving in the lowest gear moving at 0.31–0.35 m·s⁻¹.

2.3. Experimental Procedures

Each year cultivars that had high crop load and similar harvest dates were selected for the study. Due to the biennial bearing habit of cider apple cultivars, not all cultivars had sufficient fruit for harvest every year. The study included 10 cultivars in 2021, 12 cultivars in 2022, and 16 cultivars in 2023 (Table 1). All cultivars were harvested at least 2 years and three cultivars were harvested all 3 years. In 2021, only data for mechanical harvest efficiency were collected. In 2022 and 2023, data for mechanical harvest efficiency, tree damage, time required to harvest and fruit and juice quality were collected.

Table 1. Sixteen cider apple cultivars mechanically harvested using the over-the-row modified Oxbo-Korvan 930 (Oxbo International Co., Lynden, WA, USA) at Washington State University Northwestern Washington Research and Extension Center, Mount Vernon, WA, USA, in 2021–2023.

Cultivars Harvested	Historical Harvest Date ¹
2021 and 2023	
Ashmead’s Kernel	30 September
Chisel Jersey	29 September
Foxwhelp	4 October
Jouveaux	25 September
Liberty ²	- ³
Porter’s Perfection	26 September
Redfield	-
Roxbury Russet ²	21 September

Table 1. Cont.

Cultivars Harvested	Historical Harvest Date ¹
Russet King	26 September
Smith’s Cider ²	1 October
2022 and 2023	
Amere de Berthcourt	25 September
American Forestier	18 October
Brown Thorn	8 October
Brown’s Apple	-
Liberty ²	-
Roxbury Russet ²	21 September
Smith’s Cider ²	1 October
Sweet Alford	7 October
Sweet Coppin	10 October

¹ Harvest dates were recorded 2002 to 2017 in Mount Vernon, WA, USA, and the 15-year average was calculated [16].
² Indicates that a cultivar was harvested in all 3 years of this study. ³ “-” indicates no historical data for this cultivar at this site.



Figure 1. Modified Oxbo-Korvan 930 over-the-row mechanical harvester (Oxbo International Co., Lynden, WA, USA), with two vertical columns with attached, fiberglass harvest rods, during apple harvest in 2023 in Mount Vernon, WA. Photos by Kwabena Sarpong.

The orchard was sprayed with three commercial plant growth regulator (PGR) products (ReTain, Valent Biosciences, Libertyville, IL, USA; Refine, Fine Americas Inc., Walnut Creek, CA, USA; Ethephon 2SL, ADAMA, Raleigh, NC, USA) at label rates prior to harvest all 3 years. Products were applied to retain fruit on the trees (ReTain and Refine) and then to release fruit (Ethephon) to maximize fruit capture by the harvester. In 2022 and 2023, fruit maturity was measured using the Cornell Starch Iodine Index (CSII; scale 1–8) [17] for five randomly selected apples per plot. In 2022, fruit did not reach full maturity by the end of the season due to inclement weather and were harvested at ~4–6 CSII. In 2023, fruit were harvested within 2 d of half the plots reaching 7–8 on the CSII scale.

Fallen apples were removed from the orchard floor and discarded 14 d and 2 d before harvest all 3 years. Each year, all plots were harvested on the same day. Prior to mechanical harvest in 2022 and 2023, the northern-most tree in each study plot was harvested by hand and the amount of time to hand harvest was recorded. The same day as mechanical harvest, apples that fell to the ground in each plot during harvest and all fruit remaining on the trees after harvest were collected, counted, and weighed separately, then discarded. The number of limbs larger than 1 cm diameter that were partially or completely broken off the tree by the harvester were counted per plot in 2022 and 2023.

All 3 years, mechanically harvested apples were stored at ambient temperature and yield was measured within 3 d of harvest. The number of lacerated fruit were counted per plot in 2022, and for a random sample of 30 fruit per plot in 2023. The total number of fruiting spurs present with the fruit were counted in 2022 and 2023, and mean number of spurs per plot was calculated. In 2023, 30 representative fruit were randomly selected (or all fruit per plot if fewer than 30), weighed, and the diameter was measured. The mean weight and diameter per fruit were calculated for each plot.

In 2022, as all cultivars were harvested before full maturity, fruit were placed in cold storage (4.4 °C) and five fruit per plot were tested each week for maturity using the CSII test. Once the fruit in a plot reached maturity (7–8 on the CSII), fruit were milled (MultiMax 30; Zambelli Enotech, Camisano Vicentino, Italy), pressed (Carezza; Enotecnica Pillan, Camisano Vicentino, Italy), and a 500 mL subsample of juice was frozen (−15 °C) for analysis. All plots reached maturity within 21–35 d post-harvest. In 2023, 25 fruit were milled and pressed within 3 d of harvest, and a 500 mL juice subsample was frozen for analysis. Remaining fruit were placed in cold storage to assess if juice quality declined due to post-harvest storage. At 15 d post-harvest, 25 fruit were milled, pressed, and a subsample was frozen for analysis. For the 12 plots where yield was >20 kg, 25 cold-stored fruit were milled and pressed 32 d post-harvest, and juice subsamples were frozen for analysis. At both 15 and 32 d post-harvest, the number of rotten fruit was recorded for each plot and then discarded.

Each year, all frozen juice samples were thawed and juice quality was measured: total soluble solids (TSS), pH, titratable acidity (TA), specific gravity (SG), and percent tannin (a subgrouping of polyphenols relevant to cidermakers). Juice analysis followed the same procedures as outlined in Kendall et al. [18], summarized here. Juice samples were thawed at room temperature (23 °C) then hand shaken for homogenization. TSS (recorded as °Brix) were measured for two to three drops of juice per sample with a digital refractometer (Palm Abbe model #PA201; MISCO, Cleveland, OH, USA), making sure no bubbles were present and adjusted to room temperature for 30 s. pH was measured for each sample using a digital pH meter (Ohaus Starter 5000; Ohaus Corporation, Parsippany, NJ, USA) by submersing the probe in 5 mL of juice. TA (percent malic acid) was measured for each sample using titration with an auto-titrator (HI932; Hanna Instruments, Woonsocket, RI, USA) with 0.1 N sodium hydroxide (NaOH; Aldon Corp., Avon, NY, USA) to an endpoint of 8.2. TA was calculated as grams of malic acid per 100 mL, using the conversion factor mL

$\text{NaOH} \times 0.536$ (a constant based on sample volume and normality). SG was measured using a precision hydrometer (SG range 1.000–1.070; Bellwether; VeeGee Scientific, Kirkland, WA, USA) suspended in 200–250 mL of juice. Percent tannin (expressed as tannic acid equivalents) was measured using the Löwenthal Permanganate Titration method [19–21]. A blank control was calculated for each batch of potassium permanganate solution (Ward’s Science, Rochester, NY, USA) by titrating the solution into an indigo carmine indicator without juice. The volume of titrant required to turn the indicator solution yellow was recorded as the “Q” value for that batch. For each plot, the potassium permanganate was titrated into 1 mL juice samples mixed with 5 mL of indicator solution until the reference color (yellow) was reached; the volume of titrant was recorded as the “P” value. Percent tannin was calculated by $(P - Q)/10$.

2.4. Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in R (version 4.2.2., R Studio, Boston, MA, USA). The relationships between fruit damage and weight, as well as fruit damage and diameter, were analyzed using polynomial regressions with the “nlme” package. The relationships between total fruit and fruit dropped, as well as total fruit and fruit captured, were analyzed using simple linear regressions with the “nlme” package. Juice analysis data were analyzed using paired *t*-tests (2022) and repeated measures ANOVA (2023) in R. Significance of all interactions was reported at $\alpha = 0.05$.

3. Results

There were no differences among years ($p = 0.88$) or cultivars ($p = 0.43$) for the percent of apples captured by the modified Oxbo-Korvan 930 harvester for the 3 years of this study. Overall, across years and cultivars, 82% of fruit were captured by the harvester and the range was 70–95% (Figure 2). There was no correlation between total weight of mechanically harvested fruit and the percent of fruit that were captured ($p = 0.25$). There was a positive correlation between total weight of harvested fruit and the percent of fruit that were dropped on the ground during harvest (9% of fruit on average across years and cultivars) ($p < 0.001$, $R = 0.49$), but there was no difference among cultivars for percent of fruit dropped on the ground during harvest across years ($p = 0.57$). There also was no difference among cultivars for the percentage of fruit left on the tree by the harvester across years and cultivars (9% of fruit on average) ($p = 0.91$). The overall average time required to mechanically harvest one tree in this orchard (1.8 m in-row spacing, 1495 trees·ha^{−1}) was 5.3 s, averaging 2.9 s per row-meter traveled (Table 2). The average time required to hand harvest one tree in this study was 229 s (3.8 min) (Table 2).

Table 2. Amount of time to mechanically harvest cider apples using the over-the-row modified Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA), and to hand harvest one tree per plot for comparison, in Mount Vernon, WA, USA.

Year	Time (s) per Row Meter Traveled	Time (s) to Mechanically Harvest One Tree ¹	Time (min) to Hand Harvest One Tree ²
2022	3.1	5.7	4.46
2023	2.7	4.9	3.4

¹ A diesel tractor in low 1 gear moving 0.31–0.36 m·s^{−1} was used to pull the harvester, which was operated by the tractor’s 540 rpm PTO. ² In-row spacing was 1.8 m.

Overall, 8.0 spurs were removed with fruit per tree during mechanical harvest, and there was a difference among cultivars ($p = 0.03$). Only one cultivar, Sweet Alford, had high spur removal the two years it was harvested (28.7 spurs removed per tree on average) (Figure 3). When this cultivar outlier was removed from the dataset, there were no

significant differences among the remaining 15 cultivars ($p = 0.47$), and the overall mean spur removal with fruit was 6.2 per tree. A positive correlation was found between total weight of harvested fruit and total spurs removed per plot ($p < 0.001$, $R = 0.52$). The overall average number of branches broken during mechanical harvest was 1.4 per tree, and there were no differences among cultivars ($p = 0.12$) (Figure 4), but there was a difference due to year: 0.9 branches were broken per tree in 2022 and 1.8 in 2023 ($p = 0.006$).

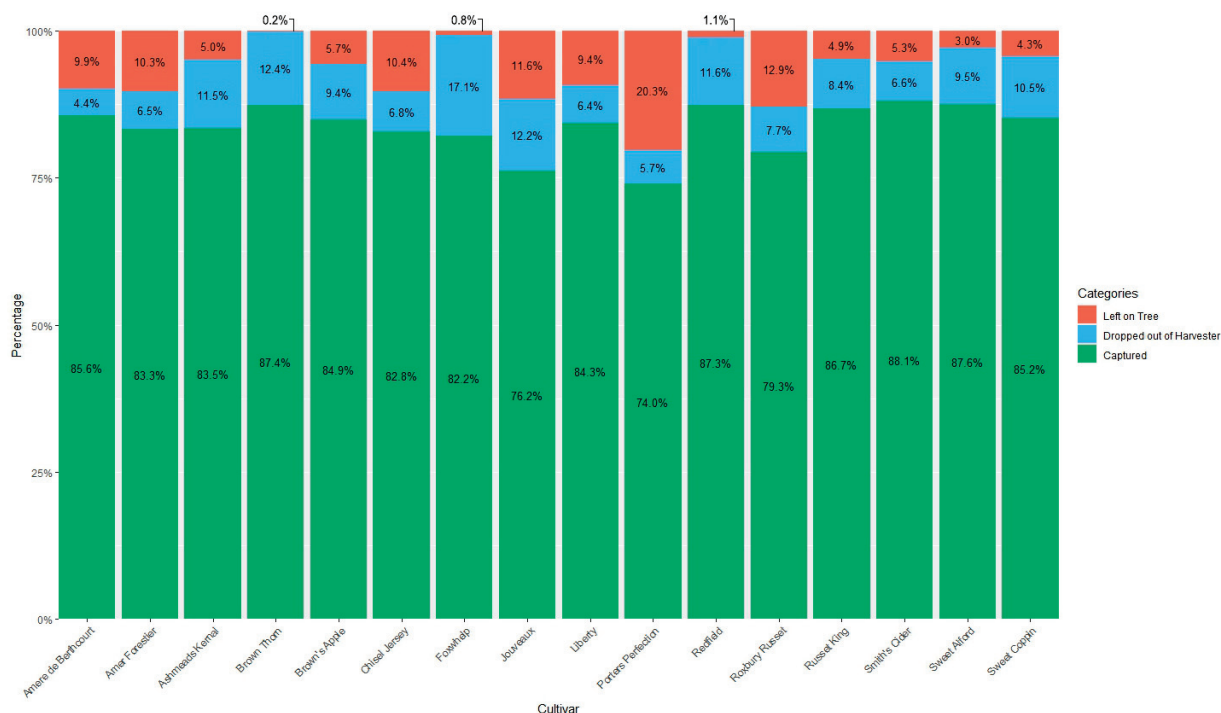


Figure 2. Harvest efficiency of the modified Oxbo-Korvan 930 over-the-row mechanical harvester (Oxbo International Co., Lynden, WA, USA) for 16 cider apple cultivars harvested in 2021, 2022, and 2023 in Mount Vernon, WA, USA.

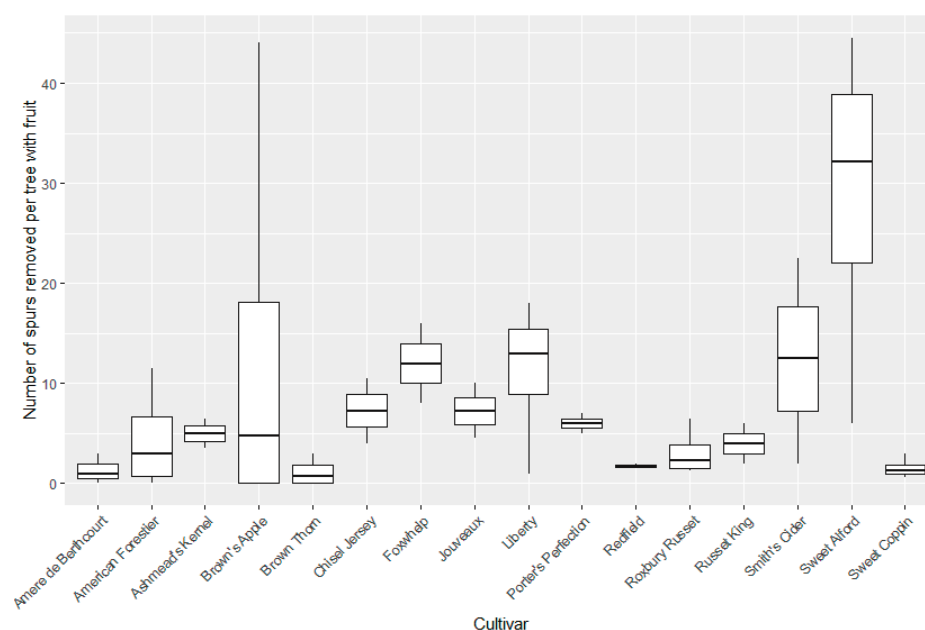


Figure 3. Spur removal with fruit per tree during mechanical harvest using the modified Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA) in 2022 and 2023 in Mount Vernon, WA, USA; boxplots display the median as the horizontal line, box indicates upper and lower quartiles, and whiskers indicate minimum and maximum.

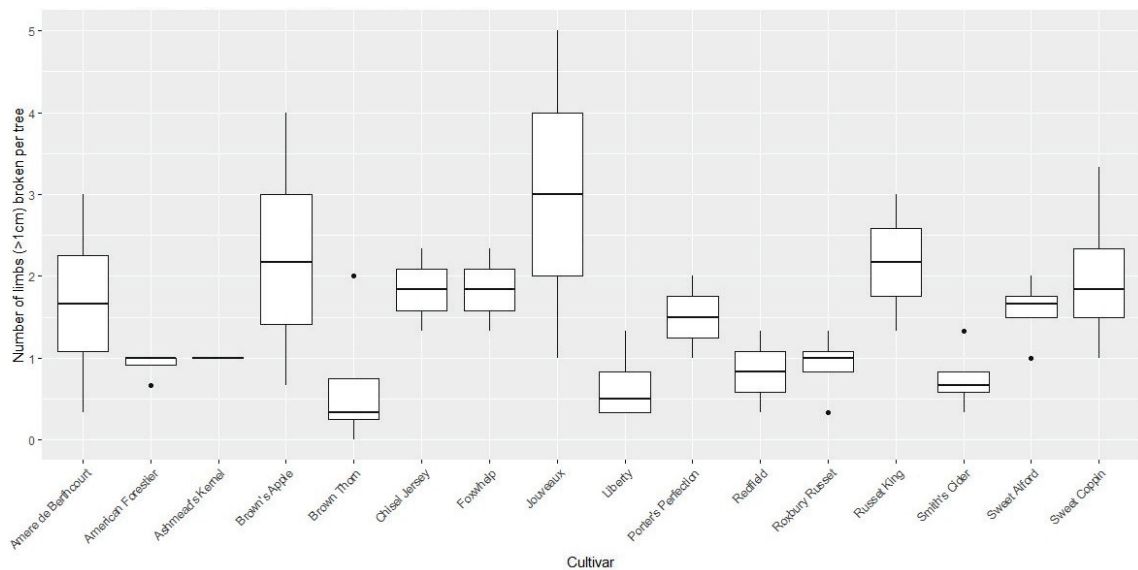


Figure 4. Branch (≥ 1 cm diameter) breakage per tree during mechanical harvest using the modified Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA) in 2022 and 2023 in Mount Vernon, WA, USA; boxplots display the median as the horizontal line, box indicates upper and lower quartiles, whiskers indicate minimum and maximum, and dots are outliers.

All of the cider apples captured by the harvester were bruised. There was a positive correlation between mean fruit weight and the percent of apples that had lacerations ($p < 0.001$, $R = 0.53$) (Figure 5). Additionally, there was a positive correlation between mean fruit diameter and the percent of apples that had lacerations ($p = 0.001$, $R = 0.39$). Juice quality characteristics (Table 3) differed between years as fruit were not fully mature at harvest in 2022 but were fully mature at harvest in 2023. In 2022, fruit matured while in cold storage and reached full maturity by 21–35 d after harvest. TSS increased 16% on average with cold storage in 2022 and 2023 ($p = 0.02$ and 0.008 , respectively), while SG increased 8% only in 2023 ($p = 0.03$) but not in 2022 ($p = 0.05$). There were no differences in pH either year after fruit were cold-stored ($p = 0.60$ and 0.25 , respectively). TA did not differ due to cold storage in 2022 ($p = 0.13$) but increased in 2023 ($p < 0.001$). There were differences in percent tannin due to cold storage in 2022 ($p < 0.001$) but not in 2023 ($p = 0.42$). In 2023, the greatest amount of fruit with rot was ~4% after 15 d in cold storage, and was ~6% after 32 d.

Table 3. Juice total soluble solids (TSS, measured as °Brix), specific gravity (SG), pH, titratable acidity (TA), and tannin (%) for cider apples mechanically harvested (modified Oxbo-Korvan 930, Oxbo International Co., Lynden, WA, USA) in 2022 and 2023 in Mount Vernon, WA, USA.

Year	Cultivar	TSS (°Brix) ¹		SG ⁴		pH ⁵		TA ⁶		Tannin (%) ⁷	
		First Press ²	Final Press ³	First Press	Final Press	First Press	Final Press	First Press	Final Press	First Press	Final Press
2022	Amere de Berthcourt	10.7	13.1	1.042	1.052	4.02	4.06	4.15	4.19	0.27	0.2
	American Forester	9.5	11.7	1.038	1.047	4.22	4.16	4.1	4.06	0.23	0.1
	Brown Thorn	10.5	11.4	1.043	1.044	4.3	4.45	4.59	4.39	0.22	0.06
	Brown's Apple	10.8	12.7	1.045	1.05	3.75	3.37	3.98	3.95	0.21	0.07
	Liberty	11.1	12.7	1.046	1.05	3.26	3.55	4.37	4.36	0.19	0.02
	Roxbury Russet	15.9	19	1.069	1.072	3.61	3.65	4.4	4.47	0.2	0.04
	Smith's Cider	10.4	11.2	1.041	1.047	3.49	3.51	4.43	4.35	0.21	0.04
	Sweet Alford	10.1	12.2	1.045	1.049	4.44	4.5	4.17	3.99	0.17	0.02
	Sweet Coppin	11.1	12.6	1.044	1.055	4.16	4.29	4.07	4.1	0.19	0.04
	<i>p</i> value ⁸	0.02		0.05		0.60		0.13		<0.001	

Table 3. Cont.

Year	Cultivar	TSS (°Brix) ¹		SG ⁴		pH ⁵		TA ⁶		Tannin (%) ⁷	
		First Press ²	Final Press ³	First Press	Final Press	First Press	Final Press	First Press	Final Press	First Press	Final Press
2023	Amere de Berthcourt	14.7	16.5	NA ⁸	1.064	3.58	3.87	4.07	3.97	NA ⁹	NA
	American Forestier	13.9	17.4	1.068	1.074	3.85	4.05	3.91	3.9	NA	NA
	Ashmead's Kernel	12.3	14.7	1.051	1.059	3.31	3.34	4.13	4.33	0.08	0.1
	Chisel Jersey	9.2	11.7	1.038	1.045	4.29	4.32	3.82	4.04	0.21	0.28
	Foxwhelp	13.5	15.1	1.055	1.059	3.23	3.25	4.15	4.32	0.17	0.21
	Jouveaux	11.8	13.6	1.047	1.052	3.95	4.06	3.88	4.13	0.16	0.18
	Liberty	11.3	12.5	1.044	1.05	3.04	3.48	4.06	4.3	0.06	0.06
	Porter's Perfection	8.7	10.3	1.035	1.041	3.32	3.46	4.16	4.3	0.22	0.25
	Redfield	11.2	12.4	1.043	1.05	3.19	3.41	4.05	4.38	0.26	0.26
	Roxbury Russet	14.1	15.8	1.058	1.063	3.57	3.63	4.01	4.32	0.06	0.07
	Russet King	13.6	15.1	1.054	1.058	3.41	3.48	4.04	4.38	0.08	0.09
	Smith's Cider	10.8	12.7	1.045	1.049	3.43	3.39	4.07	4.19	0.07	0.07
	Sweet Alford	12.2	13.5	1.049	1.056	4.64	4.48	3.75	4.03	0.07	0.08
	Sweet Coppin	14.6	17.9	1.063	1.073	3.77	4.17	3.98	3.92	NA	NA
<i>p</i> value		0.008		0.03		0.25		<0.001		0.42	

¹ Total soluble solids was measured using a digital refractometer (Palm Abbe model #PA201; MISCO, Cleveland, OH, USA). ² The first press occurred within 3 d of harvest. ³ The final press occurred after 15–35 d in cold storage, when fruit reached 7–8 on the Cornell Starch Iodine Index. ⁴ Specific gravity was measured using a precision hydrometer (Bellwether; VeeGee Scientific, Kirkland, WA, USA) suspended in ~200 mL of juice. ⁵ pH was measured using a digital pH meter (Ohaus Starter 5000; Ohaus Corporation, Parsippany, NJ, USA). ⁶ Titratable acidity was measured using an auto-titrator (HI932; Hanna Instruments, Woonsocket, RI, USA). ⁷ Tannin (expressed as tannic acid equivalents) was measured using the Löwenthal permanganate titration method [20,21]. ⁸ *p*-values were calculated for first to final press, for all cultivars combined. ⁹ NA = data not available due to low juice volume.

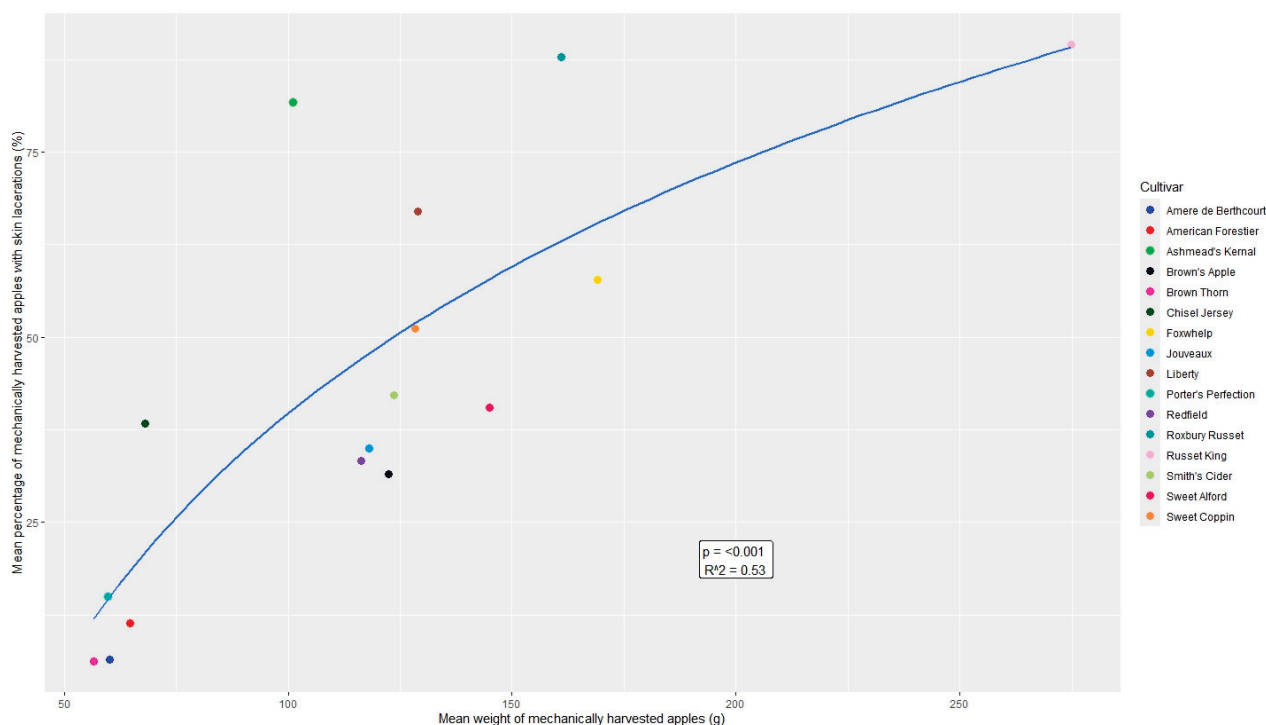


Figure 5. Correlation (blue line) between percent of fruit with lacerations and weight per fruit of 16 cider apple cultivars that were mechanically harvested using the modified Oxbo-Korvan 930 harvester (Oxbo International Co., Lynden, WA, USA) in 2022 and 2023 in Mount Vernon, WA, USA.

4. Discussion

The harvest efficiency of the modified Oxbo-Korvan 930 was ~82% (range was 70–95%) for the 16 cultivars in this study. Most of the apples that were dropped by the harvester (~9% of total yield) bounced out the front of the harvester or were shaken off before the harvester was positioned over the tree, due to the harvester shaking the trellis wires. Softening the surface of the catch plates, as has been done for mechanical harvesters for blueberries [22], would reduce the number of fruit bouncing out of the harvester. Extending the catch plates further in front of the harvester to catch apples and then funnel them back to the conveyor belts would also increase the machine's capture efficiency. The time to harvest one tree with the modified Oxbo-Korvan 930 was more than 40-fold less than by hand. Not including the time to turn around and offload/reload harvest crates, three individuals could harvest 10 ha of cider apples in less than 22.5 h with the modified Oxbo-Korvan 930 harvester.

Larger cider apples, by weight and diameter, had more lacerations due to mechanical harvest compared to smaller apples. Lacerations serve as entry points for bacteria and fungi to cause decay and rot, and thus, larger cider apples may not store as well as smaller apples after mechanical harvest. Yet after 15 d in cold storage, the greatest amount of fruit removed due to rot was ~4%, and was only ~6% after 32 d. Typically, if cider apples are harvested at maturity, they are pressed within a few days or weeks. Thus, yield loss due to fruit damaged due to mechanical harvest and developing rot would be minimal. Further, fermentation serves as a kill-step for unwanted microorganisms present in the decay of damaged fruit [12].

Spur removal during mechanical harvest was minimal. However, longer term studies are needed to determine if cultivars with high spur abscission are suitable for over-the-row mechanical harvest. The number of branches broken by the harvester was also minimal, and was consistent among cultivars and years. Branch breakage could be minimized by training new branches to be horizontal. We observed that branches that grew at steep angles, rather than horizontally, were more likely to break as they had less flexibility to move through the rotating, horizontal spindles of the harvester. It may be important to apply appropriate pesticides after harvest to reduce the introduction of disease through damaged wood.

The juice quality of mechanically harvested cider apples was minimally affected due to storage time except that sugars increased across all cultivars, as expected. While tannin concentration decreased as fruit reached full maturity [23,24], a fully mature cider apple with higher levels of sugars is more desirable for the fermentation process in cidermaking. Acidity, measured through pH and titratable acidity, was largely unaffected by time in cold storage in this study. This may have been due to the specific cultivars examined or the minimal time fruit were in storage (<35 d), as others have reported that acidity in apples tends to decrease with time in cold storage [25–27].

5. Conclusions

Mechanical harvest using the modified Oxbo-Korvan 930 required 40-fold less time than hand harvest, which is significantly more labor-efficient. The picking efficiency of the modified Oxbo-Korvan 930 harvester could be increased from 82% to >90% with relatively simple harvester improvements, such as softening the catch plates surface and extending the catch plates in front of the harvester. All of the fruit that was shaken from the trees but not captured by the harvester either bounced out of the front of the harvester or were shaken off the tree ahead of the catch plates. Softening catch plates and other hard surfaces inside the harvester to reduce fruit bouncing and bruising has been implemented for blueberry mechanical harvesters that are similar to the harvester technology used in our study. Loss due to rot of mechanically harvested fruit during 32 d post-harvest cold storage

was minimal (4–6%) and juice quality did not decline. These results demonstrate that the modified Oxbo-Korvan 930 harvester is suitable for cider apples.

Optimizing the orchard system for over-the-row harvest includes using rootstocks to produce tree height of approximately 3.0 m, pruning to a fruiting wall, and avoiding trellising systems if possible. Trellis systems add significant cost to orchard establishment and the wires are shaken by the harvester, leading to fruit loss during harvest. Cultivars that are best suited for mechanical harvest should not have pre-mature fruit drop, so they are suitable for a one-time harvest. Small-sized fruit could be especially well suited to mechanical harvest. First, they had less damage from mechanical harvest than larger fruit. Second, the amount of time to hand harvest small fruit can be up to four times greater than for larger fruit [8].

More research is needed to assess yield and tree health following multiple years of mechanical harvest. Testing is also needed to evaluate viability of over-the-row harvest in a commercial cider apple orchard setting. There is a need to assess costs and returns of medium- and high-density fruiting wall orchard systems combined with over-the-row harvest, and compare with traditional cider apple orchards with standard-size trees and current harvest techniques.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11091123/s1>. Supplemental Information: History of the Modified Oxbo-Korvan 930 Used in This Research Study

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Conflicts of Interest: Author Aidan Kendall was employed by the company 2Highland Economics LLC, Missoula, MT. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Article

CND and DRIS Methods for Nutritional Diagnosis in ‘Hass’ Avocado Production

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Abstract

The production of fruit crops plays a vital role in the agricultural sector, contributing significantly to the social and economic development of rural communities. In Brazil, fruit production is diverse due to favorable edaphoclimatic conditions, with avocado (*Persea americana* Mill.) emerging as an important crop. Its production continues to expand in both cultivated areas and yield, making it a key export to non-producing countries. However, despite its importance, nutritional management information, crucial for achieving high yields, remains limited. Current guidelines on nutrition monitoring are outdated, general, and based on data from other countries with different edaphoclimatic conditions, making them not directly applicable to Brazilian orchards. Furthermore, outdated nutritional information becomes less reliable over time, as climate change alters soil conditions and crop nutrient concentrations and requirements, reinforcing the need for the establishment of up-to-date and specific nutritional information. This study aimed to establish nutritional standards for ‘Hass’ avocado production using the Diagnosis and Recommendation Integrated System (DRIS) and Compositional Nutrient Diagnosis (CND) methodologies, and to define sufficiency ranges (SRs) and Critical Levels (CLs) for both macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (B, Cu, Fe, Mn, and Zn). The analyses were based on yield (t ha⁻¹) and leaf nutrient content data from commercial orchards, with datasets divided into younger (4–9 years) and older (10–26 years) plant groups. The DRIS effectively established nutritional standards for younger plants, explaining 11% of yield variation through nutritional balance. CND, in turn, was effective for both groups, accounting for 14% of yield variation and outperforming DRIS in associating nutritional status with productivity. SRs and CLs for ‘Hass’ avocado production were defined using both DRIS and CND. Together, these indices and diagnostic parameters offer valuable tools for enhancing nutritional monitoring and fertilization strategies in Brazil. Notably, SRs and CLs varied according to plant age.

Keywords: nutritional balance; nutritional status; leaf analysis; orchard fertilization; *Persea americana* Mill.

1. Introduction

Brazil produces a wide variety of fruits due to the diverse climate conditions across the country [1], making it the third largest producer globally, with approximately 40 million tons of fresh fruit produced in 2020 [2]. Among these, avocado (*Persea americana* Mill.) has seen significant growth in both yield and cultivated area, positioning Brazil as the seventh largest global producer, with 338 thousand tons produced in 2022 [3].

The opening of international markets in response to increasing avocado consumption has driven global demand, particularly in non-producing countries such as Japan, Canada, France, the Netherlands, and England [4]. This demand continues to rise due to the health benefits of avocados, which contribute to weight loss, combat oxidative stress, and reduce inflammation [5,6]. This trend presents significant opportunities for Brazilian producers. Recently, it was reported that avocado became the 11th most exported fruit from Brazil [7], with 25.6 thousand tons destined for international markets in 2023, more than double the amount exported in 2022 [8], primarily the ‘Hass’ cultivar.

In Brazil, São Paulo is the leading state in avocado production, accounting for approximately 43.4% of the country’s total output [9]. Although the potential for avocado production is estimated to exceed 30.0 t ha⁻¹ [10], as also observed in countries like Samoa, El Salvador, and Panama [3], the average productivity in Brazil remains at 18.6 t ha⁻¹ [3,9], indicating a gap and presenting an opportunity to further increase productivity in Brazilian orchards.

Achieving high yields is influenced by several biotic and abiotic factors [11]. Additionally, plant growth and development depend on nutritional balance, as an excess or deficiency of nutrients can negatively impact productivity and crop quality [12]. Assessing the nutritional status through leaf analysis is crucial for planning fertilization programs, thereby enhancing productivity [13].

Despite the potential for avocado production in Brazil, studies on mineral nutrition management are limited. Information on the upper and lower nutrient limits for optimal avocado cultivation has not been updated or developed for specific cultivars. The only reference available to avocado producers is the sufficiency ranges in Teixeira et al. [14], which have remained unchanged since the last publication [15] and, in addition to not specifying cultivars, are similar to those published by Embleton and Jones [16].

Moreover, climate change represents a growing challenge for agricultural systems, with projections indicating a potential reduction in global crop yields [17,18]. Nutrient management is particularly affected by climatic variability, which can alter plant physiological processes and soil dynamics [19–21]. These factors underscore the increasing need for updated and comprehensive nutritional guidelines to sustain crop performance under changing environmental conditions.

Given this, the use of diagnostic methodologies such as the Diagnosis and Recommendation Integrated System (DRIS) [22], a bivariate method, and Compositional Nutrient Diagnosis (CND) [23], a multivariate method, emerges as a valuable tool. These methods not only determine crop nutritional standards but can also generate sufficiency ranges (SRs) and Critical Levels (CLs) [24], while considering nutrient interactions [25], distinguishing them from the classical univariate method and allowing for a more precise assessment of plants’ nutritional status, especially when employed with compositional data from commercial areas [26].

Applying the DRIS and CND diagnostic methods to compositional data from commercial ‘Hass’ avocado orchards will allow the establishment of accurate nutritional standards, including SRs and CLs for macro- and micronutrients, thereby supporting more effective nutrient management and enhancing yield performance under Brazilian conditions. Therefore, the objective of this study was to establish the DRIS and CND standards in avocado

‘Hass’ production and generate the SRs and CLs of macro- and micronutrients’ leaf nutrient content for adequate ‘Hass’ avocado production.

2. Materials and Methods

2.1. Data Collection

The data used in this study consisted of leaf nutrient content (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn) and yield (t ha^{-1}), collected from 2021 to 2024 across four avocado ‘Hass’ production farms located in Avaré, Bernardino de Campos, Charqueada, and Itai, São Paulo, Brazil (Figure 1). The predominant soil types in these regions are Argisol and Latosol [27]. The climate of the region is classified according to the Köppen system as Cfa in Avaré, Bernardino de Campos, and Itai and as Cwa in Charqueada. Avaré has a mean annual temperature of 19.6 °C and rainfall of 1315.8 mm; Bernardino de Campos, 20.2 °C and 1334.6 mm; Charqueada, 20.4 °C and 1339.7 mm; and Itai, 19.7 °C and 1235.3 mm [28].



Figure 1. Geographical distribution of ‘Hass’ avocado production farms used for data collection.

The dataset consisted of commercial-age rainfed ‘Hass’ avocado plants grafted onto seed-derived rootstocks, with plant ages ranging from 4 to 26 years (176 commercial plots). Due to age differences between the plants, two separate datasets were created and analyzed: one for the 4–9-year-old plants (123 plots) with plants spaced at 7 m × 4 m, 7 m × 5 m, 8 m × 4 m, 8 m × 5 m, and 8 m × 6 m, and another for the 10–26-year-old plants (53 plots) with plants spaced at 7 m × 5 m, 8 m × 6 m, 9 m × 4.5 m, and 10 m × 5 m. To confirm the need for dividing the datasets by plant age, a *t*-test was performed on the multivariate nutrient relationship obtained in the CND calculation.

Leaf sampling followed the protocol established by Koen and Plessis [29], collecting four complete (leaf + petiole) fully expanded healthy leaves from each quadrant of the plant, located at the 3rd node of fruitless shoots without signs of new vegetative growth flushes. After sample collection, the leaves were dried in a forced-air oven at 60 °C ± 5 °C until reaching a constant weight, then ground and passed through a 20-mesh sieve. N concentration was determined using the micro-Kjeldahl method. K, Ca, Mg, Cu, Fe, Mn, and Zn were quantified by atomic absorption spectrophotometry, while P and B were determined by colorimetry and S by turbidimetry [30]. Yield (t ha^{-1}) for each plot was recorded and used as the productivity indicator for the CND and DRIS calculations.

2.2. DRIS—Calculations and Statistical Analyses

Nutrient content for macronutrients was expressed in g kg^{-1} , while micronutrients were expressed in mg kg^{-1} . The datasets consisted of 123 plots for 4–9-year-old plants and 53 plots for 10–26-year-old plants. The normality of each yield distribution was tested using the Kolmogorov–Smirnov test—4–9-year-old plants ($p > 0.24$) and 10–26-year-old plants ($p > 0.59$)—indicating that the data followed a normal distribution [31]. Each dataset was then divided into high-yield (reference population) and low-yield populations, using the mean yield (t ha^{-1}) as the criterion [32]. The mean and standard deviation (DRIS norms) of the log-transformed bivariate relationships for the reference population were calculated [33], followed by the determination of nutrient ratio functions [34]:

$$f\left(\frac{A}{B}\right) = \left[\left(\frac{A}{B}\right) - \left(\frac{a}{b}\right) \right] \cdot \left(\frac{c}{s}\right), \quad (1)$$

where $\left(\frac{A}{B}\right)$ represents the dual relationship between nutrient content in the sample; $\left(\frac{a}{b}\right)$ is the average of the dual relationships for nutrient content in the high-yield population; s is the standard deviation of the dual relationships in the high-yield population; and c is the sensitivity coefficient (set to 1).

Subsequently, DRIS indices were calculated for each nutrient [22]:

$$IA = \frac{\sum f\left(\frac{A}{B}\right) - \sum f\left(\frac{B}{A}\right)}{n + m}, \quad (2)$$

where IA is the DRIS index for nutrient A ; $f\left(\frac{A}{B}\right)$ is the directly proportional function between two nutrients; $f\left(\frac{B}{A}\right)$ is the inversely proportional function between two nutrients; n is the number of direct relationships assessed; and m is the number of inverse relationships assessed.

The Nutrient Balance Index (NBI) was calculated as the sum of the absolute values of the DRIS indices for each nutrient [22]:

$$\text{NBI} = |IN| + |IP| + |IK| + \dots + |INa|, \quad (3)$$

The Mean Nutrient Balance Index (NBIm) was then calculated by dividing the NBI by the total number of nutrients analyzed (n):

$$\text{NBIm} = \left(\frac{\text{NBI}}{n} \right), \quad (4)$$

Finally, the Critical Levels, along with the lower and upper limits for each dataset, were determined through regression analysis between each nutrient's content and its corresponding NBI [35,36].

2.3. CND—Calculations and Statistical Analyses

Nutrient content for both macronutrients and micronutrients is expressed in mg kg^{-1} . Outlier exclusion was performed using the Mahalanobis distance ($p < 0.01$) for both datasets [37]. After excluding outliers, the number of plots for the CND calculations was 120 for the 4–9-year-old plants (97.6% of the original dataset) and 45 for the 10–26-year-old plants (85% of the original dataset). Normality tests using the Kolmogorov–Smirnov test indicated that the data followed a normal distribution: 4–9-year-old ($p > 0.24$) and 10–26-year-old ($p > 0.57$) [31]. Each dataset was then divided into high-yield (reference

population) and low-yield populations, with the cumulative distribution function serving as the criterion [38].

The CND calculation for each dataset was performed following the procedure to obtain R , where leaf tissue is regarded as a closed system consisting of both quantified nutrients and non-quantified components, represented by R [23]:

$$R = 1,000,000 - \sum_{i=1}^n Nt_i, \quad (5)$$

where R represents all components not determined in the dry matter, expressed in mg kg^{-1} ; 1,000,000 represents the total dry matter in mg kg^{-1} ; and Nt_i is the quantity of each nutrient in the dry matter, also expressed in mg kg^{-1} .

The geometric mean was calculated to ensure that all components included in the R value were accounted for in the sample analysis [23]:

$$G = \left[\left(\prod_{i=1}^n Nt_i \right) \cdot R \right]^{\frac{1}{(n+1)}}, \quad (6)$$

where G is the geometric mean of nutrient content in the dry matter; Nt_i is the content of each nutrient in the dry matter; R is the value of non-determined components; and n is the number of nutrients analyzed.

Next, the multivariate relationship between the nutrients was determined using the Napierian logarithm of nutrient content to improve the data distribution [33]:

$$V_i = \ln \left(\frac{Nt_i}{G} \right), \quad (7)$$

where V_i represents the multivariate relationship between the nutrients; G is the geometric mean of the nutrient content in the dry matter; and Nt_i is the content of each nutrient in the dry matter.

The CND indices were then calculated:

$$I_i = \frac{(V_I - V_i)}{\sigma_i} \quad (8)$$

where I_i is the Nutritional Balance Index for nutrient i ; V_I is the value of the multivariate relationship I for the evaluated sample; V_i is the mean value of the multivariate relationship i in the reference population; and σ_i is the standard deviation of the variable i in the reference population.

The Nutrient Imbalance Index (CND- r^2) was determined by summing the individual indices. Critical Levels, along with the lower and upper limits for each dataset, were established through regression analysis of each nutrient's content against its corresponding CND index [35,36].

2.4. Statistical Analysis

The Pearson correlation analysis, regression tests, and t -test were performed using the R software (version 4.3.3) [39].

3. Results

3.1. T-Test of the Multivariate Nutrient Relationships

Differences in nutritional balance between the two datasets were observed through a t -test applied to the multivariate relationships among nutrients obtained from the CND calculation (Table 1). All parameters showed significant differences between the datasets, except for K, Fe, and Mn (Table 1).

Table 1. Mean $\pm$ standard deviation (SD) and *t*-test of multivariate relationship between nutrients (CND standards) for leaf nutrient content in relation to yield in the 4–9-year-old and 10–26-year-old plant datasets for ‘Hass’.

Variables	4–9-Year-Old (<i>n</i> = 120)	10–26-Year-Old (<i>n</i> = 45)	<i>t</i> -Test
	Mean $\pm$ SD	Mean $\pm$ SD	<i>p</i> -Value
V_N	2.94 $\pm$ 0.18	2.83 $\pm$ 0.20	0.0005 ***
V_P	0.22 $\pm$ 0.12	0.08 $\pm$ 0.20	<0.0001 ***
V_K	1.91 $\pm$ 0.27	1.92 $\pm$ 0.26	0.8013 ns
V_{Ca}	2.87 $\pm$ 0.25	2.64 $\pm$ 0.29	<0.0001 ***
V_{Mg}	1.69 $\pm$ 0.20	1.36 $\pm$ 0.21	<0.0001 ***
V_S	0.67 $\pm$ 0.20	0.60 $\pm$ 0.16	0.0405 *
V_B	−3.48 $\pm$ 0.31	−3.31 $\pm$ 0.44	0.0087 **
V_{Cu}	−3.08 $\pm$ 0.56	−2.84 $\pm$ 0.59	0.0148 *
V_{Fe}	−2.22 $\pm$ 0.23	−2.25 $\pm$ 0.27	0.4923 ns
V_{Mn}	−1.04 $\pm$ 0.49	−1.21 $\pm$ 0.62	0.0760 ns
V_{Zn}	−3.86 $\pm$ 0.20	−3.44 $\pm$ 0.44	<0.0001 ***
V_R	3.38 $\pm$ 0.31	3.62 $\pm$ 0.32	<0.0001 ***

ns: non-significant; significancy levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. V_i : CND standard for the i nutrient. V_R : CND standard for the non-quantified components in dry matter.

3.2. Correlation Analysis

In the 4–9-year-old plants dataset, 27 significant correlations were identified. All nutrients exhibited either positive or negative correlations with one another, and yield showed a negative correlation with N, Fe, and Mn, while being positively correlated with Mg (Figure 2a). In the 10–26-year-old plants dataset, 23 significant correlations were observed. All nutrients, except N, were correlated with each other, and yield had a negative correlation with Ca, Mg, and Mn, while being positively correlated with B, Cu, and Zn (Figure 2b).

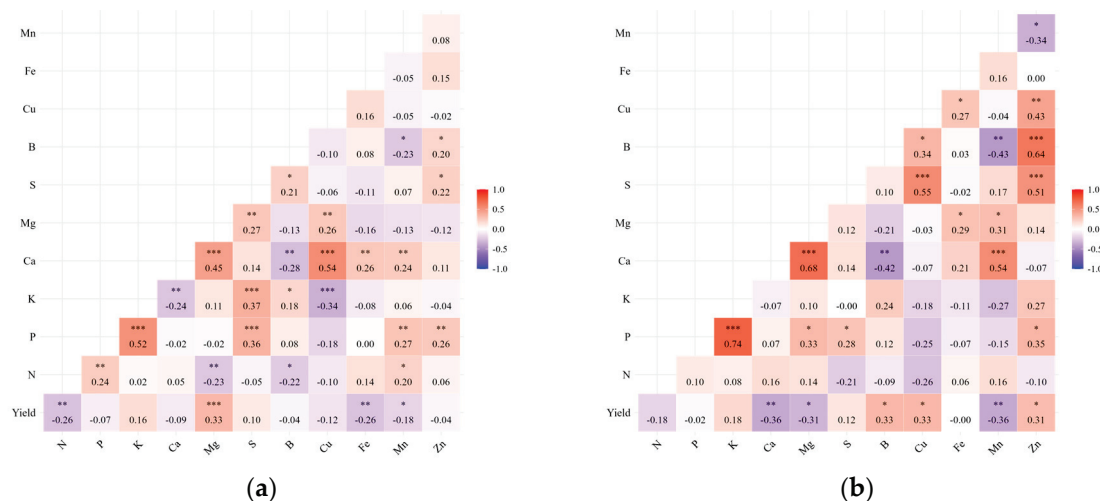


Figure 2. Pearson's correlation matrix between leaf nutrient content and yield ($t\ ha^{-1}$) in ‘Hass’: (a) 4–9-year-old plants ($n = 123$); (b) 10–26-year-old plants ($n = 53$). Significancy levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

3.3. DRIS Standards Establishment

The datasets were divided into high- and low-yield populations. In the 4–9-year-old plants' high-yield population, the yield ranged from 9.0 to 33.6 $t\ ha^{-1}$, with a mean productivity of 19.2 $t\ ha^{-1}$, and 30% of the dataset was classified as high-yield. In the

10–26-year-old plants' high-yield population, the yield varied from 9.0 to 33.3 t ha^{−1}, with a mean productivity of 15.1 t ha^{−1}, and 43.4% of the plots were classified as high-yield within the entire dataset (Table 2).

Table 2. Minimum (Min.), maximum (Max.), mean, standard deviation (SD), and coefficient of variation (CV) for leaf nutrient content and yield in the 4–9-year-old and 10–26-year-old plant datasets for 'Hass', categorized into high- and low-yield populations, for the DRIS calculation.

4–9-Year-Old—High-Yield Population (<i>n</i> = 37)						10–26-Year-Old—High-Yield Population (<i>n</i> = 23)					
Variables	Min.	Max.	Mean	SD	CV	Variables	Min.	Max.	Mean	SD	CV
Yield (t ha ^{−1})	9.0	33.6	19.2	8.0	41.9	Yield (t ha ^{−1})	9.2	33.3	15.1	5.8	38.2
N (g kg ^{−1})	15.6	33.1	21.5	4.0	18.6	N (g kg ^{−1})	15.8	47.6	21.2	6.7	31.6
P (g kg ^{−1})	1.1	1.9	1.5	0.2	12.5	P (g kg ^{−1})	1.0	3.5	1.4	0.5	38.6
K (g kg ^{−1})	4.7	13.3	9.1	2.3	25.8	K (g kg ^{−1})	5.7	23.5	9.8	3.9	39.7
Ca (g kg ^{−1})	13.6	30.0	20.3	4.1	20.2	Ca (g kg ^{−1})	6.6	28.3	15.4	4.1	26.9
Mg (g kg ^{−1})	4.4	9.5	7.0	1.3	18.1	Mg (g kg ^{−1})	2.7	8.1	4.5	1.1	23.8
S (g kg ^{−1})	1.3	3.2	2.5	0.5	20.2	S (g kg ^{−1})	0.1	3.3	2.2	0.7	34.1
B (mg kg ^{−1})	19.1	72.7	37.3	15.3	41.1	B (mg kg ^{−1})	18.8	83.9	54.8	21.2	38.7
Cu (mg kg ^{−1})	8.2	123.0	51.4	31.5	61.2	Cu (mg kg ^{−1})	12.0	232.5	94.9	71.7	75.6
Fe (mg kg ^{−1})	83.0	201.3	117.6	27.3	23.2	Fe (mg kg ^{−1})	53.0	258.0	138.6	60.5	43.6
Mn (mg kg ^{−1})	210.5	1012.0	427.2	207.7	48.6	Mn (mg kg ^{−1})	80.0	870.0	294.3	164.7	56.0
Zn (mg kg ^{−1})	16.9	57.0	26.1	7.4	28.4	Zn (mg kg ^{−1})	20.0	102.7	49.8	24.0	48.1
4–9-year-old—Low-Yield Population (<i>n</i> = 86)						10–26-year-old—Low-Yield Population (<i>n</i> = 30)					
Variables	Min.	Max.	Mean	SD	CV	Variables	Min.	Max.	Mean	SD	CV
Yield (t ha ^{−1})	0.9	8.8	4.6	1.9	41.9	Yield (t ha ^{−1})	0.2	8.7	4.6	2.6	55.5
N (g kg ^{−1})	18.2	34.4	23.4	3.4	14.3	N (g kg ^{−1})	16.2	30.2	21.0	3.5	16.7
P (g kg ^{−1})	1.1	2.0	1.5	0.2	12.4	P (g kg ^{−1})	0.9	2.1	1.3	0.2	17.6
K (g kg ^{−1})	4.8	14.6	8.0	2.3	28.6	K (g kg ^{−1})	5.1	18.3	8.5	2.8	33.5
Ca (g kg ^{−1})	9.8	41.2	22.3	7.4	33.3	Ca (g kg ^{−1})	6.2	30.6	18.5	6.0	32.7
Mg (g kg ^{−1})	3.1	9.2	6.3	1.3	20.4	Mg (g kg ^{−1})	2.4	7.6	4.9	1.2	25.2
S (g kg ^{−1})	1.2	3.2	2.3	0.5	21.1	S (g kg ^{−1})	0.6	3.2	2.0	0.6	28.0
B (mg kg ^{−1})	21.8	79.5	39.6	13.6	34.4	B (mg kg ^{−1})	20.7	104.2	44.8	20.7	46.2
Cu (mg kg ^{−1})	2.6	251.0	69.3	46.9	67.6	Cu (mg kg ^{−1})	10.3	150.0	62.9	33.7	53.6
Fe (mg kg ^{−1})	69.0	263.0	142.2	38.6	27.1	Fe (mg kg ^{−1})	58.0	233.6	130.1	39.9	30.7
Mn (mg kg ^{−1})	142.0	1639.0	512.7	320.3	62.5	Mn (mg kg ^{−1})	94.0	2328.0	499.3	427.4	85.6
Zn (mg kg ^{−1})	14.0	39.0	25.6	5.5	21.4	Zn (mg kg ^{−1})	18.0	109.3	40.8	25.2	61.7

In general, for both datasets and for both high- and low-yield populations, the coefficient of variation was higher for micronutrients compared to macronutrients (Table 2).

The mean and standard deviation of the log-transformed nutrient ratios for leaf content in the high-yield populations of both the 4–9-year-old and 10–26-year-old plant datasets were calculated (Tables 3 and 4).

Linear and quadratic regression analyses were performed between the mean Nutrient Balance Index (NBIm) and yield for both datasets (Figure 3). For the 4–9-year-old plant dataset, the regression was statistically significant, with an R^2 of 11%, indicating that 11% of the variation in yield for 'Hass' avocado production can be explained by the plant's nutritional status (Figure 3a). In contrast, the 10–26-year-old plant dataset showed no significant correlation, with an R^2 of 4%, suggesting that for these plots, yield variation is not solely dependent on the nutritional balance of the avocado trees but also influenced by other field factors (Figure 3b).

Table 3. Mean ($\bar{x}$) and standard deviation (SD) of log-transformed ratios (R) (DRIS standards) for leaf nutrient content in the high-yield population of 4–9-year-old ‘Hass’ plants.

4–9-Year-Old—High-Yield Population ($n = 37$)										
R	N/P	N/K	N/Ca	N/Mg	N/S	N/B	N/Cu	N/Fe	N/Mn	N/Zn
$\bar{x}$	1.16	0.38	0.03	0.49	0.94	−0.21	−0.31	−0.73	−1.26	−0.08
SD	0.08	0.12	0.09	0.12	0.11	0.20	0.31	0.10	0.18	0.12
R	P/N	P/K	P/Ca	P/Mg	P/S	P/B	P/Cu	P/Fe	P/Mn	P/Zn
$\bar{x}$	−1.16	−0.78	−1.14	−0.67	−0.22	−1.37	−1.47	−1.90	−2.43	−1.24
SD	0.08	0.09	0.09	0.09	0.08	0.16	0.29	0.11	0.19	0.10
R	K/N	K/P	K/Ca	K/Mg	K/S	K/B	K/Cu	K/Fe	K/Mn	K/Zn
$\bar{x}$	−0.38	0.78	−0.36	0.11	0.56	−0.60	−0.69	−1.12	−1.65	−0.46
SD	0.12	0.09	0.13	0.14	0.11	0.18	0.34	0.16	0.22	0.14
R	Ca/N	Ca/P	Ca/K	Ca/Mg	Ca/S	Ca/B	Ca/Cu	Ca/Fe	Ca/Mn	Ca/Zn
$\bar{x}$	−0.03	1.14	0.36	0.46	0.92	−0.24	−0.33	−0.76	−1.29	−0.10
SD	0.09	0.09	0.13	0.10	0.09	0.19	0.31	0.12	0.16	0.13
R	Mg/N	Mg/P	Mg/K	Mg/Ca	Mg/S	Mg/B	Mg/Cu	Mg/Fe	Mg/Mn	Mg/Zn
$\bar{x}$	−0.49	0.67	−0.11	−0.46	0.45	−0.70	−0.79	−1.22	−1.75	−0.57
SD	0.12	0.09	0.14	0.10	0.11	0.18	0.29	0.14	0.21	0.14
R	S/N	S/P	S/K	S/Ca	S/Mg	S/B	S/Cu	S/Fe	S/Mn	S/Zn
$\bar{x}$	−0.94	0.22	−0.56	−0.92	−0.45	−1.15	−1.25	−1.68	−2.20	−1.02
SD	0.11	0.08	0.11	0.09	0.11	0.16	0.32	0.13	0.18	0.12
R	B/N	B/P	B/K	B/Ca	B/Mg	B/S	B/Cu	B/Fe	B/Mn	B/Zn
$\bar{x}$	0.21	1.37	0.60	0.24	0.70	1.15	−0.09	−0.52	−1.05	0.14
SD	0.20	0.16	0.18	0.19	0.18	0.16	0.30	0.19	0.27	0.16
R	Cu/N	Cu/P	Cu/K	Cu/Ca	Cu/Mg	Cu/S	Cu/B	Cu/Fe	Cu/Mn	Cu/Zn
$\bar{x}$	0.31	1.47	0.69	0.33	0.79	1.25	0.09	−0.43	−0.96	0.23
SD	0.31	0.29	0.34	0.31	0.29	0.32	0.30	0.28	0.38	0.32
R	Fe/N	Fe/P	Fe/K	Fe/Ca	Fe/Mg	Fe/S	Fe/B	Fe/Cu	Fe/Mn	Fe/Zn
$\bar{x}$	0.73	1.90	1.12	0.76	1.22	1.68	0.52	0.43	−0.53	0.66
SD	0.10	0.11	0.16	0.12	0.14	0.13	0.19	0.28	0.18	0.13
R	Mn/N	Mn/P	Mn/K	Mn/Ca	Mn/Mg	Mn/S	Mn/B	Mn/Cu	Mn/Fe	Mn/Zn
$\bar{x}$	1.26	2.43	1.65	1.29	1.75	2.20	1.05	0.96	0.53	1.19
SD	0.18	0.19	0.22	0.16	0.21	0.18	0.27	0.38	0.18	0.20
R	Zn/N	Zn/P	Zn/K	Zn/Ca	Zn/Mg	Zn/S	Zn/B	Zn/Cu	Zn/Fe	Zn/Mn
$\bar{x}$	0.08	1.24	0.46	0.10	0.57	1.02	−0.14	−0.23	−0.66	−1.19
SD	0.12	0.10	0.14	0.13	0.14	0.12	0.16	0.32	0.13	0.20

Table 4. Mean ($\bar{x}$) and standard deviation (SD) of log-transformed ratios (R) (DRIS standards) for leaf nutrient content in the high-yield population of 10–26-year-old ‘Hass’ plants.

10–26-Year-old—High-Yield Population ($n = 23$)										
R	N/P	N/K	N/Ca	N/Mg	N/S	N/B	N/Cu	N/Fe	N/Mn	N/Zn
$\bar{x}$	1.20	0.34	0.14	0.67	1.03	−0.39	−0.53	−0.79	−1.10	−0.34
SD	0.14	0.17	0.15	0.13	0.32	0.24	0.44	0.21	0.24	0.22
R	P/N	P/K	P/Ca	P/Mg	P/S	P/B	P/Cu	P/Fe	P/Mn	P/Zn
$\bar{x}$	−1.20	−0.85	−1.06	−0.53	−0.17	−1.58	−1.72	−1.99	−2.30	−1.54
SD	0.14	0.10	0.17	0.12	0.28	0.26	0.44	0.24	0.30	0.19
R	K/N	K/P	K/Ca	K/Mg	K/S	K/B	K/Cu	K/Fe	K/Mn	K/Zn
$\bar{x}$	−0.34	0.85	−0.21	0.32	0.68	−0.73	−0.87	−1.13	−1.45	−0.69
SD	0.17	0.10	0.17	0.14	0.31	0.27	0.42	0.23	0.29	0.21
R	Ca/N	Ca/P	Ca/K	Ca/Mg	Ca/S	Ca/B	Ca/Cu	Ca/Fe	Ca/Mn	Ca/Zn
$\bar{x}$	−0.14	1.06	0.21	0.53	0.89	−0.53	−0.67	−0.93	−1.24	−0.48
SD	0.15	0.17	0.17	0.07	0.31	0.29	0.39	0.20	0.17	0.23

Table 4. Cont.

10–26-Year-old—High-Yield Population (<i>n</i> = 23)										
R	Mg/N	Mg/P	Mg/K	Mg/Ca	Mg/S	Mg/B	Mg/Cu	Mg/Fe	Mg/Mn	Mg/Zn
$\bar{x}$	−0.67	0.53	−0.32	−0.53	0.36	−1.05	−1.19	−1.46	−1.77	−1.01
SD	0.13	0.12	0.14	0.07	0.32	0.27	0.42	0.19	0.21	0.22
R	S/N	S/P	S/K	S/Ca	S/Mg	S/B	S/Cu	S/Fe	S/Mn	S/Zn
$\bar{x}$	−1.03	0.17	−0.68	−0.89	−0.36	−1.41	−1.55	−1.82	−2.13	−1.37
SD	0.32	0.28	0.31	0.31	0.32	0.34	0.35	0.37	0.38	0.29
R	B/N	B/P	B/K	B/Ca	B/Mg	B/S	B/Cu	B/Fe	B/Mn	B/Zn
$\bar{x}$	0.39	1.58	0.73	0.53	1.05	1.41	−0.14	−0.40	−0.72	0.04
SD	0.24	0.26	0.27	0.29	0.27	0.34	0.36	0.25	0.36	0.23
R	Cu/N	Cu/P	Cu/K	Cu/Ca	Cu/Mg	Cu/S	Cu/B	Cu/Fe	Cu/Mn	Cu/Zn
$\bar{x}$	0.53	1.72	0.87	0.67	1.19	1.55	0.14	−0.26	−0.58	0.19
SD	0.44	0.44	0.42	0.39	0.42	0.35	0.36	0.37	0.41	0.36
R	Fe/N	Fe/P	Fe/K	Fe/Ca	Fe/Mg	Fe/S	Fe/B	Fe/Cu	Fe/Mn	Fe/Zn
$\bar{x}$	0.79	1.99	1.13	0.93	1.46	1.82	0.40	0.26	−0.31	0.45
SD	0.21	0.24	0.23	0.20	0.19	0.37	0.25	0.37	0.28	0.26
R	Mn/N	Mn/P	Mn/K	Mn/Ca	Mn/Mg	Mn/S	Mn/B	Mn/Cu	Mn/Fe	Mn/Zn
$\bar{x}$	1.10	2.30	1.45	1.24	1.77	2.13	0.72	0.58	0.31	0.76
SD	0.24	0.30	0.29	0.17	0.21	0.38	0.36	0.41	0.28	0.37
R	Zn/N	Zn/P	Zn/K	Zn/Ca	Zn/Mg	Zn/S	Zn/B	Zn/Cu	Zn/Fe	Zn/Mn
$\bar{x}$	0.34	1.54	0.69	0.48	1.01	1.37	−0.04	−0.19	−0.45	−0.76
SD	0.22	0.19	0.21	0.23	0.22	0.29	0.23	0.36	0.26	0.37

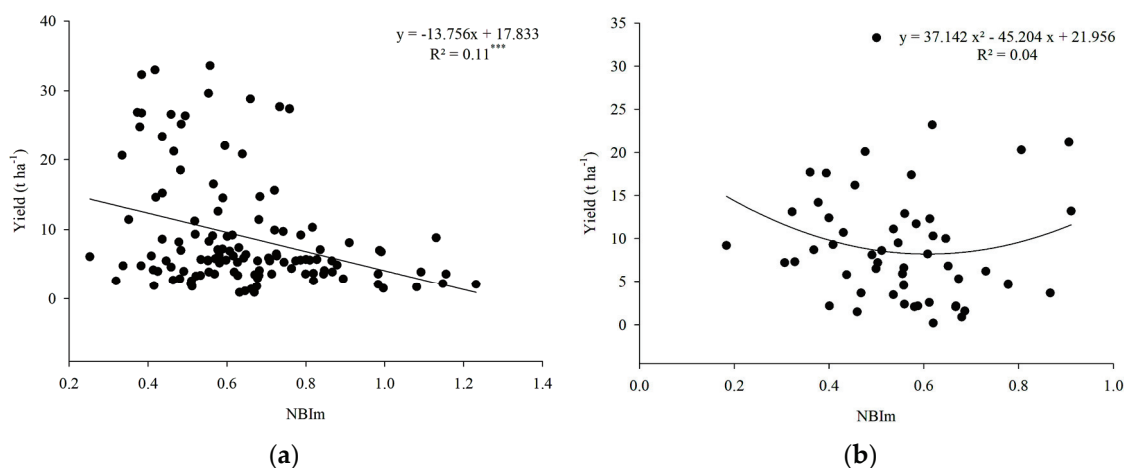


Figure 3. Relationship between the mean Nutrient Balance Index (NBIm) and yield (t ha^{-1}) in ‘Hass’ (a) 4–9-year-old plants; (b) 10–26-year-old plants. Significance level: *** $p < 0.001$.

3.4. CND Standards Establishment

The regression analysis between the cumulative function and yield was fitted to a cubic model. For the 4–9-year-old plants dataset, the inflection point occurred at 20.3 t ha^{-1} , classifying 15.0% of the dataset as the high-yield population. In the 10–26-year-old plants dataset, the inflection point was 10.0 t ha^{-1} , with 33.3% of the dataset classified as high-yield. The yield in the high-yield population of the 4–9-year-old dataset ranged from 20.6 to 33.6 t ha^{-1} , with a mean productivity of 26.5 t ha^{-1} . For the high-yield population in the 10–26-year-old plants dataset, the yield ranged from 10.3 to 33.3 t ha^{-1} , with a mean of 16.1 t ha^{-1} (Table 5). In general, both high- and low-yield populations in both datasets showed higher coefficients of variation for some types of micronutrient leaf content compared to macronutrients (Table 5).

Table 5. Minimum (Min.), maximum (Max.), mean, standard deviation (SD), and coefficient of variation (CV) for leaf nutrient content and yield in the 4–9-year-old and 10–26-year-old plant datasets for ‘Hass’, categorized into high- and low-yield populations, for the CND calculation.

4–9-Year-Old—High-Yield Population (<i>n</i> = 18)						10–26-Year-Old—High-Yield Population (<i>n</i> = 15)					
Variables	Min.	Max.	Mean	SD	CV	Variables	Min.	Max.	Mean	SD	CV
Yield (t ha ^{−1})	20.6	33.6	26.5	4.0	15.1	Yield (t ha ^{−1})	10.3	33.3	16.1	6.1	37.8
N (g kg ^{−1})	15.6	32.2	20.6	4.2	20.5	N (g kg ^{−1})	15.8	26.6	19.4	3.2	16.7
P (g kg ^{−1})	1.1	1.8	1.5	0.2	12.5	P (g kg ^{−1})	1.0	2.0	1.3	0.3	20.3
K (g kg ^{−1})	4.7	12.7	8.8	1.9	21.8	K (g kg ^{−1})	5.7	15.0	9.2	2.3	24.6
Ca (g kg ^{−1})	14.0	28.3	20.0	3.4	16.8	Ca (g kg ^{−1})	12.4	28.3	16.2	4.4	27.0
Mg (g kg ^{−1})	5.9	9.5	7.5	0.9	11.5	Mg (g kg ^{−1})	3.1	8.1	4.5	1.2	25.8
S (g kg ^{−1})	1.3	3.1	2.4	0.6	24.0	S (g kg ^{−1})	1.5	3.3	2.4	0.6	24.8
B (mg kg ^{−1})	19.1	72.7	41.1	17.0	41.4	B (mg kg ^{−1})	19.0	83.9	57.8	24.0	41.6
Cu (mg kg ^{−1})	18.0	109.2	60.4	35.3	58.4	Cu (mg kg ^{−1})	26.0	232.5	118.0	74.2	62.9
Fe (mg kg ^{−1})	83.0	149.0	111.4	20.3	18.3	Fe (mg kg ^{−1})	61.3	258.0	134.6	59.0	43.8
Mn (mg kg ^{−1})	210.5	535.0	327.3	97.7	29.9	Mn (mg kg ^{−1})	149.5	522.0	291.8	111.9	38.3
Zn (mg kg ^{−1})	16.9	34.0	25.2	5.0	20.0	Zn (mg kg ^{−1})	24.0	102.7	54.8	26.9	49.2

4–9-Year-old—Low-Yield Population (<i>n</i> = 102)						10–26-Year-old—Low-Yield Population (<i>n</i> = 30)					
Variables	Min.	Max.	Mean	SD	CV	Variables	Min.	Max.	Mean	SD	CV
Yield (t ha ^{−1})	0.9	18.5	6.0	3.7	60.7	Yield (t ha ^{−1})	0.2	9.5	4.8	2.8	58.6
N (g kg ^{−1})	17.8	33.8	23.1	3.2	14.0	N (g kg ^{−1})	16.2	26.2	20.7	3.1	15.1
P (g kg ^{−1})	1.1	1.9	1.5	0.2	11.8	P (g kg ^{−1})	1.0	1.7	1.3	0.2	14.3
K (g kg ^{−1})	4.8	14.6	8.3	2.4	29.1	K (g kg ^{−1})	5.1	14.3	7.9	1.7	22.2
Ca (g kg ^{−1})	9.8	41.2	22.1	7.0	31.7	Ca (g kg ^{−1})	6.2	30.6	18.0	5.8	32.2
Mg (g kg ^{−1})	3.3	9.4	6.4	1.2	19.5	Mg (g kg ^{−1})	2.4	7.6	4.9	1.2	25.5
S (g kg ^{−1})	1.2	3.2	2.4	0.5	20.6	S (g kg ^{−1})	1.4	3.2	2.1	0.5	22.2
B (mg kg ^{−1})	20.9	79.5	38.4	13.2	34.5	B (mg kg ^{−1})	20.7	74.1	42.4	16.7	39.3
Cu (mg kg ^{−1})	8.2	251.0	66.1	44.7	67.6	Cu (mg kg ^{−1})	10.3	150.0	69.7	34.1	48.9
Fe (mg kg ^{−1})	69.0	263.0	137.3	36.8	26.8	Fe (mg kg ^{−1})	58.0	226.0	131.3	38.0	29.0
Mn (mg kg ^{−1})	154.0	1639.0	511.5	301.3	58.9	Mn (mg kg ^{−1})	94.0	2328.0	518.5	430.1	83.0
Zn (mg kg ^{−1})	14.0	57.0	25.6	6.1	23.9	Zn (mg kg ^{−1})	18.0	109.3	38.3	24.3	63.3

The mean and standard deviation of the multivariate relationships between nutrients (CND standards) were calculated (Table 6). Based on the high values obtained, the macronutrients with the greatest requirements for ‘Hass’ avocado production, in both datasets, were ranked in descending order as follows: N > Ca > K > Mg > S > P (Table 6).

Table 6. Mean and standard deviation (SD) of multivariate relationship between nutrients (CND standards) for leaf nutrient content in relation to yield in the 4–9-year-old and 10–26-year-old plant datasets for ‘Hass’.

Variables	4–9-Year-Old (<i>n</i> = 18)		10–26-Year-Old (<i>n</i> = 15)	
	Mean	SD	Mean	SD
V_N	2.87	0.20	2.74	0.17
V_P	0.23	0.09	0.01	0.22
V_K	2.01	0.20	1.98	0.22
V_{Ca}	2.84	0.16	2.54	0.29
V_{Mg}	1.87	0.12	1.28	0.27
V_S	0.70	0.20	0.63	0.20
V_B	−3.41	0.36	−3.18	0.47
V_{Cu}	−3.12	0.63	−2.57	0.63
V_{Fe}	−2.35	0.16	−2.30	0.34
V_{Mn}	−1.30	0.30	−1.51	0.43
V_{Zn}	−3.84	0.17	−3.23	0.40

The Nutrient Imbalance Index (CND- r^2) regression in relation to yield for both datasets was fitted to a linear model. For the 4–9-year-old plants dataset, the regression was highly significant ($p < 0.001$), while for the 10–26-year-old plants dataset, it was significant at the 5% level. In both datasets, the R^2 value was 14%, indicating that, using the CND method, 14% of the variation in yield can be explained by nutritional balance (Figure 4).

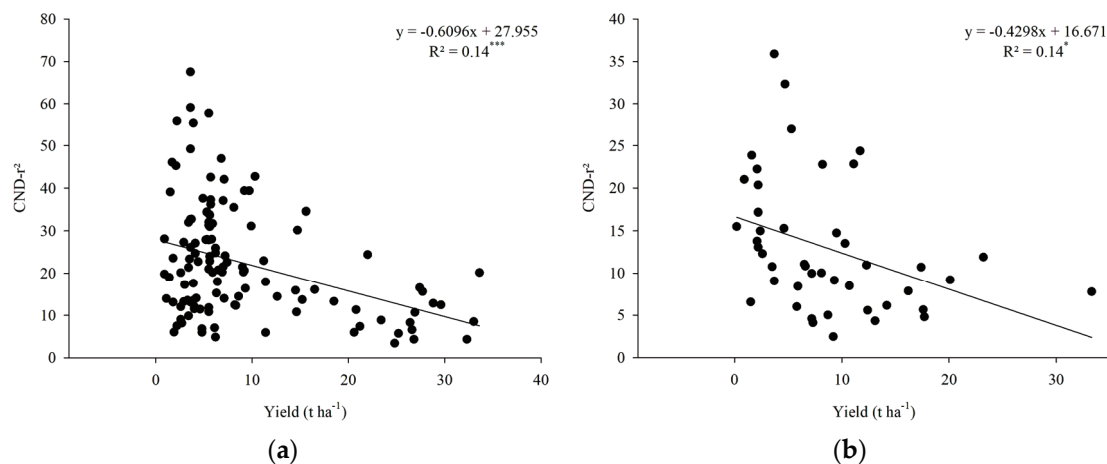


Figure 4. Relationship between the Nutrient Imbalance Index (CND- r^2) and yield in ‘Hass’ (a) 4–9-year-old plants; (b) 10–26-year-old plants. Significance level: *** $p < 0.001$, and * $p < 0.05$.

3.5. Sufficiency Range (SR) and Critical Level (CL) Development

Establishing the SR and CL using the DRIS indices for each nutrient and their relationships with the respective leaf nutrient content resulted in linear, quadratic, and logarithmic regression models (Table 7). All regressions were significant ($p < 0.001$, $p < 0.01$, and $p < 0.05$) for both datasets, with high R^2 values ranging from 38% to 96%. As a result, SRs and CLs were calculated using the DRIS method for both the 4–9-year-old and 10–26-year-old plant datasets (Table 7).

In the CND method, regressions were fitted to linear, quadratic, and logarithmic models to analyze the relationship between the CND indices and their respective nutrient content (Table 8). For both the 4–9-year-old and 10–26-year-old plant datasets, all regressions were significant ($p < 0.001$, $p < 0.01$, and $p < 0.05$), with R^2 values ranging from 35% to 98% (Table 7). SRs and CLs were successfully established using the CND method (Table 8).

Table 7. Statistical models for the relationship between DRIS indices and leaf nutrient content, sufficiency ranges (SRs), and Critical Levels (CLs) according to datasets: 4–9-year-old and 10–26-year-old plants for ‘Hass’ avocado production.

4–9-Year-Old Plants—DRIS				
Nutrients	Equations	R^2	SRs	CLs
N	$IN = 0.1404 N - 3.0464$	0.61 ***	19.3–24.1	22.0
P	$IP = 1.7071 P - 2.5472$	0.38 ***	1.4–1.6	1.5
K	$IK = 2.8461 \ln(K) - 6.277$	0.85 ***	7.5–10.6	9.1
Ca	$ICa = -0.0016 Ca^2 + 0.2089 Ca - 3.6517$	0.90 **	16.4–25.2	20.8
Mg	$IMg = 2.793 \ln(Mg) - 5.5079$	0.74 ***	6.3–8.1	7.2
S	$IS = -0.2963 S^2 + 2.659 S - 4.8205$	0.77 *	2.2–2.9	2.5
B	$IB = -0.0004 B^2 + 0.094 B - 2.8183$	0.89 ***	25.9–44.7	35.3
Cu	$ICu = 1.3711 \ln(Cu) - 5.17$	0.97 ***	14.4–72.4	43.4
Fe	$IFe = -0.00004 Fe^2 + 0.03393 Fe - 3.36924$	0.83 **	92.3–141.9	117.1
Mn	$IMn = 2.0396 \ln(Mn) - 12.198$	0.96 ***	200.5–590.9	395.7
Zn	$IZn = -0.0008 Zn^2 + 0.1493 Zn - 3.3672$	0.77 *	22.2–30.3	26.2

Table 7. Cont.

10–26-Year-old plants—DRIS				
Nutrients	Equations	R ²	SRs	CLs
N	$IN = 0.0789 N - 1.6475$	0.53 ***	17.5–24.3	20.9
P	$IP = 1.648 \ln(P) - 0.394$	0.50 ***	1.0–1.5	1.3
K	$IK = 2.0639 \ln(K) - 4.618$	0.74 ***	7.1–11.6	9.4
Ca	$ICa = 0.1098 Ca - 1.6736$	0.82 ***	11.6–18.9	15.2
Mg	$IMg = 0.3093 Mg - 1.3779$	0.64 ***	3.7–5.2	4.5
S	$IS = 1.1948 \ln(S) - 0.7657$	0.86 ***	1.5–2.3	1.9
B	$IB = -0.0005 B^2 + 0.0827 B - 2.8745$	0.87 ***	35.5–63.9	49.7
Cu	$ICu = 0.9894 \ln(Cu) - 4.2047$	0.96 ***	33.2–107.0	70.1
Fe	$IFe = 0.0114 Fe - 1.5444$	0.84 ***	101.9–167.9	134.9
Mn	$IMn = 1.4714 \ln(Mn) - 8.1397$	0.93 ***	18.0–487.2	252.6
Zn	$Izn = 1.5252 \ln(Zn) - 5.8326$	0.89 ***	29.2–62.3	45.8

Significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

Table 8. Statistical models for the relationship between CND indices and leaf nutrient content, sufficiency ranges (SRs), and Critical Levels (CLs) according to datasets: 4–9-year-old and 10–26-year-old plants for ‘Hass’ avocado production.

4–9-Year-Old—CND				
Nutrients	Equations	R ²	SRs	CLs
N	$I_N = 0.2167 N - 4.561$	0.73 ***	18.7–23.4	21.0
P	$I_P = 5.3612 P - 8.1313$	0.49 ***	1.4–1.6	1.5
K	$I_K = 4.5593 \ln(K) - 9.9646$	0.88 ***	7.3–10.5	8.9
Ca	$I_{Ca} = -0.0040 Ca^2 + 0.4277 Ca - 7.0868$	0.91 ***	16.1–25.0	20.6
Mg	$I_{Mg} = -0.069 Mg^2 + 2.0621 Mg - 11.969$	0.78 *	7.0–8.7	7.9
S	$I_S = -0.3885 S^2 + 3.5794 S - 6.3825$	0.83 **	2.1–2.8	2.4
B	$I_B = -0.0005 B^2 + 0.1026 B - 3.374$	0.92 ***	31.9–50.3	41.1
Cu	$I_{Cu} = 1.4527 \ln(Cu) - 5.742$	0.98 ***	23.2–80.9	52.1
Fe	$I_{Fe} = -0.00007 Fe^2 + 0.05894 Fe - 5.71554$	0.86 **	87.9–135.8	111.8
Mn	$I_{Mn} = 3.0393 \ln(Mn) - 17.501$	0.97 ***	125.0–508.6	316.8
Zn	$I_{Zn} = 5.0656 \ln(Zn) - 16.442$	0.81 ***	21.7–29.6	25.7
10–26-Year-old—CND				
Nutrients	Equations	R ²	SRs	CLs
N	$I_N = 0.227 N - 4.078$	0.38 ***	15.8–20.1	18.0
P	$I_P = 2.5398 P - 2.9653$	0.35 ***	1.0–1.3	1.2
K	$I_K = 0.4669 K - 4.1324$	0.62 ***	7.5–10.2	8.9
Ca	$ICa = 0.1706 Ca - 2.6455$	0.84 ***	11.9–19.1	15.5
Mg	$IMg = 0.5147 Mg - 2.147$	0.68 ***	3.4–5.0	4.2
S	$IS = 1.1318 S - 2.6719$	0.53 ***	2.1–2.8	2.4
B	$IB = -0.0008 B^2 + 0.1185 B - 3.8802$	0.89 ***	35.2–62.5	48.9
Cu	$ICu = 1.2955 \ln(Cu) - 5.9171$	0.97 ***	59.5–133.1	96.3
Fe	$IFe = 0.0159 Fe - 1.9474$	0.82 ***	92.2–152.8	122.5
Mn	$IMn = 2.1335 \ln(Mn) - 11.818$	0.94 ***	7.2–501.8	254.5
Zn	$Izn = 2.0282 \ln(Zn) - 7.9003$	0.91 ***	31.8–66.6	49.2

significance levels: *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

For comparison, the SRs established in this study using the CND and DRIS indices for both the 4–9-year-old and 10–26-year-old plant datasets are presented alongside the existing SRs for avocado production in Brazil, United States and France in Tables 9 and 10.

Table 9. Macronutrient sufficiency ranges (SRs) for avocado production obtained by the DRIS and CND methods in the present study in comparison to the SRs existing in Brazil, the United States (USA), and France.

References	N	P	K	Ca	Mg	S
	g kg ⁻¹					
4–9-year-old plants—DRIS	19.3–24.1	1.4–1.6	7.5–10.5	16.4–25.2	6.3–8.1	2.2–2.9
4–9-year-old plants—CND	18.7–23.4	1.4–1.6	7.3–10.5	16.1–25.0	7.0–8.7	2.1–2.8
10–26-year-old plants—DRIS	17.5–24.3	1.0–1.5	7.1–11.6	11.6–18.9	3.7–5.2	1.5–2.3
10–26-year-old plants—CND	15.8–20.1	1.0–1.3	7.5–10.2	11.9–19.1	3.4–5.0	2.1–2.8
Teixeira ¹	16.0–20.0	0.8–2.5	7.0–20.0	10.0–30.0	2.5–8.0	2.0–6.0
Quaggio and van Raij ²	16.0–20.0	0.8–2.5	7.0–20.0	10.0–30.0	2.5–8.0	2.0–6.0
Crowley ³	22.5–29.0	1.0–1.5	7.0–9.0	18.0–20.0	6.0–9.0	4.5–5.6
Gaillard ⁴	16.0–20.0	0.8–2.5	7.5–20.0	10.0–30.0	2.5–8.0	2.0–6.0
Embleton and Jones ⁵	20.0–26.0	1.0–2.5	7.5–20.0	10.0–30.0	2.5–8.0	2.0–6.0

¹ [14]. ² [15]. ³ [40]. ⁴ [41]. ⁵ [16].

Table 10. Micronutrient sufficiency ranges (SRs) for avocado production obtained by the DRIS and CND methods in the present study in comparison to the SRs existing in Brazil, the United States (USA), and France.

References	B	Cu	Fe	Mn	Zn
	mg kg ⁻¹				
4–9-year-old plants—DRIS	25.9–44.7	14.4–72.4	92.3–141.9	200.5–590.9	22.2–30.3
4–9-year-old plants—CND	31.9–50.3	23.2–80.9	87.9–135.8	125.0–508.6	21.7–29.6
10–26-year-old plants—DRIS	35.5–63.9	33.2–107.0	101.9–167.9	18.0–487.2	29.2–62.3
10–26-year-old plants—CND	35.2–62.5	59.5–133.1	92.2–152.8	7.2–501.8	31.8–66.6
Teixeira ¹	50.0–100.0	5.0–15.0	50.0–200.0	30.0–100.0	30.0–100.0
Quaggio and van Raij ²	50.0–100.0	5.0–15.0	50.0–200.0	30.0–100.0	30.0–100.0
Crowley ³	38.0–60.0	4.0–7.0	55.0–80.0	110.0–145.0	50.0–80.0
Gaillard ⁴	50.0–100.0	5.0–15.0	50.0–200.0	30.0–500.0	30.0–150.0
Embleton and Jones ⁵	50.0–100.0	5.0–15.0	50.0–200.0	30.0–500.0	30.0–150.0

¹ [14]. ² [15]. ³ [40]. ⁴ [41]. ⁵ [16].

The SRs established in this study showed differences between the plant datasets for both DRIS and CND. Higher values of N, Ca, Mg, Cu, and Zn were calculated for the 4–9-year-old plants dataset compared to the 10–26-year-old plants. Additionally, a wider range of Mn was observed in the older plant group (Tables 9 and 10).

When compared to the existing SRs in Brazil, the values from this study revealed some differences, narrowing the SRs for all nutrients except Mn. It is important to note that, for some nutrients, the existing SRs either overestimate or underestimate the optimal range compared to our results (Tables 9 and 10).

Some similarities were found between the SRs established by Embleton and Jones [16] and Gaillard [41] and the existing Brazilian SRs, even though these SRs were developed for different locations. For instance, our established SRs differ in value from the recently established ranges for California [40].

4. Discussion

Mineral nutrition is one of the most essential factors influencing plant development and yield [42]. Supplying macronutrients and micronutrients in a balanced way enables the crop to reach its genetic potential for growth and productivity [43]. On the other hand, an excess or deficiency of nutrients can directly affect crops' yield and quality [44],

underscoring the importance of an adequate nutrient supply. In our study, both datasets showed significant positive and negative correlations between nutrient content and yield.

The 4–9-year-old plants exhibited a negative correlation with N, Fe, and Mn and a positive correlation with Mg, while the 10–26-year-old plants showed a negative correlation with Ca, Mg, and Mn and a positive correlation with B, Cu, and Zn. However, explaining yield through univariate methods is challenging, as nutrients interact in complex multi-nutrient relationships [45]. As observed in the correlation matrix, almost all nutrients showed significant correlations with one another, highlighting that using a univariate method to explain yield is insufficient [46].

The variability in nutrient content in both datasets was higher for some micronutrients compared to macronutrients, as indicated by the high coefficient of variation. This phenomenon is not fully understood [47], but it is likely related to evolutionary and environmental factors. Additionally, macronutrients, which are required in larger quantities, tend to be more stable [47,48], a trend also observed in recent DRIS and CND studies on fruit crops [49,50]. In particular, the higher coefficients of variation (CVs) observed for Cu may be associated with the application of agrochemicals containing this micronutrient, which are routinely used. Despite careful washing of the plant material, Cu may remain adhered to suberized, non-physiologically active tissues, which are still included in analytical procedures that digest the entire leaf sample. In contrast, the high variability observed for Fe is likely due to differences in soil properties, especially at greater depths (below the arable layer). Such variability is common in tropical soils and may exert a greater influence on avocado crops, which are characterized by a deep root system.

The plots in the high-yield population showed a yield variation ranging from 9.0 to 33.6 t ha⁻¹ in the 4–9-year-old plants dataset and from 9.2 to 33.3 t ha⁻¹ in the 10–26-year-old plants dataset. The mean productivity for the high-yield populations was 19.2 t ha⁻¹ for the younger plant group, which is higher than the Brazilian mean productivity of 18.6 t ha⁻¹ in 2023 [9], and 15.1 t ha⁻¹ for the older plant group, which is lower than the national average.

Avocado yield is influenced by various biotic and abiotic factors, including climate variations, pests, soil conditions, and diseases [51–54]. Additionally, as plants age, their yield tends to decrease [55], which may help explain the yield differences observed between the two datasets in our study. It is also worth noting that the 10–26-year-old plants dataset includes plots with larger spacings, which may influence yield [56].

However, both datasets included plots yielding more than 30 t ha⁻¹, which is considered above the estimated potential for avocado production [10], suggesting that the datasets are suitable for establishing nutritional standards, as they presents high-yield orchards [57].

According to the multi-nutrient variables obtained through the CND method in this study, the greatest nutrient requirement for avocado production in both plant age groups was N > Ca > K. This differs from fruit nutrient removal studies, where in Mexico the highest nutrient export was K > N > Mg [58] and in Colombia, K > N > P [59]. This disparity emphasizes the need for site-specific studies on nutrient management in avocado, which are still lacking under Brazilian conditions.

Using the mean Nutrient Balance Index (NBIm) from the DRIS method and the Nutrient Imbalance Index (CND-r²) from the CND method, regression analysis was performed with the yield for both datasets. High values of NBIm and CND-r² indicate nutritional imbalances in the plot, which consequently reduce yields [49]. When significant, the coefficient of determination from the regression can explain, in percentage, the variation in yield affected by the plant's nutritional status [49,60].

In the present study, all regressions were significant, except for the DRIS method in the 10–26-year-old plants dataset. The reasons for DRIS method being ineffective for older trees may be derived from the fact that the subpopulation division in this method is based on an arbitrary criterion, and that the DRIS does not include the outlier exclusion as in the CND method. The R^2 values for the 4–9-year-old plants dataset were 11% (DRIS) and 14% (CND), while for the 10–26-year-old plants dataset, they were 0.4% (DRIS) and 14% (CND). This indicates that, for the 4–9-year-old plants, 11% and 14% of the yield variation can be explained by nutritional balance, while for the 10–26-year-old plants dataset, only the CND method was effective in explaining 14% of the variation in yield.

The DRIS method has been successfully and widely applied to assess the nutritional status of fruit and perennial crops, leading to the development of SRs and CLs, as demonstrated for sweet cherry [*Prunus avium* (L.) L.] [61], apple [*Malus domestica* (Suckow) Borkh.] [62], rubber tree [*Hevea brasiliensis* (Willd. Ex A.Juss.) Müll.Arg.] [22], and more recently for cashew [*Anacardium occidentale* L.] [63] and mango (*Mangifera indica* L.) [64].

However, in the present study, the CND method proved to be superior to DRIS for evaluating the nutritional status of ‘Hass’ avocado. CND is a multivariate approach that considers the relationship between nutrient content and the geometric mean of other dry matter components, including those not measured in the tissue analysis [13,25]. Additionally, unlike the arbitrary division of high- and low-yield populations in the DRIS method [65], the CND method bases its division on the cumulative function of the variance ratio derived from the indices. It also uses Mahalanobis distance prior to population division, excluding aberrant data [23], which results in a more precise diagnosis [37,66].

Using the equations of the obtained regressions between nutrient indices and leaf nutrient content, equating them to zero made it possible to obtain the Critical Levels (CLs) for both the DRIS and CND methods, and by adding or subtracting two-thirds of the standard deviation of the indices, the sufficiency ranges (SRs) were calculated [60]. In this study, for the first time, we established the CLs and SRs for ‘Hass’ avocado production in Brazil, based on plant age, from data collected in commercial orchards. Plant aging alters the nutritional status of perennial woody trees and should be considered when planning fertilization programs [67], as well as when establishing nutritional standards, as demonstrated in this study, supporting the indications by Rozane et al. [68] on the need to develop specific norms and refuting the applicability of universal standards.

Despite the existence of SRs for avocado production in Brazil [14,15], these values for leaf nutritional diagnosis, considered adequate, are not cultivar-specific and have not been updated since 1997. Additionally, the values are similar to SRs established in other countries [16,41], suggesting that they may be adaptations of those SRs and may not be suitable for Brazilian orchards. This discrepancy is reflected in the different results obtained in the present study.

A similar situation occurred in California, where SRs were established by adapting values used in citrus production, combined with researcher knowledge [69]. The new optimum ranges for California avocado production were then defined, and these values differ from those previously used [40], highlighting the necessity of updating the old SRs and generating new, specific ranges to guide producers.

The development of site-specific nutritional status guidelines, such as SRs and CLs, is essential for accurately monitoring the nutritional balance of orchards [70], challenging the validity of universal recommendations [68]. Variations in edaphoclimatic conditions such as temperature and rainfall directly influence plant nutrient requirements by altering growth patterns and physiological responses [71–73]. Additionally, soil type and fertility are key factors that shape nutrient availability and influence symbiotic associations within the crop [74–76]. These environmental conditions may induce structural and physiological

adaptations in plants, enhancing their ability to optimize their nutrient uptake and response under specific scenarios [75,76]. Building on the influence of local environmental factors, climate change introduces an additional layer of complexity to plant nutrition by affecting nutrient availability and plant physiology, largely driven by the rise in atmospheric CO₂ and global mean temperatures [19,21].

In summary, the nutritional standards for ‘Hass’ avocado production, along with the CLs and SRs, were successfully established using the DRIS and CND methods. These results will serve as a valuable tool for nutritional monitoring in Brazilian orchards, improving nutritional balance and, consequently, yields, while minimizing losses due to over- and under-fertilization. Soon, management tools based on the CND norms will be developed, including software similar to the one presented by Rozane [77].

Annual recording of leaf nutrient content and yield data is needed to improve future DRIS and CND analyses for avocado in Brazil, especially regarding other cultivation regions and different cultivars, improving the robustness of the standards and SRs and CLs for nutritional analysis in this fruit crop.

5. Conclusions

Nutritional standards for ‘Hass’ avocado production were established using the DRIS method for 4–9-year-old plants and the CND method for both 4–9 and 10–26-year-old plant groups. CND proved more efficient than DRIS in explaining the yield variation related to nutritional balance, accounting for 14% of the variation across both age groups, with this portion of variability attributed to nutritional conditions. DRIS and CND norms can be applied to assess nutrient excess, equilibrium, or deficiency for both macro- and micronutrients. The SRs and CLs generated from these methods serve as useful tools for nutritional diagnosis and support adequate ‘Hass’ avocado production in Brazil. Together, the DRIS and CND indices, along with SRs and CLs, will contribute to more precise nutrient monitoring and fertilization strategies. Importantly, SR and CL values varied according to plant age. Further data from different cultivated regions and cultivars are needed to refine nutritional guidelines and enhance the productivity of this important and expanding crop in Brazil.

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Article

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

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Abstract

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

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

1. Introduction

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

as rootstock selection and training system, which directly affect photosynthetic efficiency, secondary metabolite biosynthesis, and overall plant performance [2].

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

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

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

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

2. Materials and Methods

2.1. Location and Climatic Conditions of the Experimental Area

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

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

2.2. Experimental Design and Treatments

The experiment was conducted in a randomized block design with a 2 × 2 factorial arrangement, consisting of two rootstocks—IAC 766 Campinas' (*Riparia do Traviú* × *Vitis*

caribaea) and ‘106-8 Mgt’ [*Riparia* × (*Cordifolia* × *Rupestris*)]—and two training systems representing different trellis heights: 1.6 m and 2.0 m.

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

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

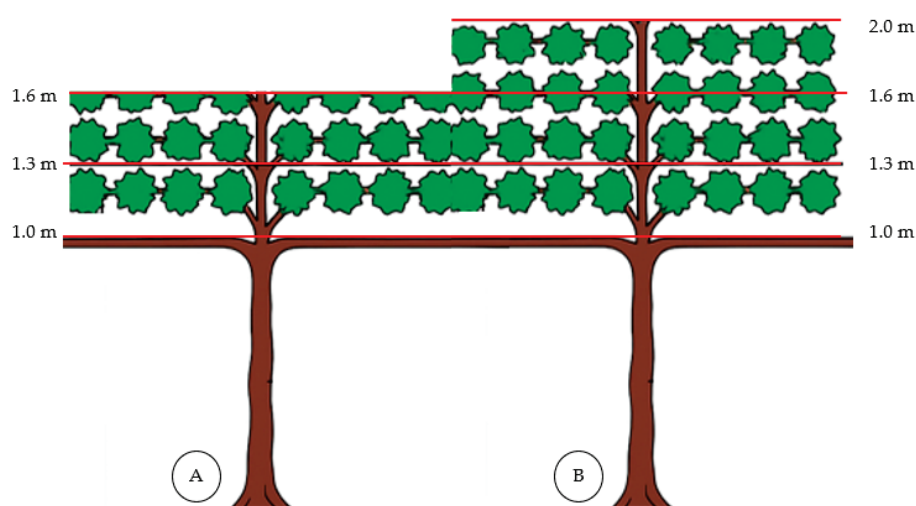


Figure 1. Schematic representation of two vertical shoot positioning (VSP) training systems with different heights: (A) Low trellis system (1.6 m) with three support wires positioned at 1.0 m, 1.3 m, and 1.6 m; (B) High trellis system (2.0 m) with four support wires positioned at 1.0 m, 1.3 m, 1.6 m,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

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

2.3. Sample Collection

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

Photosynthetic measurements were performed in situ on intact leaves. For biochemical analyses, leaves were collected, wrapped in aluminum foil, immediately frozen in liquid nitrogen, and stored in an ultra-freezer (-82°C) until analysis.

2.3.1. Photosynthetic Evaluation

Photosynthetic activity was measured using an open gas exchange system (Infra Red Gas Analyzer—IRGA, model LI-6400, LI-COR Biosciences, Lincoln, NE, USA). The following physiological variables were assessed: net CO_2 assimilation rate (A), transpiration rate (E), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s), according to the method proposed by Von Caemmerer; Farquhar (1981) [9]. Water use efficiency (WUE) was determined as the ratio of CO_2 assimilation to transpiration. Rubisco carboxylation efficiency (A/C_i) was calculated based on the ratio between CO_2 assimilation rate and internal CO_2 concentration in the leaf.

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

These measurements were used to calculate the following:

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

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

2.3.2. Biochemical Analyses

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

2.3.3. Harvest, Yield, and Must Quality

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

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

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

2.4. Statistical Analyses

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

3. Results and Discussion

3.1. Physiological and Biochemical Performance of 'Bordô' Grapevines

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

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

In addition, the high ETR values observed in grapevines trained on a high trellis and grafted onto 'IAC 766 Campinas' may reflect a more efficient electron transport capacity, possibly associated with greater light interception. However, this did not necessarily translate into higher net CO₂ assimilation (A), indicating that other physiological or biochemical factors may be limiting the overall photosynthetic capacity under these conditions. This indicates that this combination may be beneficial for maximizing the efficiency of light energy conversion, especially in regions with high solar radiation.

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

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

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

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

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

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

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

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

Stomatal conductance (gs) was significantly higher in grapevines grafted onto ‘106-8 Mgt’, reflecting greater stomatal opening and, consequently, increased CO₂ uptake for photosynthesis (Table 1). This increase in gs also resulted in a higher transpiration rate (E), demonstrating the close relationship between these processes. Since stomatal opening regulates CO₂ influx and water vapor efflux, this factor should be considered in irrigation management, especially in regions with limited water availability [28].

Water use efficiency (WUE) was higher in grapevines grafted onto ‘IAC 766 Campinas’, regardless of the training system (Table 1). This result suggests that this combination may be more suitable for conditions of low water availability, as it enables more efficient use of plant water resources. Furthermore, the lower transpiration rate observed in these grapevines indicates an efficient water regulation mechanism, which may contribute to reduced water consumption without compromising CO₂ assimilation. This characteristic may be crucial for vine adaptation in environments subject to moderate drought.

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

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

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

Table 2. CO₂ assimilation rate (A), intercellular CO₂ concentration (Ci), SPAD index, peroxidase (POD), and catalase (CAT) activity at flowering, and SPAD index and chlorophyll (Chl) b content at the berry ripening in ‘Bordô’ grapevines under different rootstocks and training systems.

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

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

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

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

3.2. Yield and Quality of ‘Bordô’ Grapevines

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

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

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

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

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

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

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

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

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

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

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

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

3.3. Physiological and Biochemical Performance of ‘Isabel’ Grapevines

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

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

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

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

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

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

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

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

The electron transport rate (ETR) was higher in the combination of high trellis and the '106-8 Mgt' rootstock, suggesting greater efficiency in the conversion of light energy into chemical energy and in electron transport through the Calvin cycle (Table 5). Additionally, this same combination resulted in a high CO₂ assimilation rate, reinforcing the positive relationship between photochemical efficiency and the plant's photosynthetic capacity. This indicates that this specific interaction maximizes the efficiency of the photosynthetic apparatus, promoting better utilization of available light energy and enhancing the production of photoassimilates.

The combined assessment of gas exchange and chlorophyll a fluorescence has been widely used to differentiate stomatal and non-stomatal limitations to photosynthesis under stress conditions [35]. The interaction between high irradiance and an inadequate training system can compromise the photosynthetic apparatus's ability to efficiently utilize incident radiation, resulting in oxidative damage and degradation of photosynthetic pigments [36]. Under adverse environmental conditions, when photon absorption exceeds CO₂ assimilation capacity, photoinhibition of photosystem II (PSII) may occur, significantly reducing photosynthetic efficiency [37]. These findings highlight the importance of proper canopy management to optimize photosynthetic efficiency and minimize potential oxidative damage in grapevines.

A significant interaction was also observed between training systems and rootstocks for stomatal conductance (gs), transpiration rate (E), Rubisco carboxylation efficiency (A/Ci), net assimilation rate (A), and intercellular CO₂ concentration (Ci) (Table 5). The high trellis system promoted greater stomatal conductance when combined with the '106-8 Mgt' rootstock, indicating greater stomatal opening and, consequently, greater CO₂ diffusion into the leaf. On the other hand, grapevines trained on a low trellis exhibited lower gs values, suggesting more restricted stomatal aperture.

Stomatal closure, particularly under adverse environmental conditions, can reduce internal CO₂ concentration, limiting photosynthetic activity due to decreased substrate availability for the Calvin cycle. This limitation reduces NADPH and ATP consumption, increases NADP⁺ concentration, and decreases the availability of electron acceptors in the transport chain, which may trigger oxidative stress.

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

Table 5. Non-photochemical quenching (NPQ), electron transport rate (ETR), stomatal conductance (gs), transpiration rate (E), Rubisco carboxylation efficiency (A/Ci), net assimilation rate (A), intercellular CO₂ concentration (Ci), SPAD index, chlorophylls a, b, and total, peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) at flowering, and superoxide dismutase (SOD) and catalase (CAT) at the berry ripening in ‘Isabel’ grapevines under different rootstocks and training systems.

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

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

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

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

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

3.4. Yield and Quality of ‘Isabel’ Grapevines

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

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

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

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

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

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

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

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

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

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

3.5. Principal Component Analysis of *Vitis labrusca* Grapevines

Principal component analysis (PCA) explained 52.51% of the total variance among treatments, with Dim 1 accounting for 32.58% and Dim 2 for 19.93% (Figure 2A,B). Dim 1 was mainly associated with higher photosynthetic efficiency and productive variables, such as Fv/Fm, soluble solids (SS), yield, productivity, total chlorophyll at flowering (ChlT.F), and Rubisco carboxylation efficiency (A/Ci). Dim 2 was positively correlated with gas exchange traits, including transpiration rate (E), stomatal conductance (gs), and net CO₂ assimilation (A), reflecting stomatal activity under varying water demand.

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

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

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

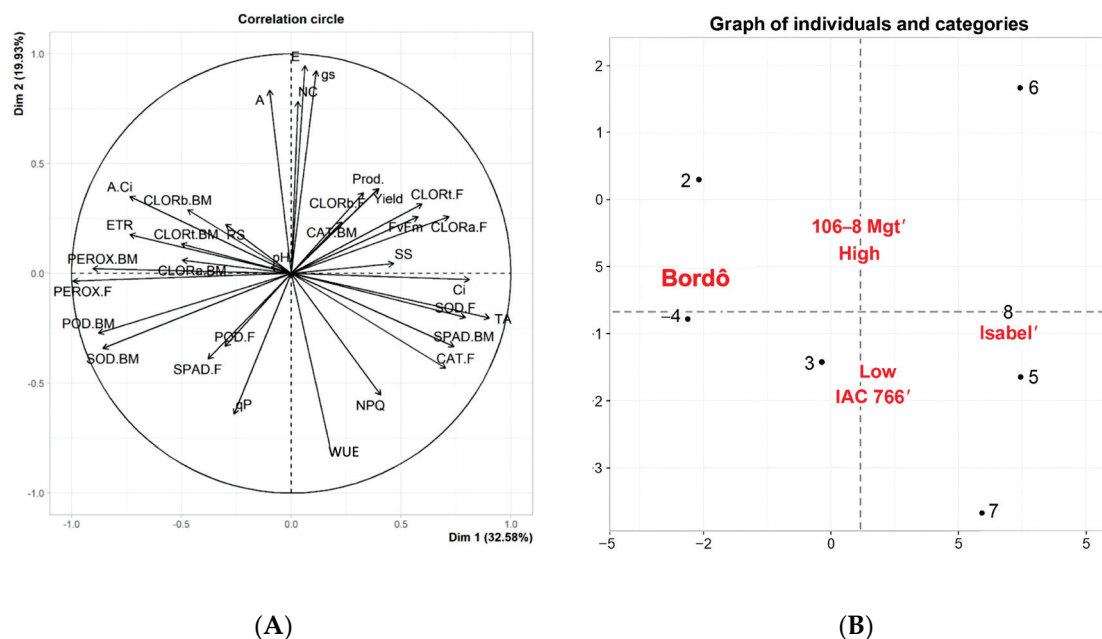


Figure 2. Principal components of photochemical, biochemical, and productive variables for the interaction between two *Vitis labrusca* grapevines ('Bordô' (B) and 'Isabel' (I)) combined with two rootstocks ('IAC 766 Campinas' (766) and 106-8 'Mgt' (106)) under two training systems (low trellis (b) and high trellis (a)). (A) Correlation circle of physiological, biochemical, and productive variables. (B) Distribution of the interaction between cultivar, rootstock, and training system along the principal components. Note: Quantum efficiency of PSII (Fv/Fm), photochemical quenching (qP), non-photochemical quenching (NPQ), electron transport rate (ETR), transpiration rate (E), water use efficiency (WUE), Rubisco carboxylation efficiency (A/Ci), stomatal conductance (gs), net CO₂ assimilation rate (A), and intercellular CO₂ concentration (Ci); SPAD index at flowering (SPAD.F) and at the beginning of berry ripening (SPAD.BM); chlorophyll a (CLORa.F; CLORa.BM), chlorophyll b (CLORb.F; CLORb.BM), and total chlorophyll (CLORT.F; CLORT.BM); peroxidase (POD.F; POD.BM), superoxide dismutase (SOD.F; SOD.BM), catalase (CAT.F; CAT.BM), and lipid peroxidation (PEROX.F; PEROX.BM) at flowering (F) and at the berry maturation (BM); soluble solids (SS), titratable acidity (TA), and yield components: production (Prod.) and yield (Yield).

4. Conclusions

The interaction between rootstocks and training systems influences photosynthetic efficiency, water use, and productivity in *Vitis labrusca* grapevines. In 'Bordô', grapevines grafted onto '106-8 Mgt' and trained on a high trellis showed a 27.1% higher net CO₂ assimilation rate (A) and a 77.8% greater Rubisco carboxylation efficiency (A/Ci) compared to the least efficient combination. In contrast, 'Isabel' grapevines grafted onto 'IAC 766 Campinas' and trained on a low trellis exhibited a 77.8% increase in water use efficiency (WUE), making it more suitable for regions with limited water availability.

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

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

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Article

Phytochemical Characterization of *Humulus lupulus* L. Varieties Cultivated in Brazil: Agricultural Zoning for the Crop in Tropical Areas

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Abstract

Hop (*Humulus lupulus* L.) is a crucial ingredient in beer, valued for its bitter acids and essential oils. Traditionally cultivated in temperate regions, hop production faces challenges from climate change, necessitating the exploration of new growing areas. This study evaluated the chemical quality of hop varieties grown in Brazil's subtropical conditions to assess their suitability for brewing. Seven hop varieties (Cascade, Centennial, Columbus, Chinook, Comet, Fuggle, and Nugget) were analyzed for total α - and β -acids and essential oil composition. Bitter acids were quantified by spectrophotometric analysis, and volatile compounds were identified using gas chromatography coupled to mass spectrometry (GC-MS). Comet exhibited the highest α -acid level (10.54%), indicating strong bittering potential. Essential oil analysis revealed β -myrcene, (*E*)-caryophyllene, and α -humulene as predominant compounds. Centennial showed a distinct (*E*)- β -farnesene profile, a characteristic of noble hops. All varieties contained α - and β -selinene, suggesting a common metabolic pattern. The results indicate that Brazil has the potential to produce high-quality hops with suitable chemical profiles for brewing. As traditional hop-growing regions face climate-related challenges, hops cultivated in Brazil may offer a viable alternative for achieving the desired chemical composition required by the brewing industry.

Keywords: Brazilian hops; essential oil; tropical conditions; bitter acids; alpha-acids

1. Introduction

Humulus lupulus L. is an angiosperm belonging to the Cannabaceae family, originating from temperate climate countries [1]. It is a herbaceous, dioecious vine capable of reaching heights up to 7 m. The plant is notably sensitive to photoperiod, significantly affecting both its vegetative growth and the production of its inflorescences [2]. Known since antiquity, the species was initially described as a horticultural plant consumed in a manner similar to asparagus [3]. Today, global hop production is destined for the cosmetic, food,

pharmaceutical, and animal industries, but approximately 97% of its use is allocated to the beer manufacturing industry [1].

Hops are one of the four key ingredients in beer production, along with water, malt, and yeast. Hops produce α - and β -acids, soft resins derived from specialized metabolism. These acids have antioxidant properties and antimicrobial actions, as well as contribute organoleptic characteristics such as bitterness and foam stability. Essential oils, on the other hand, are volatile chemical compounds related to aromatic components and impart aroma to beer [4–6].

The United States and Germany are the leading hop producers, followed by the Czech Republic, China, Poland, Slovenia, New Zealand, and Australia, where favorable climatic conditions support hop cultivation [7]. In Brazil, hop cultivation began in 2014, following the discovery of a variety that adapted well to the climatic conditions of the Mantiqueira mountain range. Since then, producers and researchers have started cultivating hops, still in an early stage, in various regions of Brazil [8]. In 2020, approximately 1400 new breweries were registered with the Ministry of Agriculture, Livestock, and Supply, with 80% located in the South and Southeast regions of the country, areas characterized as subtropical [1].

Climatic factors such as sunlight exposure, temperature, and water availability, along with genetic variability, influence the levels of active compounds in hops. This is one of the most important parameters for the brewing industry when assessing the quality of hop cones [9]. The growing focus on local production and the expansion of beer markets have increased interest in hops with unique phytochemical profiles and in new growing areas, producing local hops with characteristics specific to each region [10,11]. However, newly created and relocated varieties will bring changes in the sensory and other characteristics of hops [12]. Climate change threatens European hop production by accelerating ripening, reducing yields, and lowering α -acid content. By 2050, declines of 4–18% in yield and 20–31% in α -acid concentration are projected, underscoring the need for adaptation strategies in the brewing industry [13].

Given the increasing interest in expanding hop cultivation to new regions, this study aims to characterize the aroma and bitterness compounds of hop cones from varieties grown under subtropical conditions in Brazil to provide valuable insights into the potential of subtropical regions for hop production, despite the challenges posed by climate variability.

2. Materials and Methods

2.1. Experimental Area

The experiment was conducted in the Department of Crop Science, Division of Horticulture, School of Agricultural Sciences, São Paulo State University, located in the municipality of Botucatu, São Paulo (GPS coordinates: 22°50' S, 48°26' W, and altitude of 791 m). According to the Köppen climate classification, the region experiences a subtropical climate with hot summers (Cfa). During the cycle production period (November 2020 to March 2021), the average minimum temperature was 18.76 °C, and the average maximum temperature was 29.21 °C, with a total precipitation of 852.43 mm. The soil chemical analysis was conducted to characterize the edaphic conditions during the crop cultivation period (Table 1), including information on nutrient levels, organic matter content, and other relevant parameters for plant development.

Table 1. Complete soil chemical analysis of experimental area in Botucatu, São Paulo, Brazil at depths of 0–20 cm and 20–40 cm.

Date	Depth (cm)	pH	OM	P	K	Ca	Mg	CEC	V%	S	B	Cu	Fe	Mn	Zn
		C _a Cl ₂	G·dm ⁻³	mg·dm ⁻³	mmol _c ·dm ⁻³				%		mg/dm ³				
March 2020	0–20	5.0	25	56	3.4	39	11	77	70	71	1.00	4.7	19	5.1	3.8
	20–40	4.8	19	30	2.2	23	10	70	50	52	0.90	5.4	17	3.0	1.7

Source: Laboratories of Soil Fertility and Plant Mineral Nutrition of the Department of Soil and Environmental Resources; OM: organic matter; V%: Cation Saturation Percentage; CEC: Cation Exchange Capacity.

2.2. Experimental Design

A randomized block design was adopted, consisting of seven treatments (different hop varieties), Cascade, Centennial, Columbus, Chinook, Comet, Fuggle, and Nugget, with four blocks and two plants per plot, totalizing 28 experimental plots. The seedlings were sourced from a licensed nursery Van den Bergen, located in the state of Minas Gerais, Brazil and certified for genetic authenticity.

2.3. Cultivation Characterization

Hop seedlings were planted in November 2020, at a spacing of 4.0 m × 1.0 m., and the plants were trained in a “V” shape, with the most vigorous branches selected, following the hexagonal form [14], to be guided along the sisal. Soil preparation involved scarification, followed by plowing, harrowing, furrowing, and hole opening. Soil correction and planting fertilization were carried out based on complete soil chemical analyses (Table 1), following recommendations established for the crop in the international literature [15]. Limestone and gypsum were applied, while the planting fertilization consisted of vegetable compost, Yoorin master[®], calcitic lime, and potassium sulfate. Drip irrigation was used in the experimental area.

Green manure species were sown between the hop rows, including *Crotalaria* (*Crotalaria breviflora*), jack bean (*Canavalia ensiformis*), pearl millet (*Pennisetum glaucum*), and buckwheat (*Fagopyrum esculentum*). Phytosanitary control involved the application of Abamectin (Abamex[®]) for mites, Fipronil (Regent[®]) for ants, and Dipel[®] (*Bacillus thuringiensis*) in cases of caterpillar infestation. Green manure was mowed at the onset of flowering, and the biomass was distributed along the rows as mulch. Spontaneous vegetation was controlled mechanically, as needed, by mowing, weeding, and uprooting.

2.4. Harvest and Sample Processing

The harvest took place in March (2021), with the ideal timing determined based on qualitative sensory parameters—such as color, aroma, and cone texture [16]. Additionally, a quantitative parameter was considered by calculating the dry matter content, with the optimal harvest range set between 20% and 33% [17].

After harvesting the whole plants from the experimental area, the inflorescences were separated from the vegetative structure of the plant and placed in an oven to dry at 40 °C for 24 h until reaching a moisture content between 8% and 10%. The samples were obtained from two plants per plot following the experimental design. After that, the cones of each variety were placed in plastic bags, vacuum sealed, and stored in the freezer (−4 °C) until chemical analysis.

2.5. Essential Oil Extraction

Essential oil extraction was carried out via hydrodistillation using a Clevenger-type apparatus. For each extraction, 50 g of hop cones were utilized, and the distillation was conducted over a 90 min period [18]. Extractions were performed in triplicate for each

pooled sample of the hop varieties. The yield (%) was calculated and expressed on a dry basis ($\text{Yield (\%)} = (\text{Essential oil/Dry material mass}) \times 100$).

2.6. Chemical Composition Analysis of Essential Oils by Gas Chromatography–Mass Spectrometry and Flame Ionization (GC-MS/FID)

The essential oil samples were diluted in ethyl acetate (Tedia, chromatographic grade, Fairfield, OH, USA, 1 mg.mL^{-1}) and $1 \mu\text{L}$ of the solution was injected in triplicate. The analyses were performed using a Thermo Scientific gas chromatograph (model TRACE 1300 Series GC—Thermo Scientific—Waltham, MA, USA) equipped with a flame ionization detector (FID), a mass spectrometer (model ISQ 7000), and a Triplus RSH automatic injector (ThermoFisher Scientific). The injector was maintained at 220°C , with a helium carrier gas flow (99.9999% purity) at a split ratio of 1:20. The mass spectrometer (MS) operated in full scan mode, using electron impact ionization (70 eV), with an acquisition range from 40 to $450 m/z$. The interface temperature was maintained at 230°C , and the detector temperature was set to 250°C .

Separation of the substances was carried out using an Rtx-5MS capillary column ($30 \text{ m} \times 0.25 \text{ mm}$, $0.25 \mu\text{m}$, Restek Co., Bellefonte, PA, USA), with a carrier gas flow of 1.0 mL.min^{-1} under the following temperature program: 60°C to 240°C at 3°C.min^{-1} .

The Chromeleon software 7 (Thermo Scientific, Waltham, MA, USA) was used for data acquisition and processing. The identification of the substances was performed by comparing the mass spectra with the National Institute of Standards and Technology (NIST 14) library, the Flavour & Fragrance Natural & Synthetic Compounds (FFNSC 3) library, and the linear retention indices (LRI) of the substances with data from the literature (Adams, 2017 [19]). The linear retention indices were obtained by injecting a series of n-alkanes (C9–C24, Sigma-Aldrich, St. Louis, MI, USA, 99%) under the same chromatographic conditions as the samples, using the Van den Dool and Kratz (1963) equation [20]. The concentration of the metabolites was obtained by area normalization.

2.7. Total α - and β -Acid Content (%)

The determination of total α - and β -acid content was carried out by extracting the acids from the hop sample, followed by spectrophotometric analysis at three wavelengths [21]. Analyses were performed in triplicate for each pooled sample of the hop varieties.

Bitter acid extraction was performed using a 2.5 g sample of finely ground hop, which was added to 50.0 mL of methanol. The mixture was stirred for 30 min at room temperature, followed by a 10 min resting period. Next, membrane filtration (Millipore, $0.45 \mu\text{m}$) was performed to remove particulate material. An aliquot of $50 \mu\text{L}$ of the filtrate was placed in a 25 mL volumetric flask, and the volume was completed with the extracting solution of methanolic NaOH (0.5 mL of 6M NaOH in 250 mL of methanol). The resulting solution was placed in a quartz cuvette with a 1 cm optical path length for UV–visible spectrum analysis, using $50 \mu\text{L}$ of methanol in 25 mL of methanolic NaOH as the blank. Absorbance values were recorded at wavelengths of 275, 325, and 355 nm. From these readings, the following were determined: α -acid content (%) = $[(-51.26A_{355 \text{ nm}}) + (73.79A_{325 \text{ nm}}) - (19.07A_{275 \text{ nm}})]$; and β -acid content (%) = $[(55.27A_{355 \text{ nm}}) - (47.59A_{325 \text{ nm}}) + (5.1A_{275 \text{ nm}})]$; where A = absorbance reading at each wavelength [13].

2.8. Statistical Analysis

The data for α - and β -acid were subjected to analysis of variance (ANOVA), followed by the Scott–Knott mean test at a 1% probability level, using Sisvar software 5.4 [22]. The chemical composition data of the essential oils were analyzed using multivariate analysis with the statistical software MetaboAnalyst 5.0 [23]. Sample data were normalized, and variable values were auto-scaled. The analyzed variables were subjected to multivariate

data analysis using unsupervised methods—Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA).

3. Results and Discussion

3.1. Bitter Acids

The varieties presented different averages of α -acids (Table 2). Comet presented the highest concentration (10.54%), which was followed by the varieties Columbus (4.25%), Fuggle (2.86%), Chinook (1.41%), Centennial (1.35%), Nugget (1.15%), and Cascade (0.983%). The varieties do not show significant differences in β -acid averages at $p < 0.01$.

Table 2. Total α - and β -acids (%) of cones of *Humulus lupulus* L. varieties grown in Botucatu, São Paulo, Brazil.

Variety	Total α -Acids (%)	Total β -Acids (%)
Comet	10.54 ^a	4.30
Columbus	4.25 ^b	2.69
Fuggle	2.86 ^c	1.92
Chinook	1.41 ^d	1.79
Centennial	1.35 ^e	2.15
Nugget	1.15 ^f	1.82
Cascade	0.98 ^g	1.30

The different letters in the columns indicate significant differences ($p < 0.01$) between the groups, according to the Scott–Knott test.

Hop-derived α -acids are among the most significant components of hops. Hops have a complex composition, with α -acids comprising 2 to 12% of their weight and β -acids accounting for 1 to 10%. Both substances are precursors of bittering agents in beer. Furthermore, α -acids play a crucial role in enhancing foam stability, preventing gushing, and contributing to beer preservation [24]. Their primary function occurs during the boiling process of wort with hops, where they undergo isomerization, leading to the formation of iso- α -acids, which are responsible for the intense bitterness of beer. The bitter potential of hops is a key quality criterion, which is why varieties with a high percentage of α -acids are highly valued. However, in terms of bitterness, iso- α -acids are far more important than α -acids themselves in beer. Since iso- α -acids result from the isomerization of α -acids, high α -acids hop varieties have been extensively used to develop improved cultivars [25].

According to Barth Haas (the major global company specializing in hops and hop products), the Comet variety exhibits an α -acid content of 11.9%, closely aligning with its highest observed concentration in this study (10.54%), demonstrating its potential adaptability to tropical climates, highlighting its viability for cultivation in such conditions. In contrast, Columbus, reported to have the highest α -acid content (15.0%) by Barth Haas, presented a significantly lower concentration in this study (4.25%), indicating the influence of additional factors beyond α -acid content alone [26]. Fuggle, with a moderate α -acid content of 5.1%, demonstrated a reasonable alignment with its measured concentration (2.86%). Meanwhile, Chinook and Centennial, despite their relatively high α -acid contents of 11.4% and 9.2% [26], respectively, exhibited substantially lower concentrations (1.41% and 1.35%), suggesting the interplay of other environmental or physiological variables. Similarly, Nugget, which possesses one of the highest α -acid levels (15.2%), displayed a low concentration (1.15%), further emphasizing indications of the complex interaction with the environment and the synthesis of these compounds [27]. Cascade showed the lowest concentration in this study (0.98%). Interestingly, β -acid concentrations did not present statistically significant differences across the studied varieties ($p < 0.01$), suggesting a more uniform distribution of these compounds in contrast to the variability observed in α -acids.

Cascade and Chinook hop varieties were evaluated under tropical conditions in Brazil during the years 2020 and 2021 [28]. The average concentration of bitter acids in both

varieties was lower than the expected reference values from Barth Haas (4.22% and 9.9%, respectively). Specifically, the α -acid concentration in the Cascade variety should range between 4.5% and 9.0%, while for Chinook, it is expected to fall between 12.0% and 15.3%. The β -acid concentration in Cascade and Chinook should be within 3.6–9.0% and 3.0–4.9%, respectively. This difference might be attributed to the age of the plant, which may present a higher content and stability of compounds at maturity [16,29,30]. The quality of Cascade variety hops was also evaluated under subtropical conditions in Florida, USA, over two consecutive years and a double-season hop production system. They found that from spring to fall, the total α -acid concentration decreased by 10% in year 1 (from 5.94% to 5.35%) and by 71% in year 2 (from 8.25% to 2.39%). A similar trend was observed for total β -acid concentration, which decreased by 25% in year 1 (from 3.55% to 2.66%) and by 70% in year 2 (from 5.15% to 1.55%) [31].

The lower α -acid levels observed in this study compared to expected values from Barth Haas may be attributed to environmental factors, plant maturity, and post-harvest handling. Genetics plays a crucial role in the expression of α -acid content, and it is possible that some cultivars have already reached satisfactory levels of these compounds. Overall, the improvement and selection of hop cultivars in Brazil should focus on genotypes that either enhance or fully exploit taste and aroma, with α -acid levels below 7% [32]. On the other hand, climate conditions such as higher temperatures, increased UV exposure, and soil nutrient availability can influence α -acid biosynthesis, potentially leading to lower accumulation [33,34]. Monitoring the maturity of hop plants also becomes an important point to understand the increase in the content of compounds according to maturation, especially focused on tropical conditions. Additionally, it is important to keep the hops, pellets, or cones vacuum packed, if possible, with a modified atmosphere for N₂ and refrigerated. Anaerobic conditions at 4 °C were the best to keep bitter acids stable over the time period [35].

3.2. Essential Oil Yield and Composition

The essential oil yields measured in this study ranged from 0.70% in Cascade to 2.36% in Comet. Table 3 shows the essential oil yield for each variety.

Table 3. Essential oil yield (%) of hop varieties cultivated in Botucatu, São Paulo, Brazil.

	Comet	Columbus	Fuggle	Chinook	Centennial	Nugget	Cascade
Yield (%)	2.36 ^a	1.17 ^b	0.70 ^d	0.94 ^c	1.13 ^{bc}	1.07 ^{bc}	0.70 ^d

The different letters in the line indicate significant differences ($p < 0.01$) between the groups, according to the Scott–Knott test.

The values are within the broader interval of 0.5–3.0% (dry-cone basis) documented by Almaguer et al. (2014) for hop cultivars grown under temperate conditions [36]. This alignment suggests that the Brazilian subtropical environment does not inherently suppress overall oil production relative to standards established in temperate regions. A significant increase was observed in volatile oil content between the first and second years of hop cultivation, emphasizing the role of plant maturity in essential oil accumulation. In 2021, Cascade (0.75 mL/100 g) and Chinook (1.43 mL/100 g) fell within their expected reference ranges, indicating that oil composition stabilizes as plants mature. This supports the notion that younger hop plants yield lower essential oil amounts, with environmental factors such as temperature and soil conditions also contributing to variability [28].

In the seven analyzed varieties, 49 substances were identified in the essential oil, with variations in their relative proportions among the studied varieties. Five major compounds were consistently identified across all hop varieties analyzed. β -Myrcene content ranged from 7.98% in Centennial to 62.05% in Fuggle. Caryophyllene was found in concentrations ranging

from 2.38% in Centennial to 6.65% in Chinook. α -Humulene varied from 0.12% in Fuggle to 11.34% in Cascade. β -Selinene ranged from 0.28% in Columbus to 7.48% in Centennial, while α -selinene ranged from 0.68% in Cascade to 11.18% in Centennial. The following table presents all compounds identified, highlighting their respective relative contents (%) (Table 4).

Table 4. Chemical composition (relative content%) of hops varieties grown in Botucatu, São Paulo, Brazil.

Substance	LRI Calc.	LRI Lit.	Relative Content (%)						
			Chinook	Fuggle	Nugget	Cascade	Centennial	Comet	Columbus
isobutyl isobutyrate	915	908	–	–	0.37	–	–	–	–
α -pinene	936	932	–	0.08	–	–	–	–	–
isoamyl propionate	973	960	–	–	0.67	0.22	–	0.43	0.35
β -pinene	985	974	0.37	0.85	0.63	0.67	–	0.58	0.43
β -myrcene	992	988	34.44	62.05	57.375	51.82	7.98	56.8	56.5
isoamyl isobutyrate	1015	1007	–	–	1.34	0.36	–	–	–
isopentyl isobutyrate	1018	1014 *	0.41	–	2.2	0.70	–	0.48	0.64
methyl heptanoate	1028	1021	0.27	0.22	1.73	0.23	–	0.20	–
limonene	1034	1024	–	0.19	0.12	–	–	–	–
β -phellandrene	1037	1025	–	0.25	–	–	–	–	0.27
(<i>E</i>)- β -ocimene	1048	1044	–	–	–	–	–	2.65	–
linalol	1095	1095 *	0.47	0.50	1.54	0.30	0.31	0.90	0.99
n-nonanal	1113	1100	0.57	0.41	0.88	1.65	0.44	–	0.36
methyl octanoate	1129	1123	–	–	0.94	0.23	0.38	0.32	–
2-decanone	1200	1190	0.50	0.27	–	–	0.71	–	–
methyl-(2 <i>E</i>)-nonenoate	1217	1221	–	–	0.46	–	–	–	–
methyl nonanoate	1229	1223	0.26	–	1.35	0.36	0.92	0.35	–
3 <i>Z</i> , 6 <i>Z</i> , 9 <i>Z</i> -tridecatiene	1288	1286	–	–	–	–	0.18	–	–
2-undecanone	1300	1291 *	0.46	0.27	1.19	1.67	4.43	0.34	–
methyl geranate	1329	1322	1.08	0.24	–	–	–	0.94	0.66
methyl decanoate	1329	1323	–	–	0.84	–	0.46	–	–
geranyl acetate	1384	1379	0.53	–	–	0.98	–	1.60	2.96
2-dodecanone	1401	1388	–	0.11	–	–	0.88	–	–
(<i>E</i>)-caryophyllene	1425	1417	6.65	4.15	3.42	3.98	2.38	9.7	5.18
β -copaene	1436	1430	–	–	–	–	–	–	0.17
(<i>E</i>)- α -bergamotene	1436	1432	0.81	0.56	–	–	1.39	–	–
(<i>E</i>)- β -farnesene	1456	1454	16.73	14.49	–	4.36	31.09	–	–
α -humulene	1462	1455 *	5.49	0.12	9.64	11.34	2.00	0.51	6.14
geranyl propanoate	1475	1476	–	–	–	–	–	1.37	1.76
β -chamigrene	1479	1476	1.08	0.75	–	–	0.73	0.69	–
γ -muurolene	1481	1478	0.29	–	–	–	–	–	0.32
β -selinene	1497	1489	7.26	5.67	1.89	0.82	7.79	5.98	0.28
2-tridecanone	1502	1495	–	–	0.34	2.49	–	–	–
α -selinene	1503	1505 *	9.41	6.45	1.27	0.68	11.18	3.78	0.95
(<i>E,E</i>)- α -farnesene	1513	1505	–	0.10	–	–	0.58	–	–
geranyl isobutanoate	1513	1514	1.29	–	–	1.37	–	4.89	2.43
γ -cadinene	1521	1514 *	0.35	0.17	–	–	–	–	0.42
δ -cadinene	1525	1522	0.66	0.16	–	0.44	–	–	0.60
selina-3,7(11)-diene	1549	1545	–	–	–	–	1.07	0.85	0.21
germacrene B	1568	1559	–	–	–	–	1.79	0.26	0.28
(2 <i>E</i>)-tridecenol	1577	1568	–	–	–	–	1.06	0.58	–
caryophyllene oxide	1593	1593	–	0.10	–	–	–	–	0.24
humulene epoxide II	1622	1608	–	–	0.55	0.95	–	–	–
neo-intermedeol	1668	1658	–	0.23	–	–	–	–	–
14-hydroxy-(<i>Z</i>)-caryophyllene	1668	1666	1.50	–	1.37	–	5.45	2.20	2.12
intermedeol	1672	1666	2.04	1.17	–	–	3.28	1.74	0.81
14-hydroxy-9-epi-(<i>E</i>)-caryophyllene	1673	1668	–	–	0.28	–	–	–	–
(6 <i>Z</i>)-pentadecen-2-one	1677	1667	–	–	0.20	–	1.69	0.24	0.16
2-pentadecanone	1705	1697	–	–	–	–	0.36	–	–
monoterpene hydrocarbons			34.81	63.42	58.13	52.49	7.98	60.03	57.20
oxygenated monoterpenes			0.47	0.50	1.54	0.30	0.31	0.90	1.02
sesquiterpene hydrocarbons			48.73	32.62	16.22	21.62	60.00	21.77	14.55
oxygenated sesquiterpenes			5.50	1.70	3.57	0.95	14.56	6.14	5.53
ketones			0.96	0.65	1.73	4.16	8.07	0.58	0.16
esters			3.84	0.46	9.90	4.45	1.76	10.58	8.80

LRI Calc.: Calculated Linear Retention Index; LRI Lit.: Literature Linear Retention Index (Adams, 2017) [19]; *: Linear Literature Retention Index NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA); –: not detected.

Essential oils are chemical compounds responsible for the aromatic characteristics of hop varieties and play a key role in imparting aroma to beer. These compounds are primarily classified into three main groups: monoterpenes (β -myrcene, limonene, and α -pinene), sesquiterpenes (farnesene, α -humulene, and β -caryophyllene) and terpene alcohols (linalool). Hydrocarbons generally account for 50–80% of the total oil and include monoterpenes (such as α - and β -pinene, β -myrcene, and limonene) and sesquiterpenes (like α -humulene, β -farnesene, β -caryophyllene, α - and β -selinene, and γ -muurolene) [4]. The hop varieties exhibited a high content of monoterpene and sesquiterpene hydrocarbons. Except for Centennial, the monoterpene hydrocarbon class was the most predominant in all varieties, mainly due to the high concentration of β -myrcene, which was the most abundant compound across all samples. Notably, the Fuggle variety displayed the highest β -myrcene content (62.05%), while Centennial had the lowest (7.98%). β -Myrcene is considered the main precursor to the aroma of green hops, with a scent described as herbaceous, resinous, green, balsamic, and reminiscent of fresh hops [37].

Sesquiterpene hydrocarbons are also the class of substances most present in hops, such as (*E*)-caryophyllene and α -humulene. However, the hop varieties analyzed exhibited distinct essential oil compositions, with β -myrcene, (*E*)-caryophyllene, and α -humulene being the only substances common to all varieties. While major components, such as α -acids, tend to remain relatively stable, minor components like essential oils are more susceptible to environmental variation. However, the changes induced by edaphoclimatic and biotic conditions result in region-specific traits, collectively referred to as terroir [38,39]. Therefore, more abundant and less abundant substances are both important for characterizing hop varieties by locality. According to the HCA (Figure 1), five groups are formed based on the chemical composition of essential oil and bitter acid content, with a cutoff at 75% of the Euclidean distance for classification.

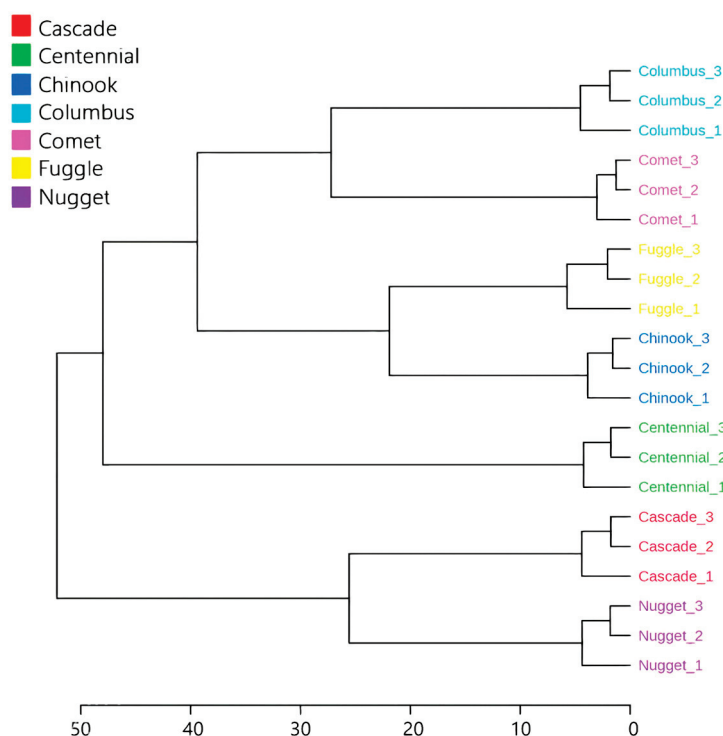


Figure 1. Hierarchical Cluster Analysis (HCA) of *Humulus lupulus* L. essential oil from different varieties grown in Brazil.

The first group consists of the Cascade and Nugget variety, which differed most among the samples. This variation could be attributed to their similar levels of α -humulene,

which are notably higher than those observed in the other varieties. The second group includes just the Centennial variety, displaying a markedly different profile, with only 7.98% β -myrcene but a substantial 31.09% of (*E*)- β -farnesene, making it the predominant compound in this variety. This hop is commonly used for dual purposes, both aroma and bittering, though farnesene is listed as one of its minor constituents on commercial pellet packaging. The third group consists of Chinook and Fuggie, the fourth group is formed by Comet variety, and the fifth is formed by Columbus. These results can be explained by PCA, where the weights of each variable for the formed groups are shown in Figure 2. The first two principal components explained 60.15% of the total variance, with PC1 accounting for 37.61% and PC2 for 22.54%.

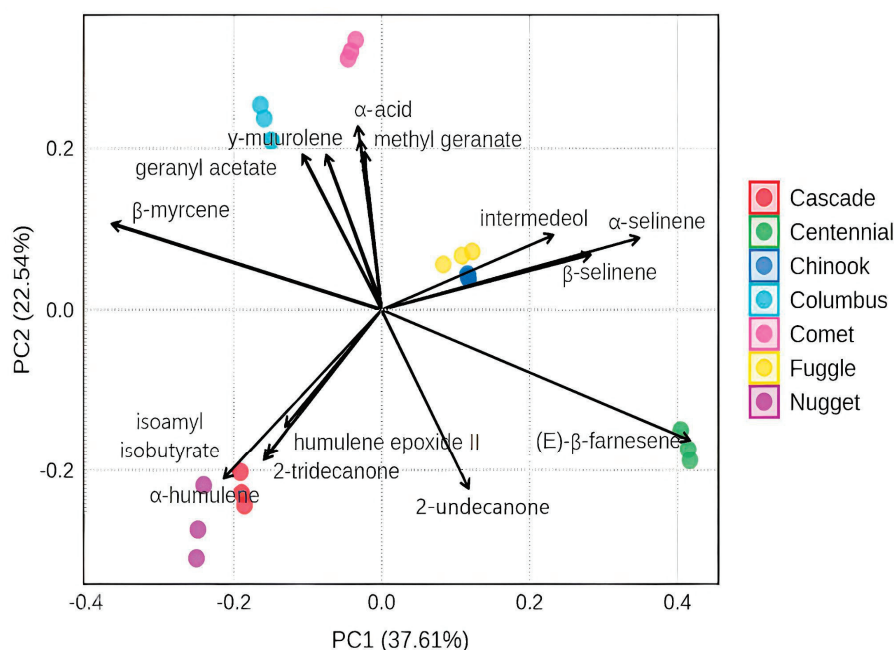


Figure 2. A biplot of the Principal Component Analysis (PCA). The vectors represent key chemical compounds contributing to the differentiation of the hop samples.

The analysis reveals the formation of five distinct groups of hop varieties, each characterized by correlations with specific chemical compounds: the first group, located towards the negative side of PC1, includes Cascade and Nugget. These varieties exhibit a strong correlation with α -humulene and humulene epoxide II in addition to isoamyl isobutyrate and 2-tridecanone, suggesting a chemical profile differentiated by these compounds. The second group, positioned on the positive side of PC1, is represented by Centennial, strongly associated with (*E*)- β -farnesene, indicating a distinct compositional pattern compared to the first group. The third group, located near the center of the biplot, consists of Fuggie and Chinook. These samples are associated with a broader distribution of compounds, including α -selinene and β -selinene, without a strong correlation to a specific subset of chemical markers.

PCA placed Comet and Columbus in distinct clusters, showing that, despite some compositional overlap, their volatile oil profiles differ enough to separate them statistically. Both cultivars exhibit very high β -myrcene levels, Comet (55.8%) and Columbus (56.5%), and a complete absence of (*E*)- β -farnesene; however, their sesquiterpene profiles differ significantly. Comet contains extremely low α -humulene (0.51%) and moderate (*E*)-caryophyllene (9.7%), whereas Columbus shows a higher α -humulene level (6.14%) but a lower (*E*)-caryophyllene fraction (5.18%). These quantitative shifts strongly influence the

loadings of PC1, which is weighted toward α -humulene and (*E*)-caryophyllene, thereby positioning the two varieties in separate regions of the score plot.

Notably, the lack of detectable farnesene in Columbus matches the reference range reported for its essential oil, and its β -myrcene content (25–55%) falls within the values documented in the literature [40]. Consequently, although Comet and Columbus share a myrcene-dominant profile, their distinct sesquiterpene balances create clearly differentiated chemometric signatures. Regarding Comet, previous studies have reported that (*E*)- β -ocimene accounts for more than 2% of its chemical composition, which aligns with our findings (2.65%), as it was the only variety to present this compound. Additionally, while Comet is not a variety rich in α -humulene, it does contain a considerable percentage of α - and β -selinene, as well as a notable amount of geranyl isobutanoate (4.89%). The variety had 56.8% of β -myrcene and the highest value of α -acids [41].

Monoterpene hydrocarbons, particularly β -myrcene, also dominate the essential oil profiles of Cascade, Chinook, and Comet varieties cultivated in Southern Italy, with β -myrcene comprising 42–62% of the total composition [41]. Similarly, significant variability was found in β -myrcene levels among Nugget, Columbus, and Chinook varieties, ranging from 15% to 52.06%, alongside consistent α - and β -acid content, which are critical for bitterness in brewing [42]. In contrast, environmental factors, such as seasonal changes in subtropical Florida, significantly impact the total essential oil content of the Cascade variety, with an 85% decrease observed over two consecutive years [31]. A study further corroborated these findings, showing that the Cascade variety cultivated in Florida exhibited seasonal and yearly variations in essential oil content and bitter acid profiles. Myrcene remained the most abundant terpene (over 31%), while α -acid content varied significantly between seasons and years, ranging from 2.7% to 6.2% [43].

Studies analyzing Brazilian hops identified α -selinene and β -selinene as potential discriminants of Brazilian varieties, along with 2-dodecanone and 2-decanone, which contribute floral aromas. The authors also found that (*E*)- α -bergamotene played a significant role in distinguishing Brazilian hops [44]. Our results showed that Centennial contained 1.39% of this compound, which is associated with floral, sweet, and citrus aromas, reinforcing its classification as a highly valued aroma variety. In another study evaluating four varieties, Centennial exhibited (*E*)- β -farnesene as the major compound, along with a high content of selinene isomers, which were also present in Fuggle varieties [45]. All varieties contained high levels of these compounds, ranging from 0.28% of α -selinene and 0.95% of β -selinene in Columbus to 7.79% of α -selinene and 11.18% of β -selinene in Centennial. Cascade and Nugget exhibited less content of these substances. Consequently, although these varieties showed a lower relative percentage of these compounds, all varieties contained α - and β -selinenes, showing that this pattern repeats for the varieties.

3.3. Climate Adaptation Implications

The difference between the values presented in this study and the values disseminated for each variety can be explained because, as these are compounds of specialized metabolism, their production is influenced by climatic factors, type of cultivation soil, hours of sunlight, and temperature [27]. Studies have demonstrated that growing conditions (such as soil composition, moisture, microclimate, etc.) significantly influence the aroma, particularly in certain cultivars. Climate change, especially adverse temperatures and more frequent droughts have already led to stagnation in crop yields and a decline in α -acid content in certain farming regions. This makes it difficult to maintain stable hop characteristics, which are essential for consistent quality in the brewing market [46]. Additionally, hops are plants more sensitive to climate warming because their adaptation is slower [47].

Climate change has significantly impacted hop cultivation in the Czech Republic, with rising temperatures, increased sunshine, and reduced humidity observed between 1951 and 2006. The Saaz yield has stagnated in recent years and so α -acid content in Saaz hops has declined at a statistically significant rate, which could affect the quality of the hops used in brewing [48]. Given that hop cultivation is concentrated in a small region, these climatic shifts may necessitate a regional redistribution of production to mitigate risks and ensure long-term sustainability. Despite global warming, hop cultivation has expanded in regions with higher temperatures and lower rainfall, supported by well-developed hop industries [12].

Climate sensitivity of European hop yield, α -acid content, and cone development was assessed from 1970 to 2050 using meteorological measurements and model projections, with a 1.4 °C temperature rise and a 24 mm decrease in precipitation. Data from Germany, the Czech Republic, and Slovenia, which represent nearly 90% of hop-growing regions, show that hop ripening started 20 days earlier, production dropped by 0.2 t/ha/year, and α -acid content fell by 0.6% after 1994. By 2050, hop yield and α -acid content are predicted to decline by 4–18% and 20–31%, respectively, highlighting the need for immediate adaptation measures [13]. Central Europe is undergoing significant climatic shifts that are expected to intensify during the 21st century, with major implications for agricultural productivity [49].

By leveraging advanced analytical methods, such as GC-MS and spectrophotometry, they have been able to monitor and maintain the chemical profiles of hops, particularly essential oils and bitter acids, to meet the stringent quality standards required for brewing. This commitment to quality, combined with innovative adaptation strategies, has not only strengthened the domestic hop supply chain but also positioned Brazil as a promising region for hop cultivation in the face of global climate challenges [8,37,50,51]. These advancements demonstrate that Brazil, with its unique climate and technological adaptations, can serve as a promising alternative for hop production, offering resilience against the climate-induced declines in quality and quantity faced by traditional hop-growing regions in Europe and North America.

In recent years, Brazilian producers and researchers have made significant efforts to establish a sustainable and high-quality hop production chain in Brazil, despite the challenges posed by the country's tropical and subtropical climates. Recognizing the growing demand for locally sourced ingredients in the craft beer industry, these stakeholders have invested in agronomic research, varietal selection, and cultivation techniques tailored to Brazil's unique environmental conditions [50]. Nevertheless, Brazil lacks rigorously validated, region-specific hop quality standards, obliging researchers to compare local material against international benchmarks that may not accurately reflect domestic agronomic and chemical realities. In this investigation, the data set was generated on a small scale at a single experimental site; future investigations conducted across multiple locations and seasons will be essential to strengthen statistical power and broaden the applicability of these findings.

4. Conclusions

Our results show that hops grown in Brazil's subtropical climate can meet key brewing requirements, especially when the right varieties are chosen. Comet delivered the highest α -acid levels alongside a favorable mix of aroma compounds, indicating that the careful matching of cultivar and environment can yield cones comparable to those from traditional temperate regions that are facing climate changes. Other varieties, although lower in α -acids, exhibited rich terpene profiles that make them attractive for aroma purposes. For local producers, this reinforces the importance of selecting varieties with desirable phytochemical profiles, particularly those adapted to tropical conditions and suited to

aroma or bitterness goals. For brewers, it opens opportunities to work with cultivars that either mimic traditional profiles or offer unique characteristics, fostering the creation of beer with a distinctive Brazilian terroir.

Future studies should prioritize understanding how environmental and management factors, such as nutrient supply, irrigation, and optimal harvest maturity, affect α -acid synthesis and overall productivity. Integrating chemical and yield data will be essential to identify hop varieties that meet both agronomic and industry standards, reducing dependence on imports and supporting the development of a competitive national hop market.

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Article

Phenological Development, Thermal Requirement, and Quality of ‘BRS Núbia’ (*Vitis vinifera* L. x *Vitis labrusca* L.) Grapes on Different Rootstocks

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Abstract

The cultivation of table grapes in Brazil is economically significant, with production influenced by edaphoclimatic factors and rootstock selection. The cultivar ‘BRS Núbia’ (*Vitis vinifera* L. x *Vitis labrusca* L.) is a promising alternative; however, its phenological behavior, thermal requirements, and compatibility with different rootstocks under subtropical conditions require further evaluation. This study aimed to assess the duration of phenological stages, thermal requirement, and ripening dynamics of ‘BRS Núbia’ grapevines grafted onto the rootstocks ‘IAC 572 Jales’, ‘IAC 766 Campinas’, and ‘Paulsen 1103’. The experiment was conducted in São Manuel, São Paulo, Brazil during the 2021 and 2022 production cycles using a split-plot experimental design (3 × 2). Evaluations included the duration of phenological stages from pruning to budburst, flowering, fruit set, onset of ripening, and harvest, as well as the ripening curve and thermal accumulation from pruning to harvest. Rootstocks did not significantly affect ($p > 0.05$) the duration of phenological stages; however, differences were observed between production cycles. The 2022 cycle was longer (167.7 days) compared to 2021 (142.6 days), with greater thermal accumulation (1871.7 GDDs vs. 1743.4 GDDs). The analysis of phenological stages revealed that, across both production cycles evaluated, the ‘BRS Núbia’ cultivar required an average accumulation of 1807.5 growing degree days from pruning to harvest. Soluble solids content ranged from 17.43 to 18.50°Brix, and titratable acidity decreased throughout maturation. The maturation index was highest in vines grafted onto ‘Paulsen 1103’, indicating its positive influence on fruit quality. The ‘BRS Núbia’ grapevine exhibited a mean thermal requirement of 1807.5 growing degree days (GDDs) to complete its phenological cycle, which lasted approximately 150 days under subtropical conditions.

Keywords: degree days; hybrid grapes; mesothermal climate; rootstocks

1. Introduction

The grapevine (*Vitis vinifera* L. x *Vitis labrusca* L.) is a perennial, climbing plant belonging to the Vitaceae family, widely cultivated in various regions of the world, especially in temperate and subtropical climates [1,2]. The grapevine exhibits a well-defined phenological cycle and is sensitive to factors such as temperature, photoperiod, and water availability [3–5]. This plant has great adaptability and is classified into different cultivars, intended both for table grape production and for processing into juice, wine, and raisins [6,7].

In addition to its agronomic relevance, the grapevine plays a significant role from both nutritional and socioeconomic perspectives. Grapes are rich in bioactive compounds such as polyphenols, flavonoids, and resveratrol—substances associated with reduced cardiovascular disease risk, antioxidant activity, and anti-inflammatory properties [8–11]. From a socioeconomic standpoint, viticulture drives important production chains, generating direct and indirect employment, fostering rural tourism, and promoting the development of communities in wine-producing regions [12–14]. Thus, grape cultivation represents not only an important source of agricultural income but also an asset for both public health and the local and global economy.

Viticulture is a strategically significant agricultural sector in South America, contributing substantially to the economies of countries such as Argentina, Brazil, Chile, Peru, and Uruguay; likewise, vineyard areas are integrated into and contribute to the landscape, providing social ecosystem services as a socio-cultural outcome [15–17]. In 2023, the global vineyard area reached 7.3 million hectares, resulting in a total grape production of 79.4 million tons, of which 43.3% was destined for fresh consumption [18]. In Brazil, viticulture accounts for approximately 1.45 million tons annually, with 52% of production dedicated to table grapes [19]. Beyond its economic relevance, table grape viticulture plays a crucial social role, generating employment in both large agro-industrial enterprises and small family-run farms [20].

Changes in environmental factors may elicit markedly different physiological and developmental responses among plant cultivars and genotypes, depending on their adaptive capacity and genetic background [21,22]. In the context of global climate change, which has been affecting orchard yields, the success of table grape production is directly related to the selection of appropriate rootstocks and cultivars that are well-adapted to local edaphoclimatic conditions and agricultural management practices [23–25]. Lack of or excess rainfall, extreme temperatures, hail, and frost affect grapevine crops worldwide [26]. For this reason, continuous assessment of the long-term evolution of climatic factors and their influence on vine production and quality is essential. The use of rootstocks is a well-established practice in viticulture, playing a crucial role in adapting vines to environmental conditions, controlling vegetative vigor, and mitigating biotic and abiotic stresses [27,28]. Furthermore, the interaction between the scion and the rootstock directly affects phenology, thermal requirements, yield, and fruit quality, serving as a key determinant for vineyard longevity and production efficiency.

In addition to their effects on phenology, vegetative vigor, and stress mitigation, rootstocks also significantly influence the physicochemical characteristics and sensory quality of grapes, particularly in warm-climate viticulture. Studies have shown that rootstock–scion interactions can affect berry size, skin thickness, sugar and acid content, phenolic composition, and aromatic profiles, thereby altering the sensory attributes and market value of grapes [28–30]. In tropical and subtropical regions, where climatic conditions accelerate ripening and increase disease pressure, selecting rootstocks that promote favorable fruit quality traits is critical for achieving commercial standards and consumer acceptance [30,31]. Therefore, assessing the compatibility of rootstocks with scions such as

‘BRS Núbia’ under these conditions is essential to ensure not only agronomic performance but also high-quality fruit production.

In recent years, Brazilian viticulture has expanded into non-traditional regions, such as the Northeast, driven by technological innovations and market demands [27,32]. In this context, selecting rootstocks compatible with different cultivars and growing environments is fundamental to ensuring productive success and the sustainability of viticultural activities. Among the widely used rootstocks in subtropical regions, ‘IAC 572 Jales’ and ‘IAC 766 Campinas’ stand out for imparting vigor to plants and providing resistance to foliar diseases [28,33–35]. Meanwhile, ‘Paulsen 1103’ exhibits high tolerance to water deficit and pest resistance, making it widely employed in regions with greater water restrictions, such as the state of São Paulo [36,37].

‘BRS Núbia’ grape, released by Embrapa in 2013, is a promising alternative for table grape production, characterized by a medium cycle of 120 days, uniform clusters, medium-sized berries, firm texture, and neutral flavor [38]. Although recommended for tropical growing conditions, its adaptation to subtropical regions still requires validation, particularly regarding compatibility with the most commonly used rootstocks in these areas. Analyzing the phenology, thermal requirements, and ripening of ‘BRS Núbia’ grafted onto different rootstocks under subtropical conditions is essential to determining its agronomic viability and establishing technical recommendations for its production.

Table grapes have specific thermal requirements to ensure proper development and high fruit quality, requiring higher temperatures during the production cycle, especially at the phenological stages of budburst, flowering, and ripening [39,40]. Heat accumulation over time is measured in growing degree days (GDDs), which is essential for the efficient completion of the crop cycle. In hot and dry climates, cultivation is favored, as these conditions reduce disease incidence and contribute to uniform ripening and market-quality fruit [39].

Characterizing grapevine phenological development allows for the refinement of management practices, optimizing input use and scheduling agricultural activities. Furthermore, understanding the cultivar’s thermal requirements enables the prediction of its production cycle duration under different climatic conditions, facilitating decision making to maximize productivity and fruit quality [41,42].

Given the above, this study aimed to evaluate the duration of phenological stages, thermal requirements, and ripening of ‘BRS Núbia’ grape grafted onto different rootstocks under subtropical conditions in the state of São Paulo, Brazil.

2. Materials and Methods

2.1. Experimental Site Location

The experiment was conducted over two production cycles (2021 and 2022) at the São Manuel Experimental Farm, located in the municipality of São Manuel, São Paulo, Brazil (22°44′28″ S, 48°34′37″ W) at an altitude of 740 m. The region has a humid subtropical climate (Cfa, according to the Köppen classification), characterized by an average annual precipitation of 1433 mm, an average annual temperature of 22.4 °C, and a relative humidity of 71%. The soil at the experimental site is classified as a Red Nitrosol [43].

For both production cycles, pruning was carried out in July, while harvest occurred in December. Throughout the study, meteorological variables were monitored, including air temperature (mean, minimum, and maximum) and rainfall, with monthly records during the production cycle. During the berry ripening period, climate measurements were intensified, with daily records for both production cycles (Figure 1a,b).

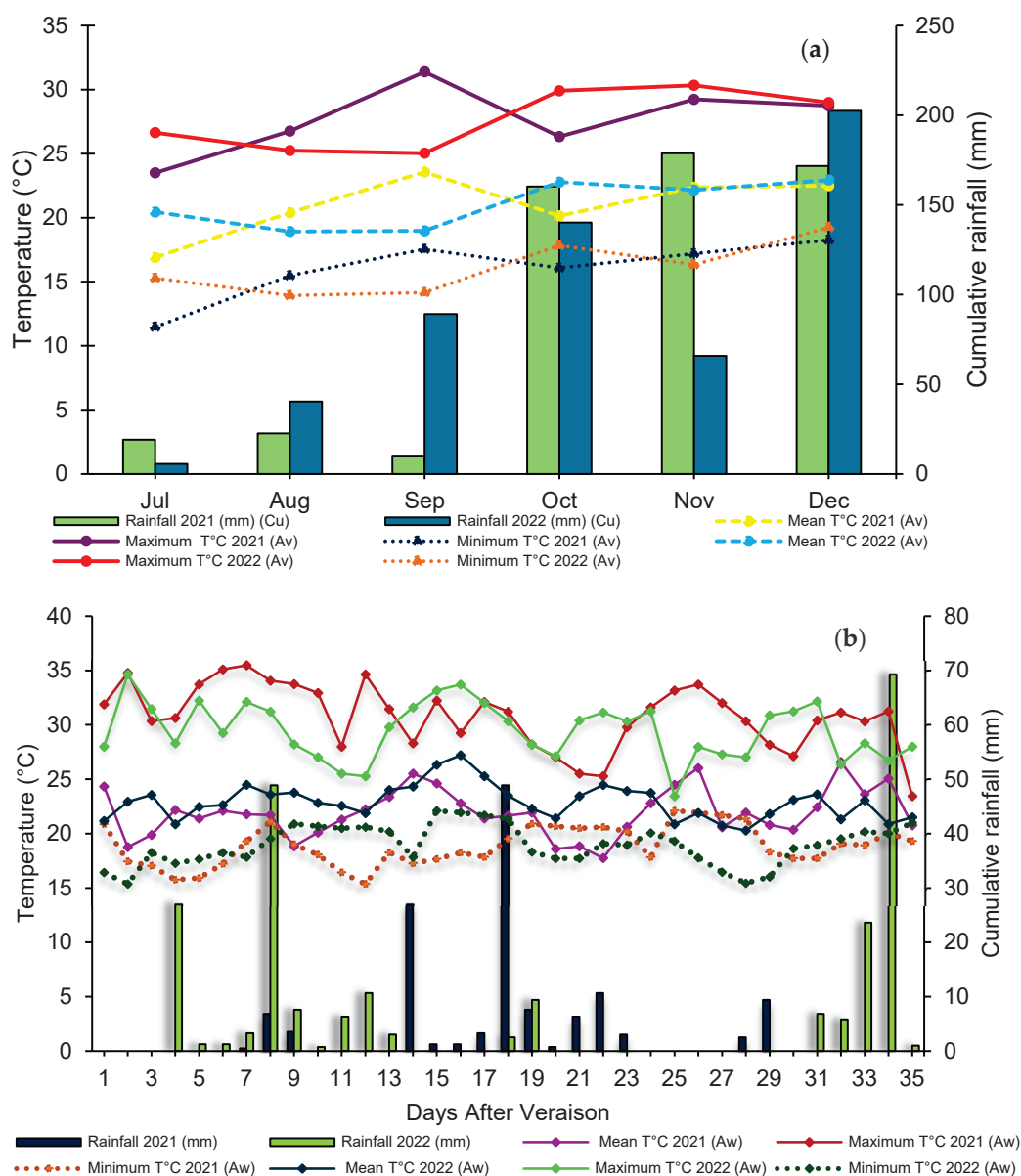


Figure 1. Annual meteorological data of maximum, minimum, and average temperature and precipitation during the production (a) and maturation (b) cycles of ‘BRS Núbia’ grapes in cycle I and cycle II, São Manuel, São Paulo, Brazil. Source: Department of Soils and Environmental Resources (FCA/UNESP).

2.2. Experimental Site Setup and Management

The planting of grafted ‘BRS Núbia’ grapevines (‘Michele Palieri’ × ‘Arkansas 2095’) was carried out in August 2020. The seedlings were grafted onto the rootstocks ‘IAC 572 Jales’ (‘101-14 Mgt’ (*Vitis riparia* × *Vitis rupestris*) × *Vitis caribaea*), ‘IAC 766 Campinas’ (*V. riparia* × *V. rupestris*) × *V. cordifolia* ‘106-8 Mgt’ × *Vitis caribaea*), and ‘Paulsen 1103’ (*V. berlandieri* × *V. rupestris*). The vines were trained in the Y-trellis system (Open Gable) using a metal structure and spaced at 3.0 m × 2.0 m.

Short pruning (two buds per cane) was adopted in both production cycles, followed by the application of 2.5% hydrogen cyanamide (Dormex®, BASF, Ludwigshafen am Rhein, Germany) to stimulate budburst. After budburst, a single productive shoot per cane was maintained, and the following cultural practices were performed: shoot thinning, tying shoots to the trellis wires, removal of lateral shoots (disbudding), and partial defoliation to enhance aeration and light interception.

Additional management practices included micro-sprinkler irrigation, weed control (mechanical and chemical), and regular applications of fungicides and insecticides for pest and disease control. Nutrient management was based on chemical fertilization, adjusted according to soil chemical analysis and recommendations from Technical Bulletin 100 of the Agronomic Institute [44].

2.3. Experimental Design and Treatments

The experiment was conducted in a randomized complete block design with split plots, where rootstocks ('IAC 572 Jales', 'IAC 766 Campinas' and 'Paulsen 1103') were assigned to the main plots and production cycles I and II (2021 and 2022) to the subplots. The experiment was structured with seven blocks, and each experimental unit comprised three 'BRS Núbia' grapevines.

2.4. Evaluated Variables

2.4.1. Phenological Stages

The phenological development of the grapevines was evaluated according to the modified Eichhorn and Lorenz (E–L) scale proposed by [45] and the methodology described by [46]. Visual observations were performed three times per week, always in the morning (between 7:00 and 10:00 a.m.) by the same trained observer to ensure consistency and reduce subjectivity. The recorded phenological stages included:

- (i) Budburst, defined as the emergence of green tissue in approximately 50% of the buds;
- (ii) Full flowering, when around 50% of the flowers in the cluster were fully open;
- (iii) Fruit set, identified by the presence of visible and developing fruitlets after petal fall;
- (iv) Onset of ripening (veraison), characterized by the beginning of berry color change and softening, depending on cultivar characteristics;
- (v) Harvest, determined when total soluble solids reached 16 to 18°Brix, according to the cultivar.

All phenological stages were expressed as days after pruning (DAP). The monitoring period extended from winter pruning until harvest, covering the full annual cycle of vine development.

2.4.2. Physiological Maturation and Physicochemical Quality of 'BRS Núbia' Grape Must

The physiological maturation of 'BRS Núbia' grapes was monitored weekly, starting when 50% of the berries reached the initial ripening stage, characterized by the onset of softening and color change. For this purpose, ten grape clusters were marked, from which six berries per cluster were collected, totaling sixty berries per experimental plot, at intervals of 0, 7, 14, 21, 28, and 35 days after the onset of ripening (DAOR). Sampling continued until harvest, when soluble solids stabilized at 17°Brix.

During ripening, the following variables were analyzed in the grape must: soluble solids content (SS), measured with a Reichert® digital refractometer (model r2i300, Depew, NY, USA); pH, determined directly in the must using a Tecnal® model Tec-10 potentiometer, Piracicaba, Brazil) [47]; and titratable acidity (TA), obtained by titration with 0.1 N NaOH, expressed as a percentage of tartaric acid per milliliter of must, according to the methodology described by [48]. The maturity index was calculated as the ratio of SS to TA.

2.4.3. Thermal Requirement and Growing Degree Days

The thermal requirement of the grapevines was determined based on the accumulation of growing degree days (GDDs) following the equation proposed by [49,50] using a base temperature of 10 °C [51] according to the phenological stages of the vine:

$$\text{GDD} = \sum (\text{Tm} - 10\text{ °C}) \times \text{number of days from pruning to harvest}$$

where:

GDD: Accumulated growing degree days;

Tm: Daily mean temperature (°C).

2.5. Statistical Analyses

Data were subjected to analysis of variance (ANOVA) to assess the effects of rootstocks and climatic conditions on the production cycles. The means of phenological stages and thermal requirements were compared using Tukey's test ($p < 0.05$). To analyze the effect of rootstocks on grape ripening and harvest timing, regression analysis was applied using Sisvar, version 6.0 software [52]. Additionally, to characterize the effects of rootstocks and assess the correlation between variables, principal component analysis (PCA) was performed using Statistical Analysis Software version 4.0 (SAS).

3. Results and Discussion

3.1. Duration of Phenological Stages and Thermal Requirement of 'BRS Núbia' Grapevine

The duration of the phenological stages of the 'BRS Núbia' grapevine did not show a significant interaction ($p > 0.05$) between the rootstocks and production cycles considering the period from pruning to harvest. However, significant differences were observed between production cycles in the duration of phenological phases, including budburst (Bu), full bloom (Fb), fruit set (St), veraison (Vs), and harvest (Hv) (Table 1).

Table 1. Duration of phenological stages (days after pruning) and thermal requirement of 'BRS Núbia' grape under different rootstocks during production cycle I and cycle II, São Manuel, São Paulo, Brazil.

Rootstocks	Duration of Phenological Stages (Days After Pruning)					Thermal Requirement
	Budburst	Full-Bloom	Fruit Set	Veraison	Harvest	
'IAC 766 Campinas'	19.3 ± 0.88	43.7 ± 1.29	51.0 ± 1.29	115.8 ± 3.29	154.3 ± 3.29	1793.4 ± 51.9
'IAC 572 Jales'	19.5 ± 1.67	44.5 ± 1.18	50.2 ± 0.70	116.2 ± 3.46	154.7 ± 3.46	1809.8 ± 50.2
'Paulsen 1103'	20.3 ± 0.93	45.0 ± 1.29	51.2 ± 1.74	117.9 ± 1.48	156.4 ± 1.48	1819.4 ± 24.9
CV (%)	5.80	3.27	2.38	3.57	2.69	3.69
DMS	1.15	1.46	1.22	4.20	4.20	67.4
Production cycle	Budburst	Full-bloom	Fruit set	Veraison	Harvest	Thermal Requirement
I	19.7 ± 1.36 b	46.5 ± 1.50 a	51.4 ± 1.56 a	106.6 ± 3.91 b	142.6 ± 3.91 b	1743.4 ± 53.1 b
II	20.3 ± 1.16 a	42.3 ± 1.03 b	50.2 ± 0.97 b	132.7 ± 4.53 a	167.7 ± 4.53 a	1871.7 ± 72.8 a
CV (%)	6.76	2.58	2.19	2.70	2.03	2.83
DMS	0.86	0.74	0.72	2.04	2.04	33.1

Means followed by different letters differ according to Tukey's test at a 5% probability level.

The analysis of phenological stages showed that the shortest duration was observed in the interval between full bloom and fruit set, whereas the longest duration occurred between fruit set and the onset of ripening. The shorter duration between flowering and fruit set can be attributed to the rapid cell growth and differentiation of berry tissues, processes driven by the mobilization of carbohydrate reserves. In contrast, the transition from fruit set to the onset of ripening involves more complex physiological processes, such as the initiation of sugar accumulation and the gradual degradation of organic acids, which may explain the longer duration of this phase.

During the berry ripening stage, the average temperature was 21.9 °C in cycle I and 22.9 °C in cycle II, with an average minimum temperature of 18.9 °C and 19.0 °C and an average maximum temperature of 30.8 °C and 29.5 °C, respectively (Figure 1b).

Maximum temperature during the ripening phase directly affects the respiration rate and metabolism of phenolic compounds in grapes, which can influence fruit coloration and chemical composition [53].

The accumulated rainfall was higher in cycle II (229.1 mm) compared to cycle I (133.4 mm), indicating a greater influence of climatic conditions on vine development (Figure 1a).

Although rootstocks did not significantly affect the duration of phenological stages (Table 1), the production cycles exhibited substantial differences in the thermal requirements of the grapevines (Figure 2).

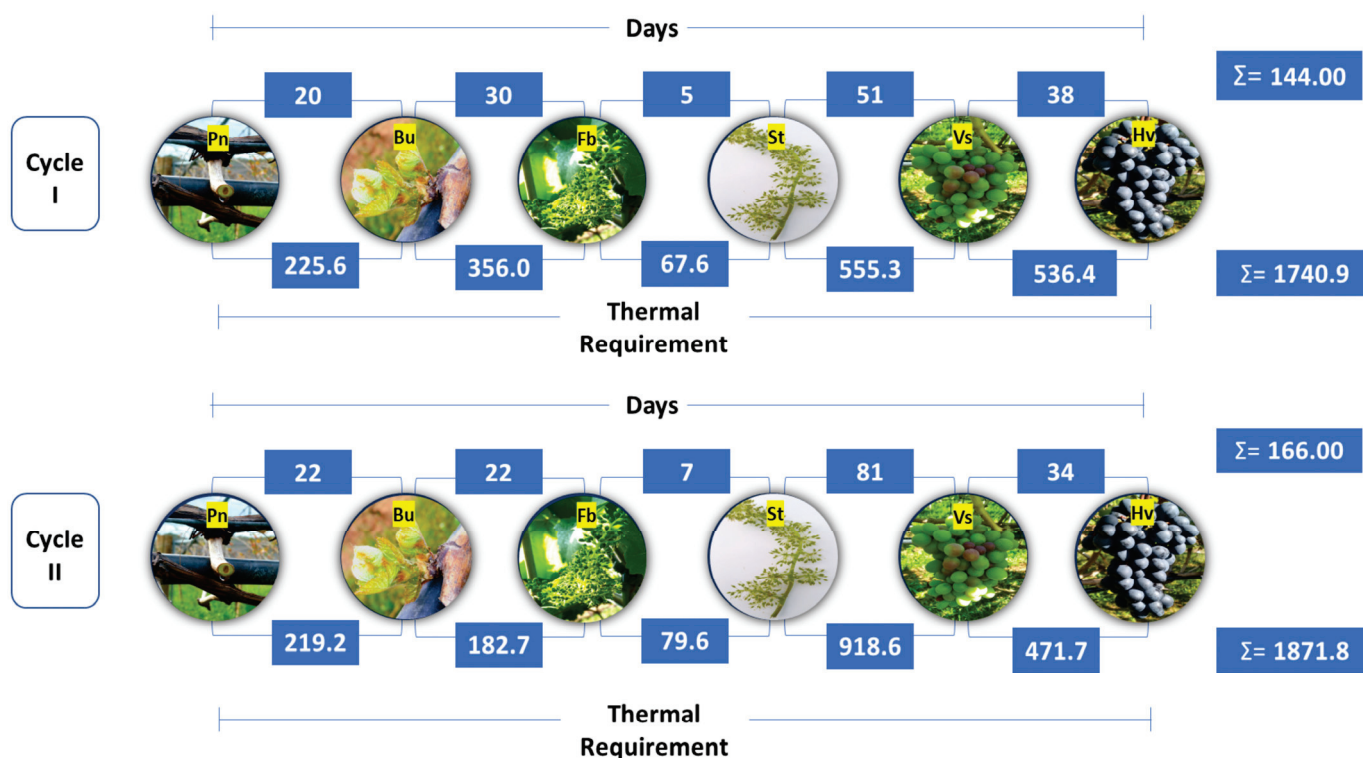


Figure 2. Duration of the phenological stages and thermal requirement of the 'BRS Núbia' grapevine on different rootstocks during production cycles I and cycle II, São Manuel, São Paulo, Brazil. Note: Pn: Pruning; Bu: Budburst; Fb: Full bloom; St: Fruit set; Vs: Veraison; and Hv: Harvest.

The period between pruning and the onset of budburst was longer in cycle II (20.3 days) than in cycle I (19.7 days), which may be related to temperature variations during this period (Figure 2). Lower temperatures can delay bud dormancy release and reduce vine metabolic activity, postponing budburst. This phenomenon is associated with the plant's hormonal regulation, particularly the balance between abscisic acid and cytokinins, which control bud induction [54].

Higher temperatures during the first cycle favored vine development, as increased temperatures after pruning can advance budburst and promote greater phenological uniformity [55]. Additionally, the effect of hydrogen cyanamide on budburst advancement may have been more efficient in the first cycle due to higher temperatures, promoting a greater hormonal stimulus for dormancy release [56].

Although temperature was the predominant environmental factor associated with the year-to-year differences observed in phenology and thermal accumulation, other physiological and environmental mechanisms may also have contributed to these variations [57]. Specifically, the depth of bud dormancy and the chilling requirement of the 'BRS Núbia' cultivar could have influenced the timing of budburst and subsequent developmental

stages, particularly in response to variable winter conditions between cycles [58–61]. Moreover, sensitivity to photoperiod may also modulate hormonal regulation and sink-source dynamics, thereby affecting the pace of berry development and ripening [62]. Considering these additional factors provides a more comprehensive and mechanistic interpretation of the phenological variability observed across growing seasons.

The first cycle had a longer duration between pruning and full bloom (46.5 days), which was 4.2 days longer than the second cycle (42.3 days). Similar differences were observed between pruning and fruit set, with durations of 51.4 days in cycle I and 50.2 days in cycle II (Table 1). Despite higher average temperatures in cycle I, the duration of these phases was not solely influenced by temperature, suggesting that other factors, such as solar radiation, also played a role in development and sugar accumulation in the fruit. Solar radiation directly influences photosynthetic rate and plant metabolism, regulating carbohydrate biosynthesis and accelerating energy reserve accumulation. Thus, greater light availability can result in more efficient plant metabolism, promoting vegetative and reproductive development [63]. The production cycle duration also varied between years. In cycle II, berry ripening began 132.7 days after pruning, while in cycle I, this stage was reached in 106.6 days, representing a difference of 32.1 days (Table 1). Harvest in cycle II occurred 167.7 days after pruning, whereas in cycle I, it was performed at 142.6 days, resulting in a 25.1-day longer production cycle in the second year.

This variation may be attributed to climatic conditions, particularly temperature. In cycle II, a cooler environment likely slowed plant development, increasing the time required for berries to reach maturity and delaying harvest. Temperature directly influences plant growth rate and phenological stage development. In general, higher temperatures accelerate plant metabolism, shortening the cycle, while lower temperatures slow this process, extending the cycle. These factors explain how thermal variations can affect the cycle length of ‘BRS Núbia’ grapevines.

The variation in ripening time between production cycles has important practical implications for vineyard management and commercial planning. Early ripening may allow harvest to occur under more favorable climatic conditions, potentially improving fruit integrity and reducing disease pressure [64,65]. It also facilitates better labor scheduling and allocation of resources during peak harvest periods. In contrast, delayed ripening, as observed in the second cycle, may increase exposure to late-season rainfall, elevate disease risk, and shorten the marketing window, particularly for table grapes with limited shelf life [66,67]. Therefore, understanding the factors that control ripening dynamics is essential not only for improving fruit quality but also for optimizing harvest logistics and aligning production with market demands.

The duration of the ‘BRS Núbia’ grapevine cycle in the two evaluated years (167.7 days in cycle II and 142.6 days in cycle I) was longer than reported in other regions. In northern Paraná, cycles of 130 to 135 days were recorded, while in northwestern São Paulo, duration ranged from 125 to 130 days [68]. Under Cerrado conditions, the production cycle lasted approximately 140 days, highlighting the influence of climate on grapevine phenological development [69].

The increase in thermal accumulation is associated with a reduction in the grapevine production cycle, advancing ripening and harvest, which may impact fruit quality due to the shorter time available for reserve compound development and the assimilation of photoassimilates and sugars [70]. During both production cycles, cumulative precipitation was 1047.7 mm and 1083.3 mm, respectively (Figure 1a).

In the critical period between October and December, the accumulated rainfall index was 510.9 mm in cycle I and 408.4 mm in cycle II. During the vegetative stage, the higher precipitation in cycle I likely favored vine growth, providing more water for root, leaf,

and shoot development, which in turn boosted the photosynthetic rate and resulted in more vigorous vegetative growth. Conversely, in cycle II, with lower precipitation, the plants exhibited more balanced growth, avoiding risks associated with excessive moisture. This condition provided a more controlled environment for fruit ripening, favoring more uniform berry maturation, which resulted in a shorter and more efficient production cycle.

Thermal requirements varied significantly between production cycles, being 7.36% higher in cycle II (1871.7 GDDs) compared to cycle I (1743.4 GDDs) (Table 1). This variation was primarily attributed to temperature differences throughout the cycle, with higher thermal requirements between fruit set and the onset of ripening as well as from ripening to harvest. Budburst, ripening, and harvest were the stages with the highest thermal requirement, regardless of the production cycle, highlighting the importance of thermal accumulation in these periods. Additionally, accumulated thermal sum during ripening can impact the phenolic compound content in the grape skin, influencing color stability and fruit flavor intensity [71].

The influence of climate on degree-day accumulation has been observed in other grapevine cultivars. For ‘Niagara Rosada’ cultivated under subtropical conditions, thermal requirements of 1465.2 to 1615.1 GD were recorded over two production cycles [72]. Moreover, there is evidence that rootstock can influence grapevine thermal requirements [73,74]. The fact that ‘BRS Núbia’ exhibited similar thermal requirements among the tested rootstocks suggests that scion genetic factors may have a greater influence on phenological regulation than specific rootstock characteristics.

In subtropical regions, the optimal average temperature for grapevine cultivation is estimated at 27 °C [75], considering its impact on phenology and thermal accumulation. An increase in minimum temperatures can reduce the vegetative phase and advance fruit ripening and harvest [55,76], which was evident in the analyzed production cycles.

The ‘BRS Núbia’ grapevine demonstrated adaptation to local climatic conditions and was classified as having an intermediate cycle. The evaluation of phenological stages indicated an average thermal requirement of 1807.5 GD between pruning and harvest, considering the two analyzed production cycles (Table 1). The phenological stability of ‘BRS Núbia’ and its ability to complete the cycle within consistent patterns reinforce its potential for cultivation in subtropical regions. However, specific management practices, such as advancing pruning in years with expected higher temperatures or adjusting irrigation during critical periods, may be effective strategies to optimize yield and fruit quality.

3.2. Evolution of ‘BRS Núbia’ Grape Ripening

The ripening of ‘BRS Núbia’ grapes began at 106.6 and 132.7 days after pruning (DAP) in production cycle I and cycle II, respectively. No significant interaction was observed between rootstocks and berry sampling periods for the ripening curve, allowing the adjustment of regression models to analyze variations in soluble solids content, titratable acidity, pH, and the maturity index as a function of days after pruning.

The soluble solids content in the must of ‘BRS Núbia’ grapes at harvest was 18.50°Brix in cycle I and 17.43°Brix in cycle II, both exceeding the minimum requirement for table grape cultivars (16°Brix) according to Brazilian regulations [68,77,78]. Under tropical conditions, ‘BRS Núbia’ grapes exhibit soluble solids content ranging from 17 to 19°Brix [35]. Temperature and rainfall frequency directly impact ripening, reducing sugar concentration in the fruit [79,80] (Figure 3).

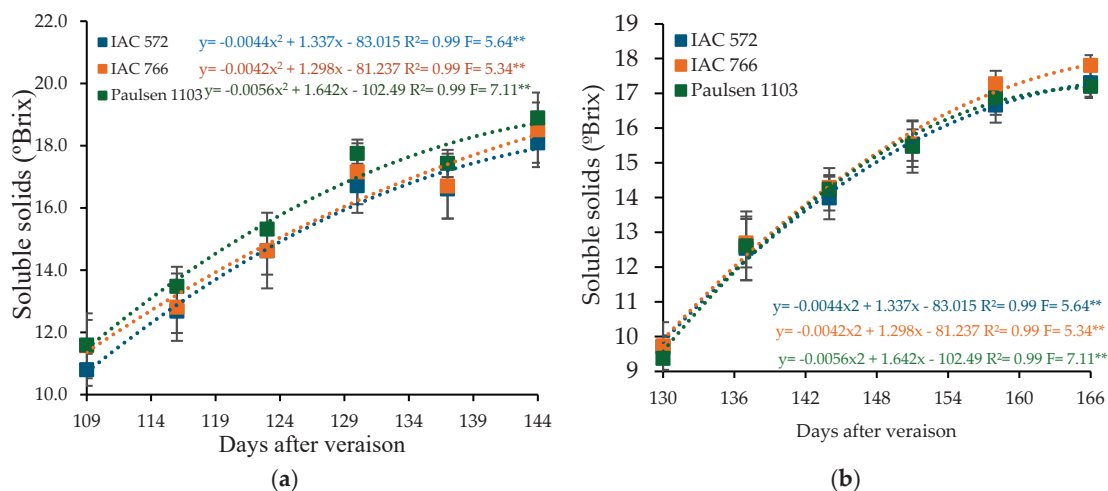


Figure 3. Soluble solids content (SS) in the must of 'BRS Núbia' grapes cultivated during production cycle I (a) and cycle II (b), São Manuel-SP, 2023. Significant F-value at $p < 0.05$ ($n = 6$). The rootstocks 'IAC 572', 'IAC 766', and 'Paulsen 1103' are represented by blue, orange, and green colors, respectively. $^{**} p > 0.05$.

Titrateable acidity decreased throughout the cycle, with quadratic regression models indicating an inverse correlation with soluble solids content (Figure 4). In the first cycle, the minimum titrateable acidity points occurred at 144, 137, and 136 DAP for the rootstocks 'IAC 572 Jales', 'IAC 766 Campinas', and 'Paulsen 1103', respectively. In the second cycle, minimum values were recorded at 138, 141, and 140 DAP (Figure 4b).

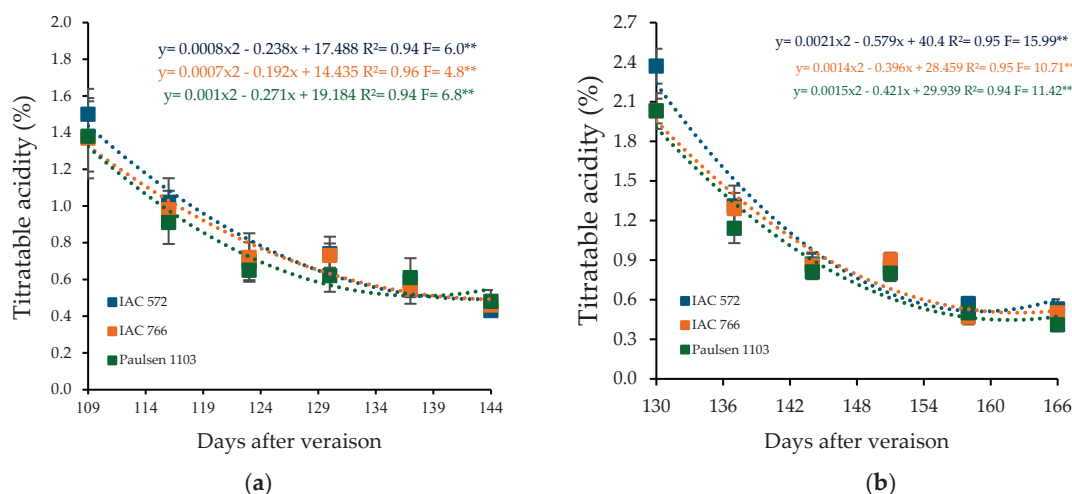


Figure 4. Titrateable acidity (TA) in the must of 'BRS Núbia' grapes cultivated during production cycle I (a) and cycle II (b), São Manuel-SP, 2023. Significant F-value at $p < 0.05$ ($n = 6$). The rootstocks 'IAC 572', 'IAC 766', and 'Paulsen 1103' are represented by blue, orange, and green colors, respectively. $^{**} p > 0.05$.

Initial titrateable acidity was 1.42% in cycle I, decreasing to 0.46% at the end of ripening. In cycle II, initial acidity was higher (2.14%) but also decreased, reaching 0.48% at harvest (Figure 4b). The reduction in acidity throughout the cycle is an expected process during grape maturation, as organic acids, especially malic acid, are metabolized and utilized in cellular respiration [81]. Cycle II, with slightly lower temperatures, may have favored the maintenance of higher acidity levels, as lower temperatures slow acid degradation and extend their retention in the fruit.

Variations in titrateable acidity between cycles were influenced by climatic conditions, including average temperatures of 28.7 °C in cycle I and 27.8 °C in cycle II as well as

accumulated precipitation (Figure 1b). The rootstock ‘IAC 572 Jales’ resulted in the highest titratable acidity values at the end of ripening (Figure 4b), directly impacting the sensory profile of ‘BRS Núbia’ grapes, as acidity influences fruit flavor perception for fresh consumption. The rootstock can affect grape chemical composition by regulating mineral absorption and translocation, particularly potassium, which directly influences must acidity. The acid-base composition of must is primarily determined by tartaric and malic acid levels as well as potassium concentration in the soil and plant, which affect grape stability and quality [82].

The lower temperatures observed during cycle II, particularly between July and September (Figure 1b), coincided with the final stage of berry filling and the onset of ripening, delaying organic acid degradation and resulting in higher acidity at harvest. This demonstrates that temperature not only affects the rate of sugar accumulation but also influences the dynamics of organic acid degradation, reinforcing the need for climate monitoring to predict the final characteristics of the harvest.

Similar results have been reported for ‘BRS Núbia’ grapes in the Brazilian Cerrado, where average titratable acidity levels were 0.52% and 0.51% tartaric acid per 100 mL of must [69,83]. These values are consistent with those obtained for other table grape cultivars grown in subtropical regions, such as ‘BRS Ísis’ [31,39], ‘BRS Melodia’ [84], ‘Niagara Rosada’ [72], and ‘BRS Vitória’ [37,85].

The pH of ‘BRS Núbia’ grape must varied according to the rootstock and production cycle. In the first cycle, the must pH values were 3.21, 3.26, and 3.28 for the rootstocks ‘IAC 572 Jales’, ‘IAC 766 Campinas’, and ‘Paulsen 1103’, respectively. In the second cycle, these values increased to 3.51, 3.50, and 3.48 (Figure 5), aligning with results obtained for grapevines cultivated in the Cerrado [69]. Quadratic regression models revealed that maximum pH values were reached at 144, 144, and 132 DAP in cycle I and at 158, 166, and 143 DAP in cycle II, depending on the rootstock used (Figure 5).

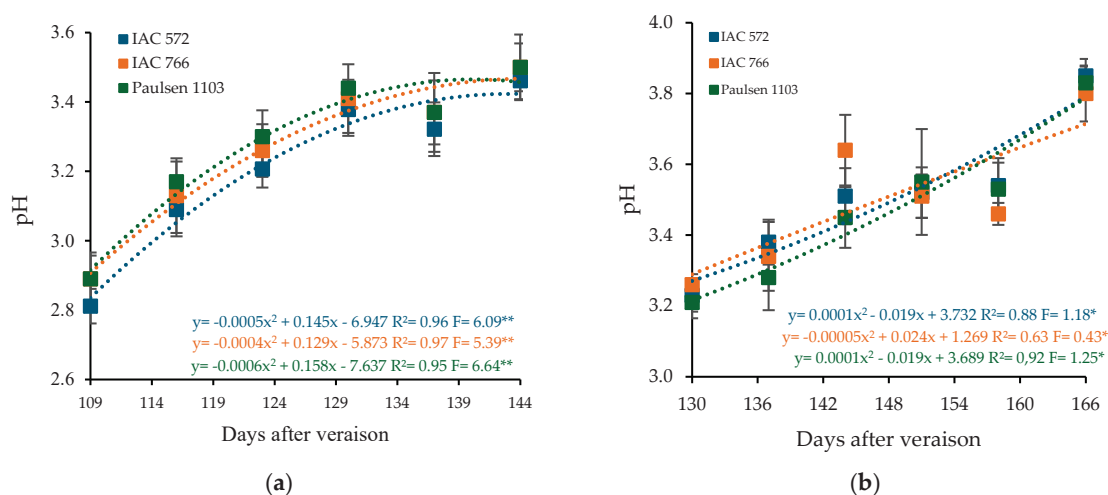


Figure 5. Hydrogen potential (pH) in the must of ‘BRS Núbia’ grapes cultivated during production cycle I (a) and cycle II (b), São Manuel-SP, 2023. Significant F-value at $p < 0.05$ ($n = 6$). The rootstocks ‘IAC 572’, ‘IAC 766’, and ‘Paulsen 1103’ are represented by blue, orange, and green colors, respectively. $^* p > 0.01$, $^{**} p > 0.05$.

The increase in pH during ripening is directly related to the degradation of malic acid and the accumulation of cations, such as potassium, in berry tissues, which reduces acidity perception and alters the acid-base balance of the must [86].

Variations in pH may be associated with climatic factors and the composition of organic acids. The increase in pH during the second cycle suggests a possible reduction in acidity due to lower temperatures and solar radiation (Figure 1b), affecting the sensory

profile of the grapes. Temperature plays a crucial role in plant metabolism, influencing must pH, as higher temperatures can reduce acidity and accelerate ripening [87,88].

The maturity index of ‘BRS Núbia’ grapes ranged from 8.21 to 41.61 in cycle I and from 4.54 to 39.20 in cycle II (Figure 6). Quadratic regression models indicated that the maximum maturity index points occurred at 144 DAP in cycle I and at 166 DAP in cycle II. The ratio between soluble solids and total acidity is a crucial parameter for determining the optimal harvest time, as it reflects the balance between sweetness and acidity in the fruit.

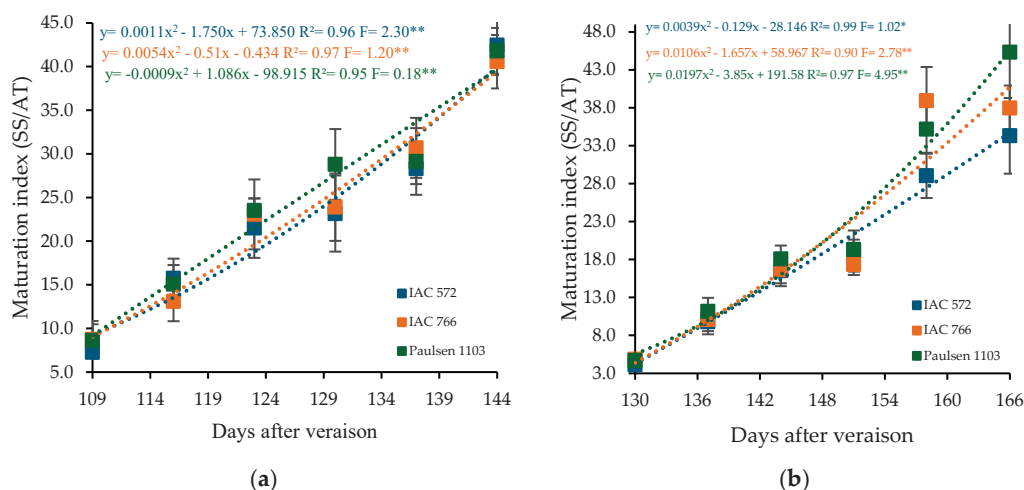


Figure 6. Maturity index (MI) in the must of ‘BRS Núbia’ grapes cultivated during production cycle I (a) and cycle II (b), São Manuel-SP, 2023. Significant F-value at $p < 0.05$ ($n = 6$). The rootstocks ‘IAC 572’, ‘IAC 766’, and ‘Paulsen 1103’ are represented by blue, orange, and green colors, respectively. * $p > 0.01$ ** $p > 0.05$.

The rootstock ‘Paulsen 1103’ resulted in the highest maturity indices, with values of 41.80 in cycle I and 45.32 in cycle II (Figure 6a,b), whereas ‘IAC 766 Campinas’ advanced full ripening to 28 DAIM in the second cycle. This superior maturation index associated with ‘Paulsen 1103’ may be linked to physiological traits of this rootstock, including its efficient water uptake capacity and vigorous root system, which enhance nutrients and assimilate translocation to the scion [89]. Furthermore, ‘Paulsen 1103’ is known to influence abscisic acid (ABA) signaling in the berries, a hormone that plays a key role in sugar accumulation and acid degradation during ripening [90–92]. The elevated maturity index may thus reflect an accelerated or more synchronized ripening process triggered by hormonal crosstalk and improved hydraulic conductance, contributing to higher °Brix and lower acidity at harvest. The maturity index is an important indicator of grape quality since soluble solids increase with ripening while acidity decreases (Figures 3 and 4). High temperatures can advance phenological stages, impacting must composition and potentially compromising final grape quality [93].

The appropriate selection of rootstocks and the monitoring of climatic conditions are essential to ensuring fruit quality. Management strategies should be adopted to mitigate the effects of climatic fluctuations on grape ripening and chemical balance [94,95]. These factors directly influence sugar content and acidity, determining the acceptance of ‘BRS Núbia’ grapes in the table grape market.

3.3. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) enabled the visualization of variability among rootstocks (‘IAC 572 Jales’, ‘IAC 766 Campinas’, and ‘Paulsen 1103’) and production cycles (cycle I and cycle II) of the ‘BRS Núbia’ cultivar. The first two principal components together

explained 94% of the total data variability (PC-1 = 73%; PC-2 = 21%), indicating a robust representation of the interrelationships among the analyzed factors (Figure 7).

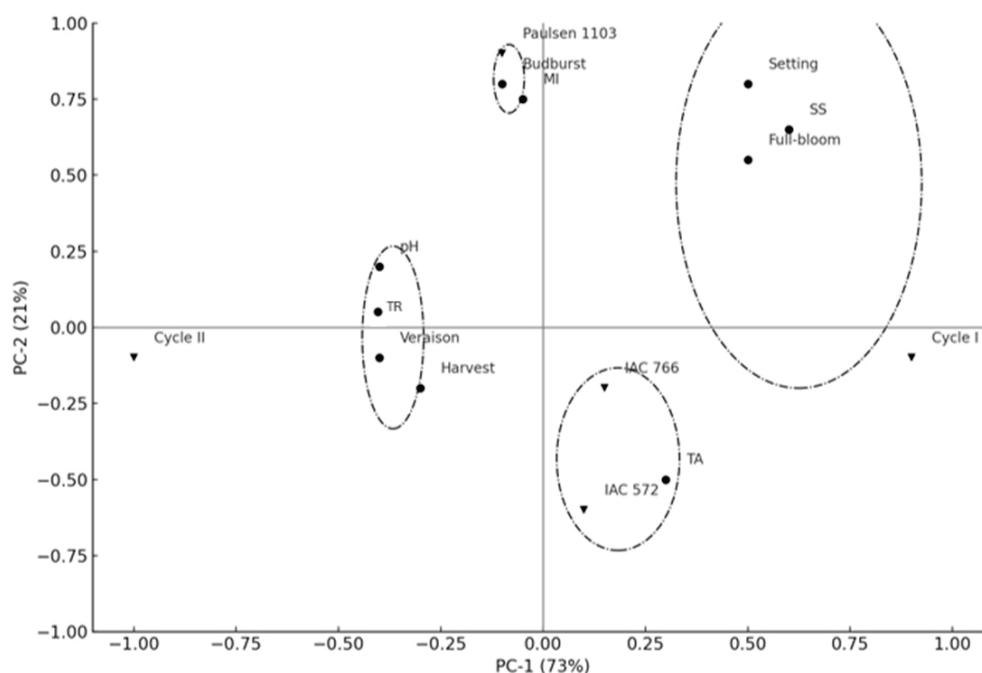


Figure 7. Principal component analysis of ‘BRS Núbia’ vines on different rootstocks. Note: SS: Soluble solids; TR: Thermal requirement; TA: Titratable acidity; MI: Maturation index. The symbols (triangles, circles, and squares) represent different combinations of cultivars and rootstocks, and are used solely for visual distinction.

The separation between production cycles indicates a strong influence of environmental conditions on phenology and fruit ripening. Cycle I (2021) showed a greater association with phenological events such as full bloom, fruit set, and soluble solids accumulation (SS), suggesting a more favorable plant development and higher sugar accumulation, possibly due to higher temperatures and lower precipitation. Conversely, cycle II did not show a strong correlation with these variables, indicating a potential delay in fruit ripening or a lower sugar accumulation rate, potentially due to differences in climatic conditions. These results highlight the importance of environmental variables in grapevine physiology, demonstrating that thermal and water fluctuations can directly impact photosynthetic efficiency and carbohydrate metabolism in fruits [96].

Rootstocks also significantly influenced grapevine phenology. ‘Paulsen 1103’ was associated with the budburst event, indicating that this scion/rootstock combination may advance the start of the phenological cycle. Meanwhile, the rootstocks ‘IAC 572 Jales’ and ‘IAC 766 Campinas’ were correlated with titratable acidity (TA), suggesting greater retention of organic acids during ripening, an important factor in maintaining the sensory balance of the fruit. Additionally, ‘IAC 766 Campinas’ was closely related to pH, suggesting an influence on the regulation of the grape’s acid profile.

The distribution of rootstocks in different quadrants reinforces their distinct influences on the metabolism of ‘BRS Núbia’ grapevines. The separate clustering of ‘Paulsen 1103’ from the other rootstocks indicates a differentiated phenological behavior, favoring early budburst and potentially influencing the duration of the production cycle. On the other hand, the rootstocks ‘IAC 572 Jales’ and ‘IAC 766 Campinas’ exhibited similar effects on acidity retention, which may directly impact the final quality of the fruit.

These findings reinforce the hypothesis that ‘Paulsen 1103’ may enhance early developmental stages due to its high hydraulic conductivity and potential stimulation of scion

vigor. This rootstock is known for promoting earlier budburst and increasing canopy activity, which can lead to greater light interception and enhanced carbohydrate biosynthesis during the initial phenological phases. These physiological traits may contribute to the higher maturity index values and earlier ripening observed for 'Paulsen 1103' in this study.

In contrast, the association of 'IAC 572 Jales' and 'IAC 766 Campinas' with higher titratable acidity may reflect their moderate vigor and potentially lower rates of abscisic acid (ABA) signaling involved in ripening onset. These rootstocks may promote a more gradual accumulation of sugars and slower degradation of organic acids, particularly under subtropical climate conditions, thereby preserving acidity and contributing to better sensory balance in the berries.

4. Conclusions

The 'BRS Núbia' grapevine exhibited a mean thermal requirement of 1807.5 growing degree days (GDDs) to complete its phenological cycle, which lasted approximately 150 days under subtropical conditions. Although the rootstocks 'IAC 572 Jales', 'IAC 766 Campinas', and 'Paulsen 1103' did not significantly alter the duration of phenological stages, they had notable effects on fruit quality parameters, particularly soluble solids content, titratable acidity, and maturation index. Differences observed in phenological progression and thermal accumulation between the two production cycles were primarily influenced by climatic variability, especially temperature and precipitation levels during key developmental stages. These findings reinforce the physiological plasticity and adaptability of the 'BRS Núbia' cultivar to subtropical environments, supporting its suitability for regions with similar agroclimatic conditions. Moreover, the results highlight the importance of integrating rootstock selection with climate monitoring to optimize fruit quality and harvest timing in subtropical viticulture systems.

Author Contributions: H.S.A.M. and M.A.T. planned and designed the experiment. H.S.A.M., S.d.N.S.B., J.C.A., D.E.F.F., P.H.H.C. and C.R.M. performed the plant physiological analyses and chemical, biochemical, and enzyme analyses. H.S.A.M., C.A.P.C.S., F.J.D.N. and M.A.T. performed data analyses. H.S.A.M., F.J.D.N. and C.A.P.C.S. created the tables and figures. H.S.A.M., F.J.D.N., C.A.P.C.S. and M.d.S.S. wrote and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Article

The Effects of Different Mineral Clay Particles on Olive Yield and Olive Oil Quality of Two Cultivars Under Rainfed or Irrigated Conditions

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Abstract

The olive tree is the emblematic tree of the Mediterranean basin, enduring intense irradiance and heat stress during prolonged dry summers. Particle film technology is a relatively new tool for mitigating both biotic and abiotic stress factors. In the present trial, two Greek olive cultivars, i.e., rainfed ‘Megaron’ and irrigated ‘Koroneiki’, were used to test the efficacy of kaolin, talc, and, for the first time, attapulgite clay particles as single and double foliar applications on the quantitative and qualitative traits of production. Clay particle treatments generally increased yield, resulting in higher olive oil production per tree. Oil quality parameters remained within the standards for extra virgin olive oil across all treatments. Talc differed from the other clay minerals, particularly in its effect on the free acid composition of the oil. Attapulgite application enhanced yield and oil production in ‘Koroneiki’, especially when compared to double kaolin application. Conversely, kaolin double application resulted in the highest yield and oil production in the ‘Megaron’ cultivar. These findings indicate that the efficacy of particle film treatments varies depending on multiple factors, yet they remain a valuable tool for mitigating the adverse effects of climate change on olive production. As this is the first study to test talc and attapulgite on olive trees, further research is required to fully elucidate the potential of particle film technology.

Keywords: antioxidant capacity; attapulgite; free acidity; free fatty acids; kaolin; phenols; talc

1. Introduction

Over the past decade, extreme weather phenomena have become increasingly frequent, largely attributed to climate change, which has escalated into a climate crisis in some areas [1]. Certain regions of the planet are particularly vulnerable to such weather extremes, including the Mediterranean basin [2,3]. According to some studies, the mean annual temperature in this region has risen by nearly 1.5 °C compared to the previous century [1,4]. This temperature increase, driven by elevated atmospheric CO₂ levels, has intensified extreme events, such as floods, prolonged droughts, heat waves, reduced rainfall, and increased vapor pressure deficit, which now occur with growing frequency [1].

The agricultural sector is highly susceptible to weather extremes, with significant losses in agricultural productivity recorded worldwide. Water scarcity, often coupled with

high irradiance and temperatures in summer, induces various physiological malfunctions in plants. These include stomatal closure, reduced photosynthesis, increased production of reactive oxygen species (ROS) leading to oxidative damage, leaf wilting and loss, yield reduction, and quality degradation. In more severe cases, plant mortality has also been documented [1].

Perennial species are particularly vulnerable, as they endure multiple abiotic stress factors throughout their lifespan and cannot escape unfavorable conditions [1,5]. Among the most significant trees in the Mediterranean region is the olive tree, one of the oldest and most emblematic species of the region, holding immense economic and cultural value for the people of the corresponding countries [6,7]. The olive is a xerophytic, sclerophyllous species capable of tolerating harsh conditions, such as drought, salt stress, and high temperatures. However, under conditions of water scarcity combined with extreme heat and high irradiance, olive trees experience significant stress, resulting in reduced stomatal aperture, impaired photosynthesis, and, ultimately, yield losses [7,8]. Therefore, developing eco-friendly strategies to enhance plant adaptation to such challenging conditions is essential.

At the same time, consumer awareness of pesticide residues is increasing, fueling a global trend toward reduced pesticide use and greater reliance on products that enhance plant defense mechanisms, such as biostimulants and biopesticides [9,10]. One of the most exciting and emerging multi-functional, environmentally friendly technologies for alleviating abiotic stresses while functioning as pest protection is the adaptation in the agricultural sector of particle film technology (PFT) [9,11–15]. This technology involves the use of aqueous formulations of chemically inert mineral particles treated in such a way as to produce a continuous coating over plant surfaces, functioning as protective films [14,16].

Effective PFT products possess beneficial properties, including high transmission of photosynthetically active radiation (PAR), while reflecting ultraviolet and infrared radiation. This reduces heat accumulation and solar damage to leaves and fruit [12,14,17]. Additionally, these particles can camouflage plant tissues, making them less recognizable to pests [14]. By forming a white mineral barrier on plant surfaces, they create an unfavorable environment for pests, leading to reduced infestation rates [18].

Among the most used minerals in PFT are clays, such as kaolinite, attapulgite, montmorillonite, talc, and pyrophyllite [14]. Kaolinite has been extensively used after proper treatment, giving rise to kaolin, a commercially available product. There are numerous studies on the use of kaolin clay particles in various plant species (pomegranate, olive, apple, grapefruit, grapes, coffee, walnut, tomato, etc.), aiming at alleviating the adverse effects of abiotic and/or biotic stress factors. In olive trees, kaolin has demonstrated promising results in enhancing both physiological and yield-related parameters under stress conditions. These improvements are achieved through an increased photosynthetic rate, improved water use efficiency, and the alleviation of oxidative stress, among other beneficial effects [6–8,14,19]. To our knowledge, talc has not been tested on olive trees, although it has been used on pomegranates to prevent fruit sunburn [20]. Similarly, attapulgite has not been reported to have been applied to olive trees or any other species as a foliar application, and thus no data exist on its efficacy.

This study aimed to evaluate the impact of particle film technology on olive tree productivity, both quantitatively and qualitatively, in terms of olive oil yield and quality. Three commercially available agricultural products containing kaolin, talc, and attapulgite particles—two of which were tested on olive trees for the first time—were applied to two olive oil cultivars in central and southern Greece.

2. Materials and Methods

2.1. Test Site Location—Plant Material

The trial was conducted in two different regions in Greece in monovarietal olive groves. The first one took place in central Greece, Viotia County, in the region of Vathi Avlidas ($38^{\circ}24'33.06''$ North, $23^{\circ}36'49.11''$ East, altitude 65 m) in 2019–2020 (Figure 1). The mean annual temperature in the region is approximately 16.7°C , while the average temperature is around 27.2°C in July and around 27.1°C in August with minimal rainfall. The mean monthly total solar irradiation on a horizontal level in the area is 220.0 kWh m^{-2} in July and 204.0 kWh m^{-2} in August (data from the Greek Ministry of Environment and Energy, <https://www.helapco.gr/ims/file/installers/totee-klimatika.pdf>, visited on 6 March 2025). The second one took place in southern Greece, on the island of Crete in Heraklio County, in the region of Kato Kastelliana ($35^{\circ}1'56.81309''$ North, $25^{\circ}16'10.86''$ East, altitude 450 m) in 2022–2023. The mean annual temperature in the region is approximately 17.8°C , while the average temperature is around 26.1°C in July and around 25.9°C in August. The mean monthly total solar irradiation on a horizontal level in the area is 227.1 kWh m^{-2} in July and 207.0 kWh m^{-2} in August (data from the Greek Ministry of Environment and Energy, <https://www.helapco.gr/ims/file/installers/totee-klimatika.pdf>, visited on 6 March 2025). The cultivars used were ‘Megaron’ (synonym ‘Megareitiki’ or ‘Megaritiki’) in Viotia (60-year-old rainfed trees) and ‘Koroneiki’ in Crete (30-year-old drip-irrigated trees), both olive oil cultivars of significant importance for Greece as well as for the entire world (regarding ‘Koroneiki’). All trees in each olive grove were selected based on uniform growth and similar expected yield, without any visible symptoms of either nutrient deficiencies or disease infection. All the necessary cultivation practices were carried out by the farmers (weed, pest, disease control, etc.) to ensure unhindered tree growth and fruit production.

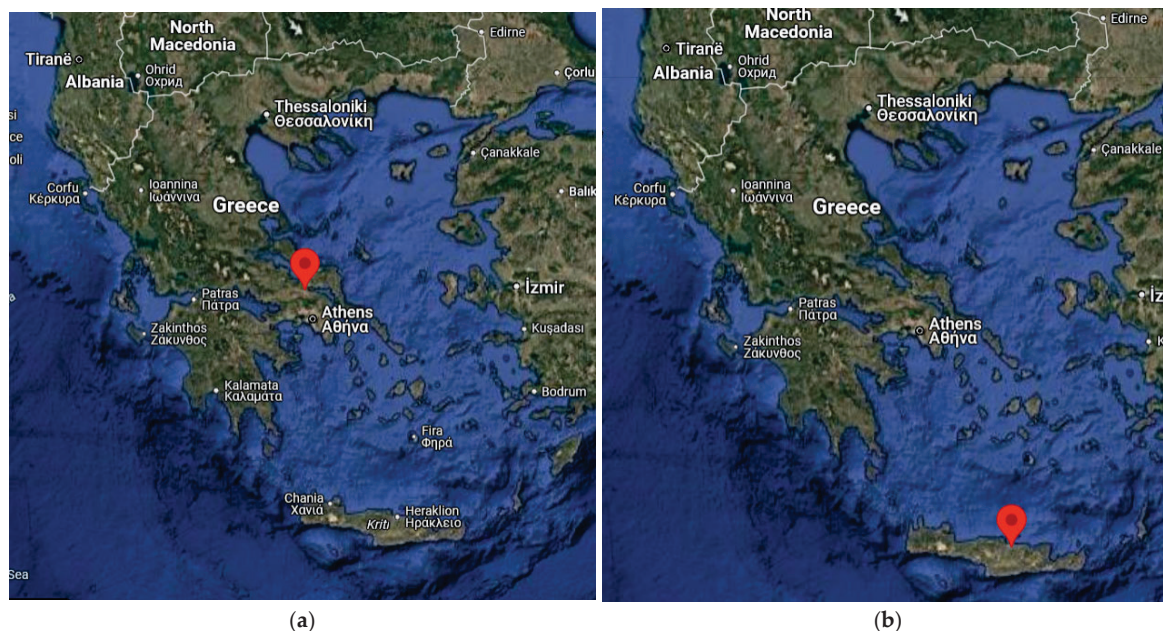


Figure 1. The location (red tags) of the two olive groves in Greece: (a) in Viotia County (on the left) and (b) in Heraklio County (on the right) (produced by Google Maps).

During the first year in Viotia County, two commercial products were used, i.e., Surround[®] WP (kaolin—aluminum silicate 95% *w/w*) ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ 95%) (Tessenderlo Kerley, Inc., Phoenix, AZ, USA), distributed in Greece by Hellafarm SA, as recommended by the supplier at a registered dose rate of $3\text{ kg } 100\text{ L}^{-1}$, and Invelop[®] White Protect (Talc E553b) ($\text{Mg}_3\text{Si}_4\text{O}_{21}\text{H}_{20}$ 100% *w/w*) (COMPO EXPERT GmbH, Münster/Westphalia,

Germany), distributed in Greece by Compo Hellas, as recommended by the supplier at a registered dose rate of $3 \text{ kg } 100 \text{ L}^{-1}$. Two schemes of spray application took place, i.e., a single application in August (18 August 2019—BBCH crop growth stage 77–78) and a double application both in July (BBCH crop growth stage 76–77) and August (13 July 2019 and 18 August 2019); thus, a total of five treatments were applied (including control) (Figure 2). Four trees were used per treatment, i.e., a total of twenty (20) trees.

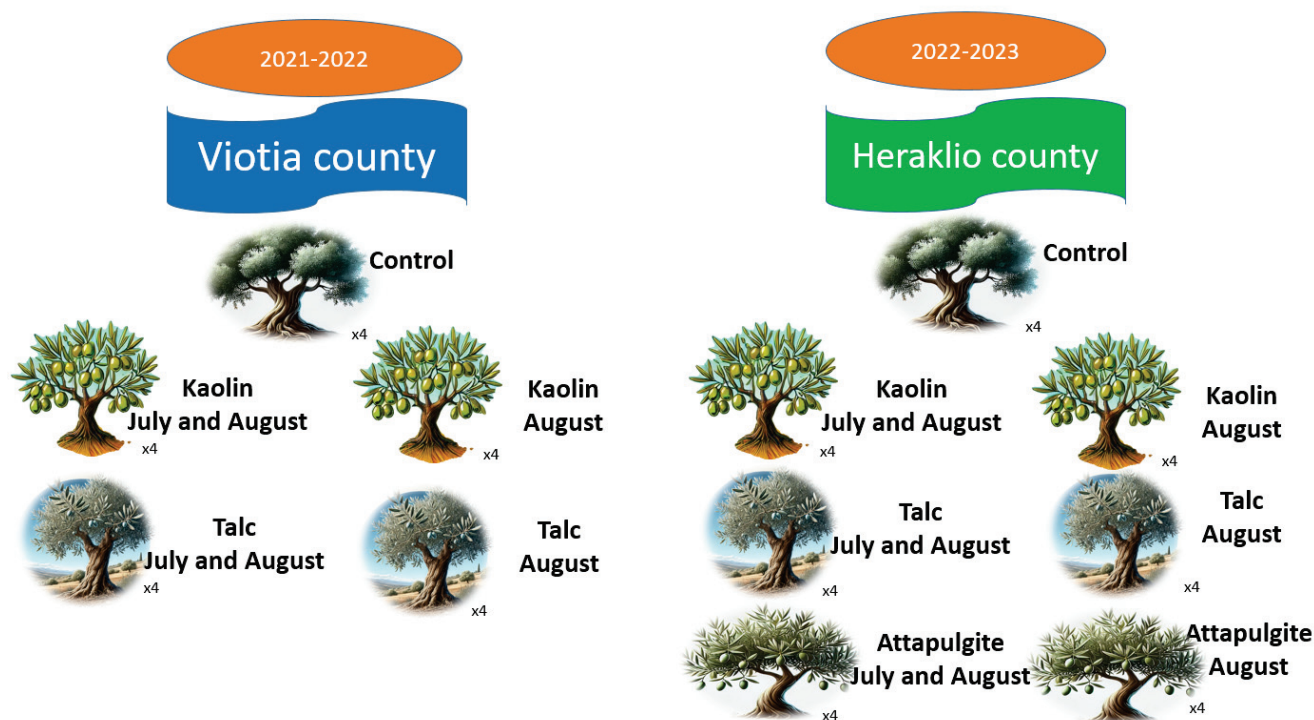


Figure 2. A graphical presentation of the treatments that took place in the two regions.

During the second year in Crete (Heraklio County), three commercial products were used. Along with the two previously described ones, i.e., Surround WP and Invelop, a third one, Aglev® SI 300 (attapulgit 100%) ($(\text{Mg}, \text{Al})_2 \text{Si}_4 \text{O}_{10}(\text{OH})_4(\text{H}_2\text{O})$) (Geohellas SA, Athens, Greece), was applied as recommended by the supplier at a registered dose rate of $2 \text{ kg } 100 \text{ L}^{-1}$. The timing of spray applications was similar to those described above, i.e., a single application in August (21 August 2022—BBCH crop growth stage 76–77) and a double application both in July (BBCH crop growth stage 74–75) and August (21 July 2022 and 21 August 2022). Thus, a total of seven treatments were applied (including control). Four trees were used per treatment, resulting in a total of 28 trees.

The schedule of two applications in July and August was chosen for two reasons. First, it ensured that the trees remained covered by particle films during the harsh summer conditions in Greece, a period when olive fruits are growing and oil accumulation in the fruit is occurring (Figure 3). Typically, no rainfall is expected in these areas during this time, as was the case throughout the experimental period. Second, a single application was tested to evaluate its efficacy, potentially reducing the cost of particle film application for farmers. This was timed for August, when oil accumulation in the fruit begins to increase. In all spray applications, a surfactant (Haiten Plus 15SL, Phytorgan SA, Kato Kifissia, Greece, at the dose rate of $25 \text{ mL } 100 \text{ L}^{-1}$) was added to the tank mix. It is not expected to have an influence on the efficacy of the treatments, as the dose rate is too small to expect any effect.

At harvest (24 November 2019 and 19 November 2022 in Viotia and Heraklio County, respectively), each tree was harvested separately, and the yield was recorded. Approximately 1.5 kg of free-from-leaves, healthy olive fruits were randomly sampled from each

plot and transferred to the laboratory for further processing. A total of four samples (1.5 kg each) per treatment were assayed.



Figure 3. Aspect of the foliage of the olive trees after the second application of kaolin (on the left) and talc (on the right).

2.2. Olive Oil Extraction Procedure

The maturity index of the fruits was determined in the laboratory in a sample of 100 randomly selected olive fruits based on the color of their skin and pulp. The free-from-leaves and other debris olives were crushed using an Abencor-type olive mill (Callis S.A., Athens, Greece). The crusher operated at 2800 rpm, and the paste produced was malaxed for 30 min at 50 rpm and 25–27 °C in the malaxer. Following malaxation, 50 g of the paste was transferred to a 50 mL Falcon tube and centrifuged at $2500 \times g$ for 15 min to accurately determine the oil percentage in the paste. The remaining paste was centrifuged at $1400 \times g$ for 3–4 min using the semi-industrial centrifuge of the Abencor-type olive mill, and the oil produced was stored in filled bottles at 4 ± 2 °C until analysis.

2.3. Olive Oil Analyses

The determination of olive-oil-free acidity, peroxide index, and ultraviolet absorption at 232 and 270 (K232 and K270, respectively), as well as ΔK (delta kappa), was conducted according to the European Official Methods of Analysis 2016/1784.

2.4. Determination of Total Phenols

Phenolic compounds were extracted by adding 5 mL of hexane into 2.5 g of olive oil. After vortexing, 5 mL of methanol–water solution (60:40) was added, and the mixture was vortexed for 2 min. The sample was then centrifuged at $2500 \times g$ for 10 min, and the upper phase was discarded. The lower phase was used for the determination of the different phenol fractions. The total phenols, the total o-diphenols, and the total flavonoids were determined as described by Roussos et al. [21] and expressed as mg gallic acid equivalents (GAEs) kg^{-1} olive oil, mg caffeic acid equivalents (CAEs) kg^{-1} olive oil, and mg catechin equivalents (CtEs) kg^{-1} olive oil, respectively.

Individual phenolic compounds were detected in the same phenolic extract by high-performance liquid chromatography (HPLC) (Shimadzu Nexera X2, Shimadzu Europa GmbH, Albert-Hahn-Str. 6-10, Duisburg, Germany) using a diode array detector operating at 260 nm, 280 nm, 325 nm, and 360 nm. A sample of 40 μL was injected in a Luna LC column 250 $\times$ 4.6 mm 5 μm C18 (2) 100 A (Phenomenex, 411 Madrid Avenue, Torrance, CA 90501-1430, USA) thermostated at 20 $^{\circ}\text{C}$. The different phenols were eluted by a gradient program using two mobile phases, i.e., (A) methanol:acetonitrile (1:1) and (B) 1% *v/v* acetic acid in water. The flow rate was adjusted at 1 mL min^{-1} , and the gradient profile was as follows for solvent A: 10% from 0 to 5 min, 20% from 10 to 35 min, 25% from 45 to 55 min, 30% at 70 min, 40% at 100 min, and 70% from 106 to 123 min. A total of eleven (11) phenolic compounds were detected based on their retention time and spectra similarity with the corresponding standards. The phenolic compounds detected were hydroxytyrosol, tyrosol, vanillic acid, caffeic acid, vanillin, p-coumaric acid, ferulic acid, oleacein, oleocanthal, luteolin, and apigenin. The standards were purchased from Sigma-Aldrich (Sigma-Aldrich, Inc., PO Box 14508, St. Louis, MO, USA), and their concentration was expressed in mg kg^{-1} olive oil.

2.5. Antioxidant Capacity

The antioxidant capacity of the oils was determined in the same extract of phenolic compounds based on two different assays, i.e., the diphenyl picryl hydrazyl (DPPH) and the ferric reducing antioxidant power (FRAP) assays, according to Roussos et al. [21]. The antioxidant capacity was expressed as $\mu\text{mol Trolox equivalent kg}^{-1}$ olive oil.

2.6. Fatty Acids and Squalene Determination

Fatty acid composition, expressed as a percentage of corresponding methyl esters (FAMES), was determined by diluting 0.5 g of olive oil in 2 mL of GC-grade hexane and vigorously shaking, followed by the addition of 1 mL of a 0.2 N solution of KOH. The methyl esters were analyzed by gas chromatography (Shimadzu GC-2030) (Shimadzu Europa GmbH, Albert-Hahn-Str. 6-10, 47269 Duisburg, Germany) equipped with an autosampler (AOC-20i+s), and a flame ionization detector (FID). The analysis was performed using a fused silica capillary column (Supelco SP[®]-2340 60 $\times$ 0.32 mm $\times$ 0.2 μm film thickness) (Sigma-Aldrich, Inc., PO Box 14508, St. Louis, MO 63178, USA). A split ratio of 80.0 was used, and the injector temperature was set at 249 $^{\circ}\text{C}$, while the detector temperature was set at 249 $^{\circ}\text{C}$. Then, 10 μL of the sample was injected at an initial column temperature of 150 $^{\circ}\text{C}$, which was held for 18 min. The temperature was then increased at a rate of 2 $^{\circ}\text{C min}^{-1}$ to 175 $^{\circ}\text{C}$, held for 5 min, and then raised at a rate of 5 $^{\circ}\text{C min}^{-1}$ to a final temperature of 200 $^{\circ}\text{C}$, which was held for 22 min. In oils from both cultivars, the following free fatty acids were determined: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), and gadoleic (C20:1) acids. In the 'Koroneiki' oil samples, heptadecanoic (C17), heptadecenoic (C17:1), behenic (C22), and lignoceric (C24) acids were also detected in small amounts. Squalene (Sigma-Aldrich, Inc., PO Box 14508, St. Louis, MO 63178, USA) concentration was determined by a five-point standard curve of the corresponding analytical standard and expressed as $\text{mg } 100 \text{ g}^{-1}$ of olive oil.

2.7. Statistical Analysis

The trial was designed with four replicates of one tree in each orchard, i.e., four trees per treatment. The four plots (trees) within each orchard were randomly selected in each orchard's area (the trial followed the completely randomized design). Significant differences among treatments for the same trial area (Viotia and Heraklio County) were determined based on the Tukey HSD test at $\alpha = 0.05$ after checking the normal distribution of raw data using standard skewness, standard kurtosis, and the homogeneity of variances,

and a suitable transformation was performed in those datasets that did not follow normal distribution. Hierarchical cluster analysis (Ward method) of raw data (olive oil quality indexes, FAMES content, phenolic compounds concentration, squalene, and antioxidant capacity as well as yield, oil percentage per fruit, and oil quantity per tree) was performed to obtain descriptive information on the effects of the various treatments. Constellation plots were also constructed to depict possible similarities of the various treatments based on the measured variables. The statistical software JMP 13.0 (SAS Institute) and Statgraphics Centurion XV (Statgraphics Technologies, Inc., The Plains, VA, USA) were used for the aforementioned analyses.

3. Results

3.1. Effects of the Treatments on Yield Components

The treatments applied in cv. ‘Megaron’ in Viotia County had a significant effect on the yield components (Table 1). Kaolin application in August resulted in the highest yield and oil production per tree, reaching 48 kg of olive fruits and 11.95 kg of oil per tree. The lowest oil production per tree was determined under control conditions (only 6.50 kg per tree). The highest oil percentage was achieved in trees treated with talc both in July and August (33.22%), followed by talc treatment only in August (29.20%). The maturity index was highest under talc applied in August.

Table 1. Effects of the various treatments on olive fruit yield, olive oil percentage, oil produced per tree, and fruit maturity index at harvest separately per cultivar.

Treatment	Yield (kg Tree ⁻¹)	Oil Percentage per Fruit	Oil per Tree (kg)	Maturity Index
cv. ‘Megaron’ in Viotia County				
Control	23.0 b	28.35 b	6.50 c	3.01 b
K-JA	30.0 b	27.47 b	8.25 bc	2.76 b
K-A	48.0 a	25.20 b	11.95 a	2.52 b
T-JA	26.9 b	33.22 a	8.91 b	2.52 b
T-A	24.3 b	29.20 ab	7.01 cd	4.13 a
cv. ‘Koroneiki’ in Heraklio County				
Control	36.1 ab	25.5 a	9.12 ab	2.66 a
At-JA	57.9 a	24.1 a	13.90 a	2.51 a
At-A	43.4 ab	24.3 a	10.67 ab	2.91 a
K-JA	30.1 b	22.3 a	6.52 b	3.06 a
K-A	46.2 ab	21.5 a	9.92 ab	2.83 a
T-JA	41.4 ab	20.9 a	8.72 ab	2.91 a
T-A	43.8 ab	25.5 a	10.77 ab	2.63 a

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: K, kaolin; T, talc; JA, application in both July and August; A, application in August; At, attapulgit.

The treatments in cv. ‘Koroneiki’ in Heraklio County had a slightly different effect. The highest yield of fruits and the highest olive oil per tree was produced after applying attapulgit both in July and August, followed by the single application of kaolin in August (fruit yield) and a single talc application in August (olive oil production). The maturity index as well as the oil percentage per fruit was similar under all treatments.

3.2. Effects of the Treatments on Olive Oil Quality Indices

The single kaolin application in July resulted in the lowest free acidity of ‘Megaron’ oil, followed by the double kaolin application (Table 2). Peroxide number was highest

under control conditions and lowest under the single kaolin application, while there were no other differences regarding K232, K270, and ΔK .

Table 2. Effects of the various treatments on oil-free acidity, peroxide index, K232, K270, and ΔK separately per cultivar.

Treatment	Free Acidity (g Oleic Acid 100 g ⁻¹)	Peroxides (meq O ₂ kg ⁻¹)	K232	K270	ΔK
cv. 'Megaron' in Viotia County					
Control	0.59 a	15.62 a	1.82 a	0.15 a	0.006 a
K-JA	0.38 bc	13.12 ab	1.76 a	0.12 a	0.009 a
K-A	0.35 c	10.0 b	1.64 a	0.12 a	0.005 a
T-JA	0.55 ab	10.62 ab	1.81 a	0.14 a	0.011 a
T-A	0.59 a	12.50 ab	1.88 a	0.14 a	0.005 a
cv. 'Koroneiki' in Heraklio County					
Control	0.40 a	8.75 a	1.61 ab	0.16 a	−0.06 bc
At-JA	0.40 a	12.5 a	1.90 a	0.20 a	0.005 ab
At-A	0.39 a	12.5 a	1.84 a	0.19 a	−0.025 abc
K-JA	0.42 a	8.75 a	1.97 a	0.20 a	0.025 ab
K-A	0.40 a	10.0 a	1.11 b	0.11 a	−0.127 c
T-JA	0.40 a	11.25 a	2.04 a	0.22 a	0.052 a
T-A	0.44 a	11.25 a	1.68 a	0.20 a	−0.015 ab

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: K, kaolin; T, talc; JA, application in both July and August; A, application in August; At, attapulgate.

The treatments did not differ regarding free acidity, peroxide number, and K270 of the oil produced by 'Koroneiki', while the lowest K232 was determined under the single kaolin application in August.

3.3. Effects of the Treatments on Olive Oil Phenol Content and Antioxidant Capacity

The double kaolin application (July and August) in 'Megaron' resulted in oil rich in total phenols and with high antioxidant capacity based on the FRAP assay (Table 3). On the contrary, control oil exhibited the lowest total phenol concentration and antioxidant capacity (based both on FRAP and DPPH assays). Kaolin single application resulted in oil rich in flavonoids and of high antioxidant capacity (based on DPPH assay).

On the other hand, 'Koroneiki' control trees produced oil rich in total phenols and flavonoids and of high antioxidant capacity (based on FRAP assay). There was no significant difference among treatments regarding antioxidant capacity measured by the DPPH assay, while the double attapulgate application resulted in high o-diphenol concentration in the oil.

The phenolic compound with the highest concentration in olive oils from both cultivars was oleocanthal, followed by oleacein, tyrosol, and hydroxytyrosol (Table 4). Olive oil from the control treatment of cv. 'Megaron' exhibited the highest concentration of hydroxytyrosol, tyrosol, p-coumaric acid, and oleacein. The double application of kaolin clay particles resulted in high concentrations of vanillic acid, p-coumaric acid, ferulic acid, oleocanthal, and apigenin, while the single application produced oils rich in vanillic acid, oleocanthal, and luteolin.

Table 3. Effects of the various treatments on olive total phenols, o-diphenols, and flavonoids concentration and antioxidant capacity separately per cultivar.

Treatment	Total Phenols (mg GAE kg ⁻¹)	o-Diphenols (mg CAE kg ⁻¹)	Total Flavonoids (mg CtE kg ⁻¹)	FRAP (μmol Trolox Equiv. kg ⁻¹)	DPPH (μmol Trolox Equiv. kg ⁻¹)
cv. 'Megaron' in Viotia County					
Control	245.5 b	139.5 a	127.0 b	1300.5 b	195.6 b
K-JA	500.9 a	159.8 a	254.3 ab	2153.9 a	636.6 ab
K-A	446.4 ab	150.9 a	271.7 a	1871.4 ab	739.2 a
T-JA	469.1 ab	109.1 a	250.1 ab	1883.2 ab	607.1 ab
T-A	415.5 ab	102.0 a	222.7 ab	1492.4 ab	665.9 ab
cv. 'Koroneiki' in Heraklio County					
Control	595.2 a	61.4 ab	590.1 a	2345.1 a	2618.2 a
At-JA	525.6 ab	124.7 a	473.4 ab	1768.7 ab	2134.8 a
At-A	497.2 ab	64.7 ab	404.5 ab	1747.9 ab	2069.7 a
K-JA	521.8 ab	55.0 b	369.2 ab	1642.2 b	1788.8 a
K-A	480.0 b	36.5 b	390.9 ab	1505.6 b	1898.1 a
T-JA	468.0 b	69.1 ab	355.7 ab	1598.0 b	1997.9 a
T-A	468.1 b	38.5 b	349.8 b	1525.4 b	1764.7 a

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: K, kaolin; T, talc; JA, application in both July and August; A, application in August; At, attapulgate.

Table 4. Effects of the various treatments on the concentration of individual phenolic compounds detected in olive oils in mg kg⁻¹ separately per cultivar.

Treatment	Hydroxytyrosol	Tyrosol	Vanillic Acid	Caffeic Acid	Vanillin	p-Coumaric Acid	Ferulic Acid	Oleacein	Oleocanthal	Luteolin	Apigenin
cv. 'Megaron' in Viotia County											
Control	23.38 a	28.00 a	0.27 b	0.02 a	0.09 a	0.98 a	0.15 bc	42.7 a	152.7 ab	9.5 ab	1.23 ab
K-JA	13.61 b	23.78 ab	0.76 a	0.03 a	0.07 a	0.87 a	0.25 a	23.5 bc	185.6 a	11.2 ab	1.54 a
K-A	13.72 b	16.84 c	0.89 a	0.02 a	0.06 a	0.59 ab	0.20 ab	19.1 bc	178.1 a	11.7 a	1.52 a
T-JA	16.14 ab	18.77 bc	0.29 b	0.03 a	0.06 a	0.71 ab	0.13 c	36.1 ab	92.3 bc	6.5 bc	0.84 bc
T-A	7.54 b	8.11 d	0.15 b	0.02 a	0.08 a	0.30 b	0.06 d	13.2 c	49.8 c	1.9 c	0.19 c
cv. 'Koroneiki' in Heraklio County											
Control	13.49 a	24.75 a	0.43 a	0.02 ab	0.03 ab	0.39 a	0.05 a	18.0 a	169.0 a	15.7 a	2.95 a
At-JA	11.91 a	23.71 a	0.70 a	0.01 b	0.04 a	0.26 bc	0.05 a	28.4 a	158.3 a	9.1 ab	2.54 ab
At-A	13.17 a	20.63 ab	0.34 a	0.01 b	0.03 ab	0.20 c	0.04 a	26.1 a	142.7 a	9.4 ab	1.99 ab
K-JA	8.16 a	21.27 ab	0.52 a	0.04 a	0.02 b	0.33 ab	0.03 a	14.0 a	185.6 a	10.3 ab	1.76 ab
K-A	10.13 a	18.47 b	0.72 a	0.01 b	0.03 ab	0.37 a	0.06 a	22.0 a	137.9 a	8.5 b	2.08 ab
T-JA	9.47 a	17.29 b	0.76 a	0.01 b	0.03 ab	0.32 ab	0.04 a	18.6 a	160.7 a	10.0 ab	1.66 b
T-A	7.34 a	21.21 ab	0.69 a	0.01 b	0.03 ab	0.34 ab	0.04 a	20.1 a	141.6 a	9.0 ab	1.82 ab

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: K, kaolin; T, talc; JA, application in both July and August; A, application in August; At, attapulgate.

There were not many differences regarding the concentration of phenolic compounds found in oils derived from 'Koroneiki' under the influence of the treatments imposed. Oils from the control treatment presented high concentrations of tyrosol, luteolin, and apigenin, while the double attapulgate application resulted in oils with high tyrosol and vanillin concentrations. Kaolin double application resulted in a high caffeic acid concentration in the oil, while the single one resulted in a high p-coumaric acid concentration. As with 'Megaron', oils produced after talc application in most cases presented low phenolic compound concentrations.

3.4. Effects of the Treatments on Olive-Oil-Free Fatty Acid Content

Significant differences were detected among the olive oils of cv. 'Megaron' produced under the influence of the different treatments regarding the free fatty acid content (Table 5).

Single talc application resulted in oils rich in C16, C18, C18:2, and C20 and low in C18:1. Similarly, the single kaolin application resulted in oils also rich in C18 and C20, but they were rich in C18:1 (without any difference from the control or the double application of either kaolin or talc). As a result of these differences, oils produced under the single talc application were rich in SFAs and PUFAs, with a high ratio of SFA/UFA but a low C18:1/C18:2 one. All other treatments resulted in oils rich in MUFAs and UFAs with a high ratio of MUFA/PUFA (except for the double kaolin application). There were, however, no differences concerning squalene concentration.

Table 5. Effects of the various treatments on the content of free fatty acids and on various groups of free fatty acids (determined as FAMES—%) and squalene concentration in the oil of cv. ‘Megaron’ in Viotia County.

Treatment	C16	C16:1	C18	C18:1	C18:2	C20	C18:3	C20:1
Control	16.99 b	1.62 a	2.05 b	63.65 a	14.71 b	0.23 b	0.59 a	0.14 a
K-JA	17.33 ab	1.70 a	2.16 b	62.22 a	15.59 ab	0.27 ab	0.59 a	0.15 a
K-A	16.74 b	1.72 a	2.46 a	64.33 a	13.85 b	0.29 a	0.53 a	0.13 a
T-JA	17.14 b	1.59 a	2.20 b	63.13 a	14.85 b	0.25 ab	0.58 a	0.16 a
T-A	17.87 a	1.68 a	2.52 a	59.59 b	17.27 a	0.28 a	0.59 a	0.15 a
Various groups of free fatty acids and squalene								
	SFA	MUFA	PUFA	UFAs	MUFA/PUFA	SFA/UFA	C18:1/C18:2	Squalene (mg 100 g ^{−1})
Control	19.3 b	65.4 a	15.3 b	80.7 a	4.27 a	0.24 b	4.32 ab	414.4 a
K-JA	19.8 b	64.0 a	16.2 ab	80.2 a	3.99 ab	0.25 b	4.03 b	398.6 a
K-A	19.5 b	66.0 a	14.4 b	80.5 a	4.59 a	0.24 b	4.66 a	386.3 a
T-JA	19.6 b	65.0 a	15.4 b	80.4 a	4.23 a	0.24 b	4.26 ab	402.8 a
T-A	20.7 a	61.4 b	17.9 a	79.3 b	3.44 b	0.26 a	3.145 c	344.9 a

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: K, kaolin; T, talc; JA, application in both July and August; A, application in August.

There were not so many differences in fatty acid concentration in the oils of the ‘Koroneiki’ cultivar (Table 6). Oils produced under control conditions presented a high content of C16:1, while the double talc application resulted in high C18, C18:2, and C20 content. As a result, the PUFA content was high in oils produced under the influence of the double talc application compared to the double attapulgit application, while the ratios MUFA/PUFA and C18:1/C18:2 were higher under the double attapulgit application, with a significant difference from the double talc application.

Table 6. Effects of the various treatments on the content of free fatty acids and on various groups of free fatty acids (determined as FAMES—%) and squalene concentration in the oil of cv. ‘Koroneiki’ in Heraklio County.

Treatment	C16	C16:1	C17	C17:1	C18	C18:1	C18:2	C20	C18:3	C20:1	C22	C24
Control	12.74 a	0.86 a	0.026 a	0.036 a	2.69 b	76.32 a	5.96 ab	0.41 c	0.54 a	0.25 a	0.12 a	0.03 a
At-JA	12.44 a	0.77 bc	0.028 a	0.036 a	2.74 ab	77.05 a	5.51 b	0.43 abc	0.56 a	0.27 a	0.12 a	0.03 a
At-A	12.71 a	0.81 ab	0.022 a	0.036 a	2.76 ab	76.17 a	6.11 ab	0.42 bc	0.54 a	0.26 a	0.12 a	0.03 a
K-JA	12.34 a	0.74 bc	0.029 a	0.036 a	2.93 ab	75.90 a	6.56 ab	0.44 ab	0.58 a	0.26 a	0.13 a	0.02 a
K-A	12.38 a	0.71 c	0.023 a	0.035 a	2.93 ab	76.34 a	6.08 ab	0.45 ab	0.59 a	0.27 a	0.13 a	0.03 a
T-JA	12.52 a	0.75 bc	0.025 a	0.040 a	3.05 a	75.52 a	6.64 a	0.46 a	0.58 a	0.26 a	0.12 a	0.02 a
T-A	12.61 a	0.78 abc	0.028 a	0.042 a	2.83 ab	75.83 a	6.41 ab	0.44 abc	0.58 a	0.27 a	0.12 a	0.03 a

Table 6. Cont.

Treatment	C16	C16:1	C17	C17:1	C18	C18:1	C18:2	C20	C18:3	C20:1	C22	C24
Various groups of free fatty acids and squalene												
	SFA	MUFA	PUFA	UFAs	MUFA/PUFA	SFA/UFA	C18:1/C18:2	Squalene (mg 100 g ⁻¹)				
Control	16.03 a	77.46 a	6.50 ab	83.96 a	11.92 ab	0.16 a	12.82 ab	170.6 a				
At-JA	15.79 a	78.12 a	6.07 b	84.20 a	12.89 a	0.19 a	14.02 a	209.2 a				
At-A	16.07 a	77.27 a	6.65 ab	83.92 a	11.66 ab	0.19 a	12.52 ab	185.5 a				
K-JA	15.90 a	76.94 a	7.15 ab	84.09 a	10.79 b	0.19 a	11.59 ab	174.0 a				
K-A	15.95 a	77.36 a	6.68 ab	84.04 a	11.58 ab	0.19 a	12.55 ab	188.0 a				
T-JA	16.20 a	76.57 a	7.22 a	83.79 a	10.64 b	0.19 a	11.42 b	151.1 a				
T-A	16.07 a	76.93 a	6.98 ab	83.92 a	11.16 ab	0.19 a	12.03 ab	185.1 a				

Means within the same column and for the same county/cultivar followed by the same letter do not differ significantly based on the Tukey HSD multiple range test at $\alpha = 0.05$. Abbreviations: At, attapulgit; K, kaolin; T, talc; JA, application in both July and August; A, application in August.

3.5. Hierarchical Cluster Analysis Results

Taking into account all the harvest parameters and oil quality indices of the ‘Megaron’ cultivar, it is obvious that the single talc application separates from the other treatments, characterized by high levels mainly of free fatty acids (C16, C18:2, PUFAs, SFAs, C18, and C20), low levels of phenolic compounds (such as o-diphenols, oleocanthol, oleacein, apigenin, etc.) and squalene, and low yield and low oil production (Figure 4). On the other hand, kaolin, either single or double, seems to give similar results. Interestingly, when the analysis took place, taking into consideration only the products used and not the number of spray applications, talc was separated from kaolin and control treatments, which were located close to each other in the constellation plot (Figure 5). Talc application, regardless of the times it was applied, resulted in a high olive percentage and fruit maturity index as well as a high content of some free fatty acids, as previously described for the single talc application. At the same time, it resulted in oils with low phenolic compounds and squalene as well as oleic acid (C18:1).

In the ‘Koroneiki’ cultivar, the hierarchical clustering analysis produced less distinctive results (Figure 6). Talc treatments seem to lead to similar results concerning harvest data and oil quality indices, as both treatments resulted in low levels of many of the attributes tested in this trial. The double application of attapulgit was grouped quite close to the control treatment. Irrespective of the application number a product was applied, the analysis produced quite clear results (Figure 7). All treatments were separated from each other, as depicted by the constellation plot. It is quite interesting to see that attapulgit treatment resulted in high yield, peroxide number, oil production, oleacein, and o-diphenols concentration, as well as high content of some free fatty acids (C18:1, MUFAs, and UFAs) and squalene. The control treatment was characterized by a high oil percentage in the fruits and some phenolic compounds (vanillin, total phenols, total flavonoids, apigenin, and luteolin) and antioxidant capacity (DPPH and FRAP). Kaolin resulted in high maturity index olive fruits and oils high in some free fatty acids (C22, C18, C20, C20:1, and C18:3). Application of talc, on the other hand, resulted in oils with high free acidity, such as K232 and K270, and free fatty acids, like C18:2, C17:1, and SFAs.

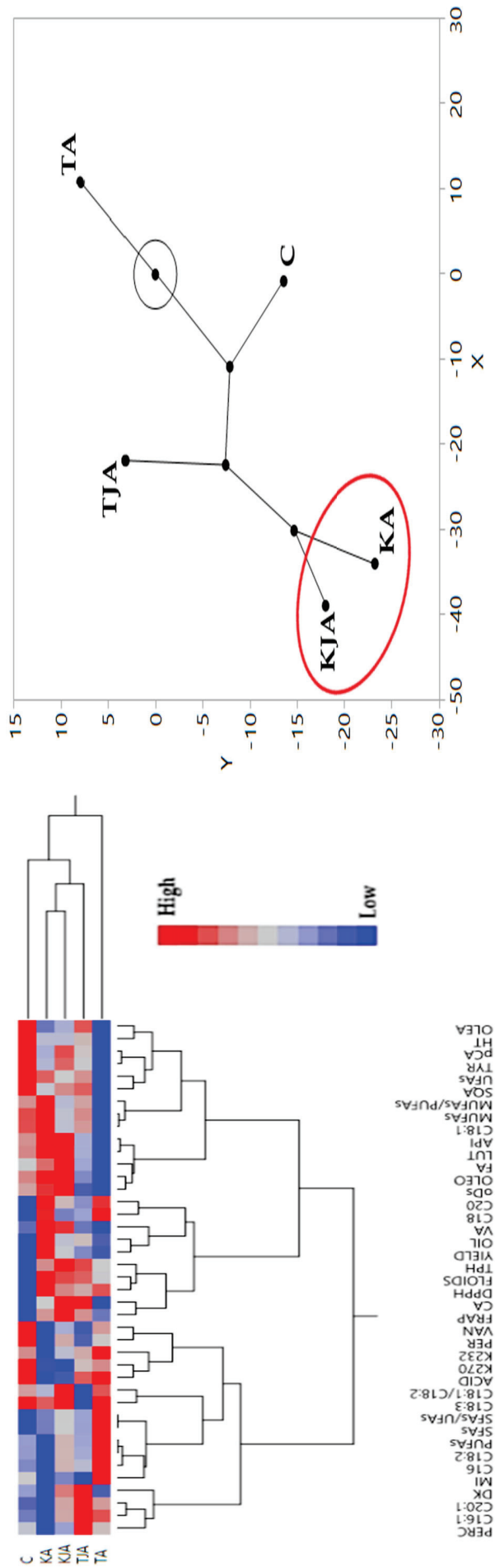


Figure 4. Hierarchical cluster analysis results (on the left) and constellation plot (on the right) of the raw data per treatment in cv. ‘Megaron’ in Viotia County. Abbreviations: PERC, oil percentage per fruit; ΔK, delta kappa; MI, maturity index; ACID, oil-free acidity; PER, peroxide number in oil; VAN, vanillin; FRAP, antioxidant capacity based on ferric reducing antioxidant power assay; CA, caffeic acid; DPPH, antioxidant capacity based on diphenyl-picryl hydrazyl assay; FLOIDS, total flavonoids; TPH, total phenols; OIL, oil produced per tree; VA, vanillic acid; oDs, total o-diphenols; OLEO, oleocanthal; FA, ferulic acid; LUT, luteolin; API, apigenin; SQA, squalene; TYR, tyrosol; pCA, p-coumaric acid; HT, hydroxytyrosol; OLEA, oleacein; K, kaolin; T, talc; JA, application in both July and August; A, application in August; C, control. The ellipse indicates the similarity in the efficacy of kaolin treatments, regardless of the time of application.

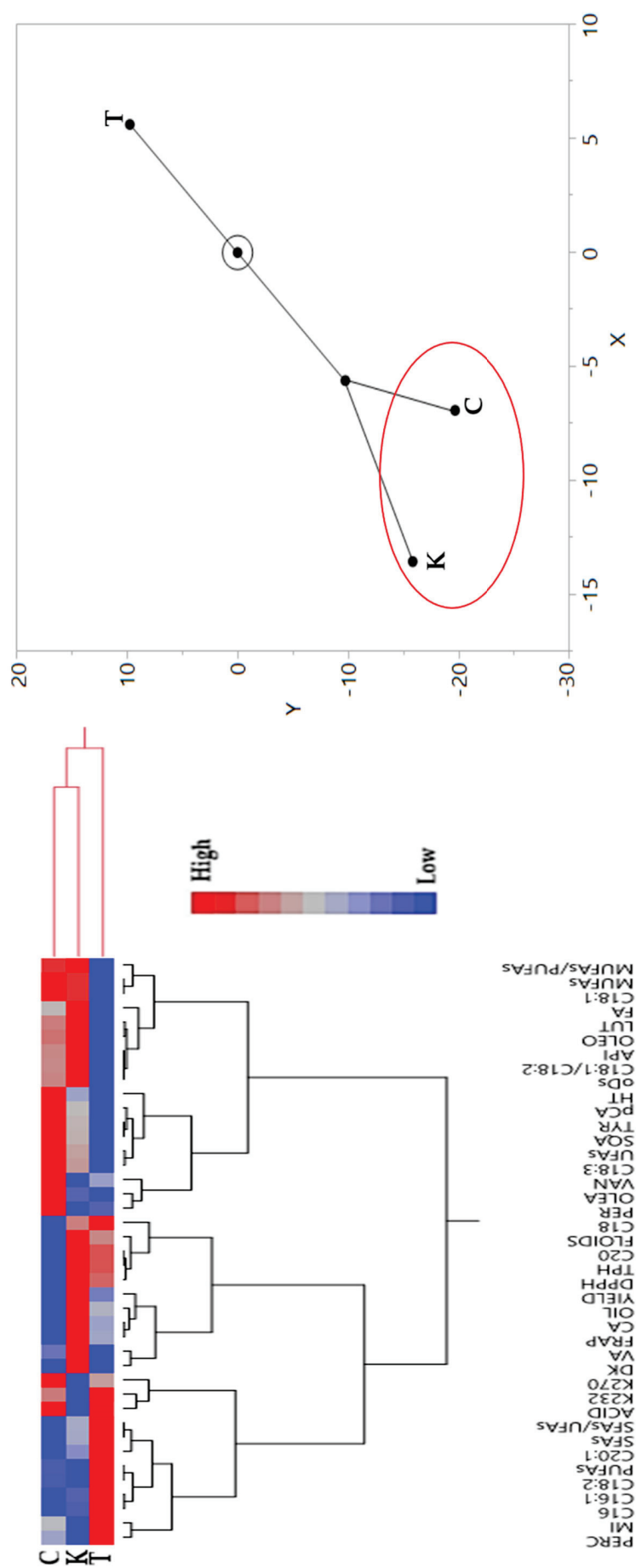


Figure 5. Hierarchical cluster analysis results (on the left) and constellation plot (on the right) of the raw data per product used in cv. 'Megaron' in Viotia County. Abbreviations: PERC, oil percentage per fruit; ΔK, delta kappa; MI maturity index; ACID, oil-free acidity; VAN, vanillin; FRAP, antioxidant capacity based on ferric reducing antioxidant power assay; CA, caffeic acid; DPPH, antioxidant capacity based on diphenyl-picryl hydrazyl assay; FLOIDS, total flavonoids; TPH, total phenols; OIL, oil produced per tree; VA, vanillic acid; oDs, total o-diphenols; OLEO, oleocanthal; FA, ferulic acid; LUT, luteolin; API, apigenin; SQA, squalene; TYR, tyrosol; pCA, p-coumaric acid; HT, hydroxytyrosol; OLEA, oleacein; K, kaolin; T, talc; C, control. The eclipse indicates the similarity in the efficacy of kaolin and control treatments.

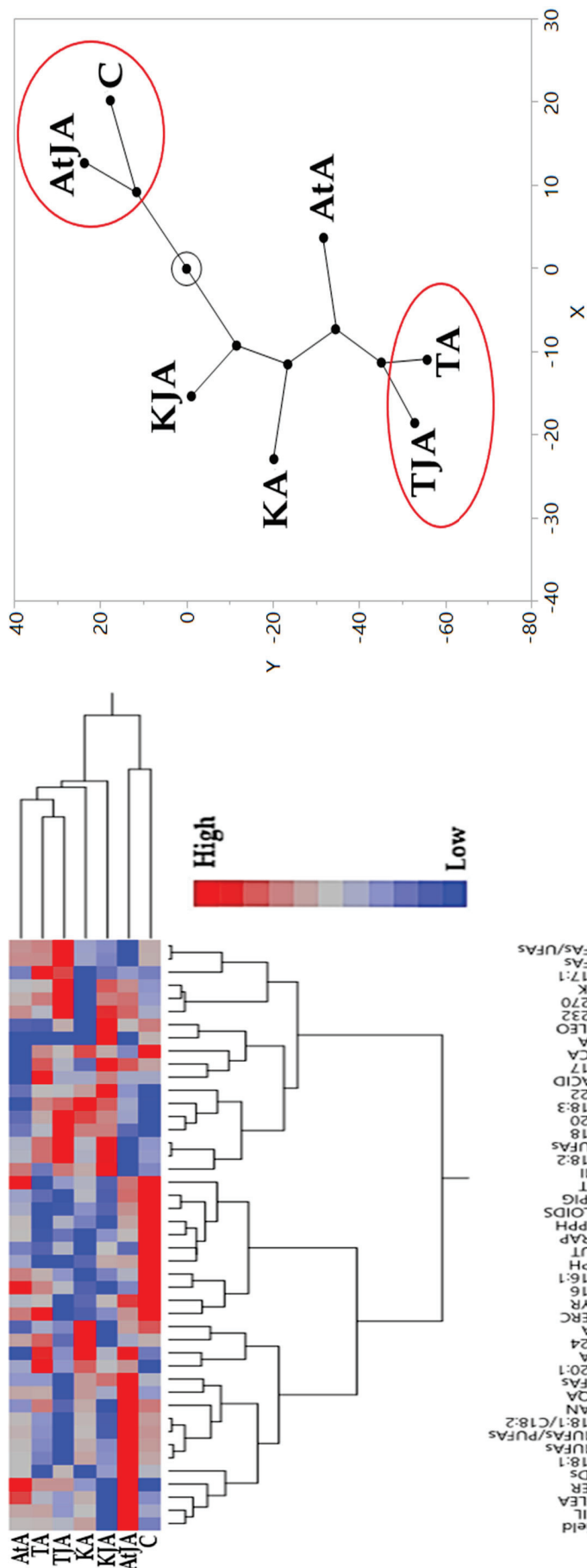


Figure 6. Hierarchical cluster analysis results (on the left) and constellation plot (on the right) of the raw data per treatment in cv. ‘Koroneiki’ in Heraklio County. Abbreviations: PERC, oil percentage per fruit; ΔK, delta kappa; ML, maturity index; ACID, oil-free acidity; PER, peroxide number in oil; VAN, vanillin; FRAP, antioxidant capacity based on ferric reducing antioxidant power assay; CA, caffeic acid; DPPH, antioxidant capacity based on diphenyl-picryl hydrazyl assay; FLOIDS, total flavonoids; TPH, total phenols; OIL, oil produced per tree; VA, vanillic acid; oDs, total o-diphenols; OLEO, oleocanthal; FA, ferulic acid; LUT, luteolin; API, apigenin; SQA, squalene; TYR, tyrosol; pCA, p-coumaric acid; HT, hydroxytyrosol; OLEA, oleacein; K, kaolin; T, talc; JA, application in both July and August; A, application in August; At, attapulgit; C, control. The eclipses indicate the similarity in the efficacy of the double attapulgit application with control and the similarity of the two talc treatments regardless of the time of application.

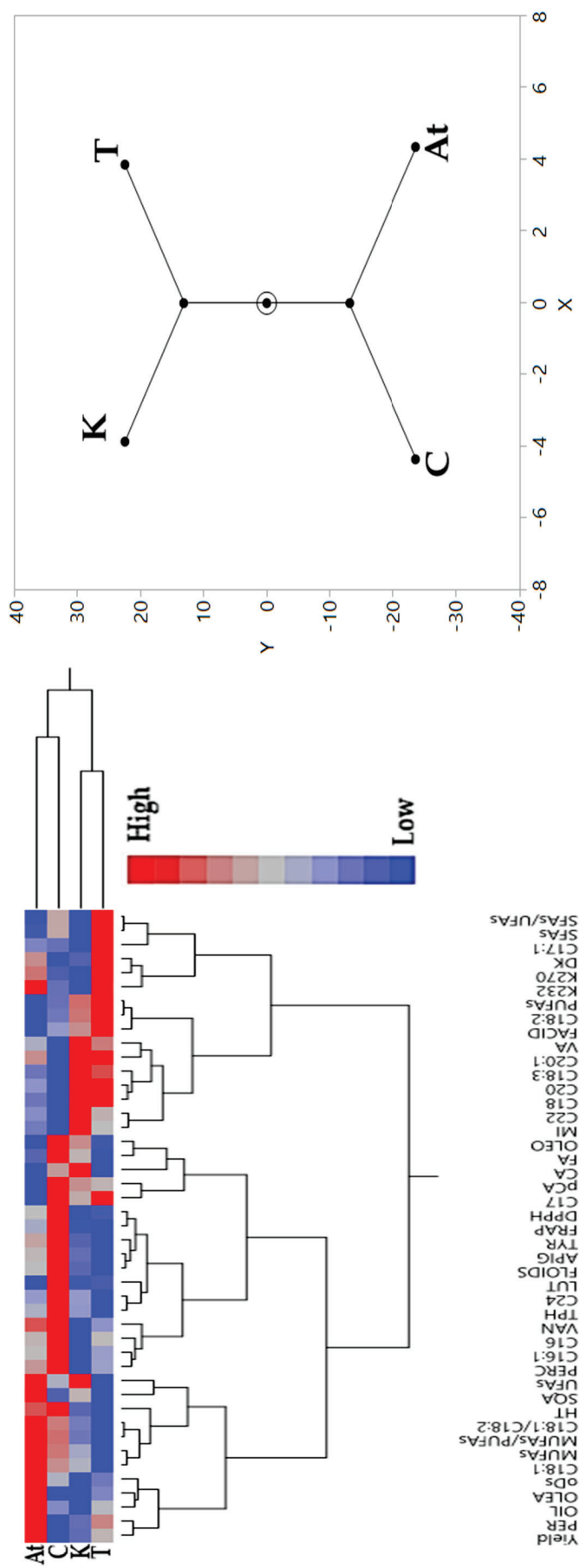


Figure 7. Hierarchical cluster analysis results (on the left) and constellation plot (on the right) of the raw data per product used in cv. ‘Koroneiki’ in Heraklio County. Abbreviations: PERC, oil percentage per fruit; ΔK, delta kappa; MI, maturity index; ACID, oil-free acidity; PER, peroxide number in oil; VAN, vanillin; FRAP, antioxidant capacity based on ferric reducing antioxidant power assay; CA, caffeic acid; DPPH, antioxidant capacity based on diphenyl-picryl hydrazyl assay; FLOIDS, total flavonoids; TPH, total phenols; OIL, oil produced per tree; VA, vanillic acid; oDs, total o-diphenols; OLEO, oleocanthal; FA, ferulic acid; LUT, luteolin; API, apigenin; SQA, squalene; TYR, tyrosol; pCA, p-coumaric acid; HT, hydroxytyrosol; OLEA, oleacein; K, kaolin; T, talc; At, attapulgitte; C, control.

4. Discussion

The hierarchical clustering of the two trials revealed both differences and similarities among treatments in terms of production traits and olive oil quality. As this is the first report on the use of talc and attapulgite on olive trees, interpretations of their mode of action are based on current knowledge of how PFT works.

Under rainfed conditions, where the ‘Megaron’ cultivar was grown, kaolin—followed by talc to a lesser extent—significantly improved fruit yield and oil production per tree. Additionally, double talc application enhanced oil percentage per fruit mass. In the irrigated ‘Koroneiki’ cultivar, clay particle treatments slightly increased yield and oil production, except for double kaolin application. The maturity index remained unchanged in ‘Koroneiki’ but was advanced in ‘Megaron’ with a single talc application, probably a result of the low efficacy of the single talc application to alleviate stress, resulting in an acceleration of maturation compared to other particle films [22] or a result of the chemical and/or optical properties of talc, which should be further examined.

Particle film technology reduces leaf temperature, maintaining stomatal function under conditions that would otherwise impair photosynthesis and transpiration [9]. Kaolin applications have been shown to decrease olive leaf temperature by approximately 2.5 °C, with irrigated trees generally exhibiting lower leaf temperatures than rainfed ones [7,8], alleviating the heat stress and allowing the unhindered function of the photosynthetic mechanism. Such a function of kaolin particle film has been described in several species, such as apple, pomegranate, tomato, coffee, etc. [9,14,23–25]. The increase in photosynthesis rate under stress conditions, due to the protective film formed on leaves, results from a multi-level alleviation effect. It is expected that, as stomata remain functional to a certain degree, this will influence transpiration rates and, consequently, water and nutrient uptake. Improved [9,14,23–25] hydration and nutrition could explain the higher yields recorded under rainfed conditions in the present experiment. Furthermore, it has been found that a kaolin film mitigates oxidative stress under high heat loads, enabling plant functions to operate more effectively than under control conditions, thus supporting growing fruit and enhancing yield [26]. Similar benefits are anticipated from other clay particles, such as talc and attapulgite, which form reflective films on leaf surfaces. However, as this is the first report on the effects of talc on olive trees, it would be premature to assume that the reflective capacity and overall properties of films formed by these clay materials are identical. However, under irrigated conditions, the effectiveness of particle films tends to be lower [11,12], as irrigation itself helps alleviate heat stress to some extent. This was observed in the present trial, where particle clay treatments slightly increased yield and oil production, suggesting that irrigation alone may not sufficiently mitigate heat stress under Crete’s summer conditions [27].

The lower yield and oil production in ‘Koroneiki’ under double kaolin application is consistent with prior reports of reduced growth or biomass accumulation when particle films are applied under optimal irrigation conditions [7,11,12,26]. However, genotype-specific responses to particle film treatments should also be considered, as previously reported in both olive [7,8,28,29] and other species [11,30]. The ‘Megaron’ cultivar is characterized by medium-sized, longitudinally shaped leaves, while ‘Koroneiki’ has smaller leaves and is considered more tolerant to drought stress [31]. These morphological differences may have influenced the varying effectiveness of the clay particles in this study.

Regarding oil quality, a single kaolin application in ‘Megaron’ resulted in superior olive oil attributes, including lower free acidity and peroxide values, compared to both the control and talc treatments. Low free acidity is highly desirable in the olive oil industry, and kaolin has been shown to contribute to reduced acidity in multiple cultivars such as ‘Konservolia’ [26], ‘Zard’ [32], and ‘Zeity’ [19]. These improvements in oil quality could

be attributed to kaolin's ability to reflect UV radiation, reduce fruit temperature, and enhance tissue hydration. According to several authors [33,34], under warm conditions with low precipitation, the olive oil produced is characterized by higher acidity than that produced under cooler and more humid environments. Thus, kaolin-treated olive canopies, as mentioned above, could have been cooler than the canopies of control trees [7,8], thereby preserving low oil acidity. At the same time, kaolin protects the fruits from olive fruit fly infestation [18], which is known to be a major factor contributing to increased oil acidity due to hydrolytic and oxidative reactions following fly attacks [35]. Furthermore, as previously suggested, kaolin application on olive trees alters the plant's oxidative status [36,37], resulting in olive oils with reduced free acidity [26].

This trend was evident in the irrigated 'Koroneiki' cultivar, where no significant effects of clay treatments were observed on free acidity or peroxide values, except for slight reductions in K232 and ΔK under single kaolin application. This could be partly attributed to the already alleviated heat stress by irrigation, combined with the additional alleviation provided by kaolin clay particles, as discussed above and by other researchers [33,34]. Similar non-significant changes in acidity [13,19] and peroxide number [6,13] have been reported under clay particle applications, likely due to factors such as environmental conditions, genotype, spray application timing, frequency, and severity of abiotic stress. Regardless, all produced oils met the criteria for extra virgin olive oil (EVOO).

Kaolin treatments also enhanced the functional properties of 'Megaron' oil, leading to increased total phenol and flavonoid concentrations and higher antioxidant capacity, as reported in previous studies [13,32]. This effect is likely due to kaolin's UV-reflecting properties, which lower fruit temperatures. Particle films applied to stressed olive trees, such as rainfed 'Megaron', significantly influence phenol biosynthesis, though the specific impact varies based on the mineral clay used [13].

Nonetheless, there are reports where olive oil phenolic content is related more to environmental conditions than to kaolin treatment [11]. Interestingly, the irrigated 'Koroneiki' oils from control and attapulgitite-treated trees had the highest phenol concentrations and antioxidant capacities, while talc-treated trees produced oil with the lowest phenolic content. This suggests that both genotype and cultivation conditions (irrigation) significantly affect phenol content and antioxidant capacity. Phenolic compounds act as non-enzymatic antioxidants under stress, mitigating oxidative damage [30,38]. This could justify the increase in phenol concentration in oils from control 'Koroneiki' trees grown under irrigated conditions, as the applied water was probably not enough to minimize leaf and fruit heat load under summer conditions in Crete. Applying clay particles on the trees resulted in an alleviation of heat load stress, leading to the generation of fewer phenolic compounds [28,30]. This was most evident in irrigated 'Koroneiki' under talc and kaolin treatments, suggesting a potentially higher efficacy of these two clay materials in alleviating heat stress. Nonetheless, further research is needed to elucidate the distinct effects of these clay particles and their impact on leaf and whole-plant physiology. Conversely, when stress exceeds the plant's capacity to regenerate antioxidant molecules, the rate of phenolic compound oxidation surpasses their rate of biosynthesis, leading to a reduction in their tissue concentration. This may be the case with the 'Megaron' cultivar under control conditions. Trees likely experienced severe drought and heat stress, unable to maintain phenol production rates sufficient to match phenol oxidation rates, resulting in oils with low phenolic content. Supporting this hypothesis are the results following kaolin application, where the oils produced exhibited higher phenolic content than those from control trees, indicating that kaolin effectively reduced heat and drought stress in 'Megaron' trees. Under these conditions, it could be expected that the need for phenols acting as antioxidant

substrates to protect physiological functions would be diminished, thereby resulting in a higher concentration in the oil produced.

The applied clay particles had a significant impact on the concentration of the individual phenolic compounds detected in the produced olive oils. A high concentration of hydroxytyrosol, tyrosol, oleacein, and p-coumaric acid was determined in oils derived from the control treatment in the 'Megaron' cultivar. Kaolin treatment, on the other hand, resulted in high concentrations of vanillic and ferulic acids and oleocanthal, while talc application resulted in the lowest values. These differences align with previous findings [13], which attribute variations in phenolic profiles to the mineral properties of the clay particles, their ability to filter UV radiation, and their effects on leaf and fruit temperature and hydration [13].

This study is the first to evaluate attapulgite as a foliar spray, and its efficacy appears distinct from other clay minerals. Similarly, talc was less effective in irrigated 'Koroneiki' trees under Crete's summer conditions. However, it must be noted here that the pulp moisture of the fruit greatly affects phenol transferring from the olive paste to the oil [6] and phenols' loss in the aqueous phase after centrifuge. Future studies should measure fruit water content under different treatments to better explain the observed differences in phenolic content and antioxidant capacity. Additionally, it would be interesting to test, apart from the reflecting and physical properties of the clay minerals on plant tissue surfaces, their ability to supply mineral nutrients (such as aluminum, sulfur, and magnesium, which is not a component of kaolin in contrast to talc and attapulgite) to the plants, minerals found in different concentrations and forms in these products. Possible differences in mineral nutrient concentration and availability may have an effect on plant metabolism and phenol biosynthesis and explain the detected differences.

A noticeable effect of the single talc application on 'Megaron' was the lowest concentration of C18:1 in the oil produced, with a simultaneous increase in C16, C18, C18:2, C18:3, and C20, compared to the control. It seems that talc treatment increased the saturated fatty acids of the oil and decreased the mono-unsaturated fatty acids, also resulting in the lowest ratio of C18:1/C18:2. Many pre-harvest factors influence this ratio, favoring either C18:1 or C18:2 [39,40]. As all 'Megaron' trees were cultivated in the same orchard, cultivation practices and pedoclimatic factors should be ruled out. However, based on data regarding the effect of air temperature on these two fatty acids, it has been reported that under warm conditions, the C18:1 and monounsaturated fatty acid (MUFA) content decreases while the C18:2 content increases, whereas under cooler conditions, the opposite occurs [22,40–44]. Given this evidence, it is reasonable to conclude that a single talc application in August failed to sufficiently lower fruit and canopy temperatures to prevent the observed decrease in C18:1 content and the subsequent increase in C18:2 content. This further supports the advanced maturity index observed with the single talc application, likely resulting from severe stress that accelerates peel color development and fruit maturation [22]. On the other hand, the differences induced by the application of clay particles were quite few in 'Koroneiki'. The double talc application resulted in higher C18 and C20 concentrations compared to the control and kaolin single application in lower C17:1. According to Khaleghi et al. [32], kaolin and, to our belief, all the other mineral clay particles influence fatty acid composition by modulating the expression of genes involved in the biosynthesis of fatty acids through the alleviation of heat stress. Consequently, kaolin clay particles altered the composition of fatty acids in olive oil, decreasing C16:0, C18:2, and C18:3 and increasing [22,40–44] C18:1, MUFAs, and the ratio of C18:1/C18:2 [32]. On the other hand, Rotondi et al. [13] reported an increase in C18:2 and decreased C18:3 content. Such effects of kaolin were not observed in the present trial, similar to that reported by Taskin and Ertan [17] and by Karaat and Denizhan [45] in almonds treated with kaolin and other

reflecting materials. These discrepancies may be attributed to multiple factors, including genotype, environmental conditions during growth [13], application timing, fruit maturity at harvest, and the severity of stress [46]. The olive tree is an emblematic species for the Mediterranean basin, and although quite tolerant, climate change greatly influences its growth and production by disrupting key physiological mechanisms (photosynthesis, transpiration, nutrition, and antioxidant mechanisms), with substantial impacts on fruit production and product quality [47–51]. Therefore, further research is needed to refine the application of particle film technology in olive cultivation and optimize its effects on olive fruit and oil quality, particularly in the context of the unfolding climate crisis scenarios.

5. Conclusions

This study marks the first investigation into the use of talc and attapulgite as a foliar spray and their effects on olive tree production. The application of all three mineral clay particles led to varying degrees of improvement in olive yield and oil production while influencing oil quality and functional properties to some extent. However, further research is necessary to evaluate the effectiveness of these mineral clays across a wider range of cultivars, pedoclimatic conditions, and cultivation practices, as it is evident that no single clay material performs optimally under all conditions.

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Article

Effects of Meteorological Factors and Frost Injury on Flowering Stage of Apples and Pears Across Regions at Varying Altitudes

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Abstract

Recent meteorological variability in winter and spring complicates predicting and managing frost damage in apples and pears. This study investigated the relationship between frost injury during the flowering stages of apples ('Hongro' and 'Fuji') and pears ('Wonhwang' and 'Niitaka') and weather conditions across regions at varying altitudes. Orchards were categorized into coastal, inland, mid-mountainous, and mountainous regions, and flowering stages and frost injury were analyzed. The flowering period of apples, from the onset of blooming to full bloom, averaged approximately 15 days, which was about 3 to 5 times longer than that of pears. Furthermore, the total flowering duration of apples was 1.5 to 2.0 times longer than that of pears. Additionally, flowering exhibited a tendency to be delayed at higher altitudes. However, orchards situated in mid-mountainous regions experienced earlier flowering compared to those in other regions. Among the two apple cultivars, the average frost damage was more severe in central flowers than in lateral flowers and was relatively higher in 'Fuji' than in 'Hongro'. In pears, frost damage was most prevalent in the first to fourth flowers within the inflorescence, with 'Wonhwang' exhibiting relatively greater susceptibility than 'Niitaka'. Across different cultivation regions, severe frost damage was observed in orchards located in mid-mountainous areas for both fruit species and cultivars. The severity of frost damage followed the order mid-mountainous, mountainous, plain, and coastal regions. Minimum temperatures were lowest in mid-mountainous and mountainous regions, while daily temperature differences were largest in mid-mountainous regions. Meteorological analysis (1981 to 2022) revealed consistent trends, with an increase in daily temperature range in recent years. These findings highlight the vulnerability of mid-mountainous orchards due to flowering stages overlapping with coastal and plain regions, exposing them to lower temperatures and larger temperature differences. Further studies on temperature variability are essential to mitigate frost damage risks.

Keywords: daily temperature difference; flowering period; *Malus domestica*; altitudes; minimum temperature; *Pyrus pyrifolia*

1. Introduction

Cold resistance in fruit trees is influenced by factors including water content, stored nutrients such as carbohydrates [1], and meteorological conditions. Extensive research on minimizing freeze injury has been conducted, with strategies categorized into preventive

and emergency measures [2]. For preventive measures, the selection of location and terrain before establishing an orchard is paramount. This includes considerations such as cultivating on slopes [3], installing windbreaks [4], deciding whether to use cover crops [5], and assessing soil characteristics [6] in relation to planting styles and soil management methods. Emergency measures include techniques such as burning [4], irrigation [5], air blowing [7,8] and trunk covering [9].

Frost injury during the flowering period is a type of damage that occurs at the lowest temperatures during the early growth stages of fruit trees, with the extent of injury varying annually. The response to methods applied to mitigate this injury has a significant impact on both the quality and yield of fruit. For pears, it is advantageous for the third to fifth flowers in the inflorescence sequence to set fruit, while for apples, fruit setting on the central flower results in a higher proportion of marketable produce [10]. However, the flowering period is short, and the number of blossoms that need to be prioritized for protection is predetermined, which limits the application of preventive or emergency measures [11]. Additionally, methods for predicting the flowering period are primarily based on the influence of spring temperatures, making it difficult to fully account for the effects of winter temperatures on the dormancy and germination variability of flower buds [12]. Therefore, as immediate countermeasures for frost injury during the flowering period are challenging, it is important to adopt a preventive approach through prior measures. Furthermore, establishing secondary emergency measures could serve as an effective complement to these preventive actions.

The apple cultivation area in Jeonbuk State in Republic of Korea spans approximately 2003 ha, with 74.6% (1495 ha) concentrated in mountainous regions (Jangsu and Muju) above 300 m in altitude. In contrast, pears are predominantly cultivated in coastal and plain regions such as Jeonju, Gimje, Wanju, and Gochang, accounting for about 65.8% (335 ha) [13]. However, frost injury during the flowering period has been reported as particularly severe in mid-mountainous regions, such as the Imsil and Namwon areas. Moreover, recent climate changes have raised concerns regarding low-temperature injury to fruit trees during various growth stages (dormancy, germination, and blooming). While research related to cold resistance, such as studies on winter minimum extreme temperatures and temperature variations [14], has been conducted, studies on frost injury during the flowering period in relation to specific cultivation regions remain limited.

In general, temperature decreases with increasing altitude, implying a greater probability of flowering-stage frost damage in apples and pears at higher elevations. However, severe frost damage has been consistently reported over several years in specific regions of Jeonbuk State, Republic of Korea. To elucidate the underlying causes, we classified cultivation altitudes into four categories for two apple and two pear cultivars. Additionally, we sought to determine the meteorological factors influencing the regional variations in frost damage severity. Therefore, this study was conducted to investigate the timing and severity of frost injury during the flowering periods of apples and pears in the cultivation regions of Jeonbuk State in Republic of Korea. This study aims to analyze the correlation between the meteorological characteristics of each cultivation region and to utilize the data as foundational material for identifying areas at risk of frost injury during the flowering period and for establishing countermeasures.

2. Materials and Methods

2.1. Experimental Design

Recurrent low-temperature damage during the flowering period of apple and pear orchards has been documented in specific regions of Jeonbuk State, Republic of Korea. This study focuses on research sites situated within major apple and pear production zones,

as depicted in Figure 1, to identify the underlying causes of low-temperature damage by analyzing its relationship with meteorological factors. Geographically, Jeonbuk State is bordered by the sea to the west and mountain ranges to the east. The areas most susceptible to low-temperature damage are primarily located in the central regions between these two geographic features, at elevations ranging from 100 to 300 m above sea level.

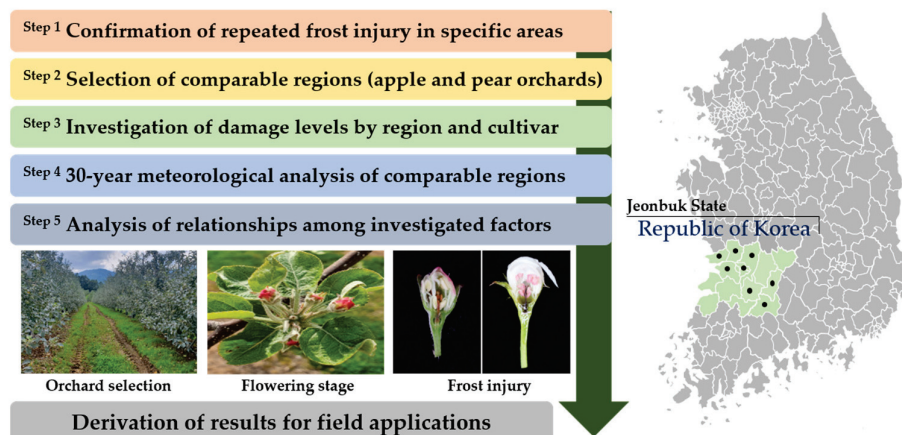


Figure 1. A schematic representation of the experimental process for result derivation.

2.2. Division of Cultivation Regions and Selection of Surveyed Orchards

In 2013, the cultivation regions in Jeollabuk-do Province in Republic of Korea were classified by altitude into coastal (altitude below 100 m), inland (below 100 m altitude and away from the coastline), mid-mountainous (100–300 m altitude), and mountainous regions (above 300 m altitude) [15]. The location of the surveyed orchards for each altitude-based cultivation region is shown in Table 1, with eight orchards selected for apples and seven orchards for pears. The surveyed apple orchards were located at altitudes of 12 m (35°49′54″ N, 126°49′43″ E), 42 m (36°00′33″ N, 127°02′42″ E), 138 m (35°29′51″ N, 127°21′47″ E), 140 m (35°31′25″ N, 127°21′00″ E), 141 m (35°30′48″ N, 127°21′39″ E), 174 m (35°30′43″ N, 127°22′14″ E), 277 m (35°33′32″ N, 127°25′20″ E), and 520 m (35°37′12″ N, 127°30′44″ E). The surveyed pear orchards were located at altitudes of 14 m (35°35′34″ N, 126°41′39″ E), 25 m (35°51′37″ N, 127°01′56″ E), 38 m (35°50′13″ N, 127°01′52″ E), 141 m (35°30′48″ N, 127°21′39″ E), 170 m (35°30′43″ N, 127°22′14″ E), 290 m (35°40′44″ N, 127°17′09″ E), and 358 m (35°36′21″ N, 127°25′57″ E). The test orchards were selected based on their similar cultivation styles and distinct blooming characteristics relative to their cultivation regions.

Table 1. The locations of the apple and pear orchards surveyed for flowering stages and meteorological factors.

Fruit Species	Region Classification by Altitude	Location (Sampling Points)	Altitude (m)	Distance from Coast (km)
Apple	Coastal	Seongdeok-myeon, Gimje-si (1)	12	12
	Inland	Geumma-myeon, Iksan-si (1)	17	30
	Mid-mountainous	Deokgwa-myeon, Namwon-si (4)	121, 138, 141, 148	75
		Beonam-myeon, Jangsu-gun (1)	275	90
	Mountainous	Doosan-ri, Jangsu-gun (1)	520	92

Table 1. Cont.

Fruit Species	Region Classification by Altitude	Location (Sampling Points)	Altitude (m)	Distance from Coast (km)
Pear	Coastal	Seongsan-myeon, Gunsan-si (1)	10	9
	Inland	Won-dong, Jeonju-si (1)	25	34
		Iseo-myeon, Wanju-gun (1)	38	35
	Mid-mountainous	Deokgwa-myeon, Namwon-si (2)	141, 170	75
		Gwanchon-myeon, Imsil-gun (1)	252	63
	Mountainous	Sanseo-myeon, Jangsu-gun (1)	358	82

2.3. Characteristics of Apple and Pear Cultivars and Their Cultivation Management

The apple cultivar ‘Hongro’ was developed in Republic of Korea through the hybridization of ‘Tsugaru’ and ‘Jonathan’. It is an early-maturing variety with a harvest period extending from late August to mid-September. In contrast, ‘Fuji’ was developed in Japan through the hybridization of ‘Red Delicious’ and ‘Ralls Janet’ and is a late-maturing cultivar, typically harvested from late October to early November. The pear cultivar ‘Wonhwang’ was developed through the hybridization of ‘Hwangkeumbae’ and ‘Nijisseiki’ and is classified as an early-maturing variety, typically harvested in August. In contrast, ‘Niitaka’ was developed through the hybridization of ‘Kikusui’ and ‘Nijisseiki’ and is a late-maturing variety, generally harvested from late September to early October.

The apple cultivar ‘Fuji’ and the pear cultivar ‘Niitaka’ are among the predominant commercially cultivated varieties in Republic of Korea. Additionally, in Republic of Korea, ‘Hongro’ and ‘Wonhwang’ have a relatively short distribution period, typically being consumed within 2 to 3 months after harvest. In contrast, ‘Fuji’ and ‘Niitaka’ are stored under controlled conditions and consumed until June or July of the following year.

In all surveyed orchards, apple trees were grafted onto M9 rootstock, whereas pear trees were grafted onto *Pyrus pyrifolia* rootstock. The test trees were 10 to 15 years old for apples and 15 to 25 years old for pears, and all orchards were equipped with irrigation systems. Additionally, the apple trees were all pruned in a spindled shape, and except for the mountainous areas (Y-shaped pruning), the pear trees were managed in a palmette shape. Notably, orchards in mid-mountainous areas, where frost injury during the flowering period is chronically severe, were intensively studied.

2.4. Determination of Flowering Period and Frost Injury Assessment

For this study, specific varieties of apples and pears were selected: ‘Hongro’ and ‘Fuji’ for apples; ‘Wonhwang’ and ‘Niitaka’ for pears. According to the Agricultural Science and Technology Research Survey Analysis Standard [16], the stages of flowering were defined as follows: first flowering occurs when about 10% of flowers are open, full bloom is when approximately 70–80% of flowers are open, and flower dropping is when 70–80% of flowers have fallen.

To evaluate the extent of frost damage in apples, each surveyed orchard was divided into five sections. From each section, 15 bearing branches were collected from three trees that were centrally located within the canopy and exhibited similar vigor. Frost damage was assessed in inflorescences that bloomed from terminal buds, with 15 inflorescences examined per replication across five replications. Additionally, frost damage was analyzed separately for central and lateral flowers within the inflorescence. For pears, the same methodology was applied, with 10 lateral branches collected per orchard. From each lateral branch, 3 inflorescences positioned in the middle were selected, and 30 inflorescences were examined per replication across five replications. Since pear inflorescences typically

bloom sequentially from the first to the eighth flower, frost damage rates were calculated separately for flowers 1–4 and flowers 5–7.

To determine the presence of frost injury during the flowering period, a cutter blade was used to horizontally slice through the peduncles of inflorescences in sequence. This allowed for the visual inspection of internal browning in both the pistils and the peduncles. Specifically, the pistil condition was assessed by bisecting it, and flowers were classified as frost-damaged if complete browning was observed.

2.5. Meteorological Data Collection and Analysis

The regional meteorological data were collected from the Korea Meteorological Administration's (KMA) Automatic Weather System (AWS) at the following locations: Jeonju (station number 146, altitude 60 m), Gunsan (station number 140, altitude 23 m), Buan (station number 243, altitude 12 m), Imsil (station number 244, altitude 247 m), Namwon (station number 247, altitude 133 m), and Jangsu (station number 248, altitude 407 m). These data were used for the analysis. An analysis of average climatic conditions was conducted using long-term average (1981 to 2010), along with minimum temperature and diurnal temperature range data over the past 12 years (2011 to 2022).

2.6. Data Analysis

The arithmetic means of the meteorological data and the degree of injury were calculated using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA), and data visualization was performed using SigmaPlot 14.0 (Systat Software Inc., Chicago, IL, USA). A multiple mean comparison test was conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to analyze differences in frost damage across cultivation regions.

3. Results and Discussion

3.1. Flowering Characteristics by Cultivation Region

The study categorized the orchards by altitude and investigated the stages of first flowering, full bloom, and flower dropping [17], as outlined in Table 2. 'Hongro' showed a first flowering period from April 16 to 19, with a variation of 1–3 days across different cultivation regions, while 'Fuji' bloomed from April 17 to 19, varying by 1–2 days. The duration from first flowering to full bloom was 13 days for 'Hongro' in coastal and plain areas, and 14 days in mid-mountainous and mountainous areas; for 'Fuji', it took 16, 17, 17, and 19 days, respectively. Additionally, differences in the progression of flowering stages were observed on the same day (April 20) across various cultivation regions ('Hongro', Figure 2(A0–A3); 'Fuji', Figure 2(B0–B3)).

For both pear varieties, the mid-mountainous areas experienced the earliest flowering, while the mountainous areas had the latest. Specifically for 'Wonhwang', blooming commenced earliest in the mid-mountainous areas, and the period from first flowering to full bloom tended to extend by 1–2 days as the altitude of the cultivation region increased. For 'Niitaka', the total flowering period lasted 10–11 days, with no significant differences across regions. Similarly to 'Wonhwang', the first flowering in mountainous areas started 1–3 days later. The duration from the start of first flowering to full bloom for both varieties ranged from 2 to 5 days, and the time for the full flower to flower dropping was 8–11 days. For 'Wonhwang' (Figure 2(C0–C3)) and 'Niitaka' (Figure 2(D0–D3)), the first flowering stages on 30 April matched the survey data, similar to the apples. Notably, the average temperatures from 1 to 15 April (Figure 5A), which is before the stage of first flowering, showed a trend of decreasing with increasing elevation similar to apples [18], though there were some differences for pears. These differences may be attributed to factors such as the nutritional status of the pear trees and slope cultivation affecting the orchards [19,20].

Table 2. The differences in the flowering periods of ‘Hongro’ and ‘Fuji’ apples and ‘Wonwhang’ and ‘Niitaka’ pears at various regions in 2013. Black circles represent first flowering, full bloom, and flower dropping, respectively. The white circle indicates the progression status of two distinct flowering stages. Coastal: altitude of less than 100 m ASL; inland: altitude of less than 100 m; mid-mountainous: altitude of 100–300 m; mountainous: altitude above 300 m.

Various Altitudes	Recorded Flowering Stages																							
	April (Day)												May (Day)											
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9
‘Hongro’ Apple																								
Coastal	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	○	○
Inland		●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●	○	○	○	○	○	○	○
Mid-mountainous			●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mountainous				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
‘Fuji’ Apple																								
Coastal		●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Inland		●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mid-mountainous			●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mountainous				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
‘Wonwhang’ Pear																								
Coastal				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Inland				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mid-mountainous				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mountainous					●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
‘Niitaka’ Pear																								
Coastal				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Inland				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mid-mountainous				●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Mountainous					●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

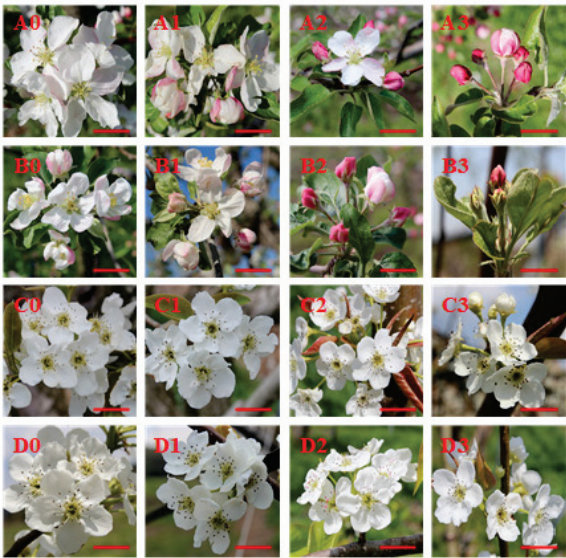


Figure 2. The differences in flowering stage at various region of ‘Hongro’ (A0–A3) and ‘Fuji’ (B0–B3) apples and ‘Wonwhang’ (C0–C3) and ‘Niitaka’ (D0–D3) pears in 2013. Coastal (altitude of less than 100 m ASL): (A0,B0,C0,D0); inland (altitude of less than 100 m): (A1,B1,C1,D1); mid-mountainous (altitude of 100–300 m): (A2,B2,C2,D2); mountainous (altitude above 300 m) regions: (A3,B3,C3,D3). Apples and pears were examined on 20 April and 30 April, respectively. The red solid scale bars indicate 2 cm.

With increasing altitude in the cultivation regions, the onset of first flowering was delayed by approximately 1–3 days more noticeably in apples than in pears [21]. For both fruit types and all four varieties studied, the period from full bloom to flower dropping lasted 4–6 days. Apples took on average about 15 days from the start of first blooming to full bloom, which is 3–5 times longer than pears, and the total first flowering period was also about 1.5–2.0 times longer [17]. While there may be variations in the first flowering period depending on the region and variety, ‘Wonhwang’ and ‘Nittaka’ exhibit shorter flowering periods than ‘Hongro’ and ‘Fuji’. This condition suggests a lower risk of frost injury during the blooming of the pears, yet there is a potential increase in the risk of cold exposure during the fruit-setting phase.

3.2. Analysis of Injury Severity During the Flowering Period of Apples by Cultivation Region

Weather analysis from five areas identified significant temperature fluctuations and lower minimum temperatures during the pre-blooming phase, specifically on April 12 (−3.8 to 0 °C), 13 (−4.1 to 1.9 °C), and 15 (−3.5 to 0.4 °C), as well as during the flowering period on April 19 (−1.0 to 3.1 °C) and 22 (−2.0 to 3.1 °C). These conditions, especially noted in the mid-mountainous (Imsil and Namwon) and mountainous (Jangsu) areas, are estimated to have consistently caused frost injury during these periods. Frost injury during the flowering period, as shown in Figure 3, appeared as a browning of the pistils and peduncle tissues compared to the normal inflorescences. The injury patterns of apples and pears exhibited similarity. An investigation into the extent of injury across the cultivation regions revealed that for ‘Hongro’ (Figure 4A), the injury to the central flowers blooming first was significantly higher at 18.1%, compared to 3.0% for lateral flowers. A similar pattern was observed in ‘Fuji’, with central flowers showing 23.6% injury versus 8.6% for lateral flowers. The injury rates for the central flowers of ‘Hongro’ varied by cultivation region, with 0.0% in coastal areas, 16.9% in plains, 24.9%, 23.1%, 13.8%, and 40.0% in mid-mountainous orchards labeled A–E, and 6.7% in mountainous areas. ‘Fuji’ (Figure 4B) showed similar patterns with injury rates of 11.1%, 20.2%, 16.9%, 36.4%, 48.4%, 32.0%, 20.0%, and 4.0%, respectively, indicating the highest injury rates in mid-mountainous orchards. Similar tendencies were observed in the injury rates of lateral flowers and the average injury within the inflorescences. Specifically, ‘Hongro’ had an average inflorescence injury rate of 6.0%, which was relatively lower than ‘Fuji’ at 11.6%. This result aligns with the findings of previous studies [20].

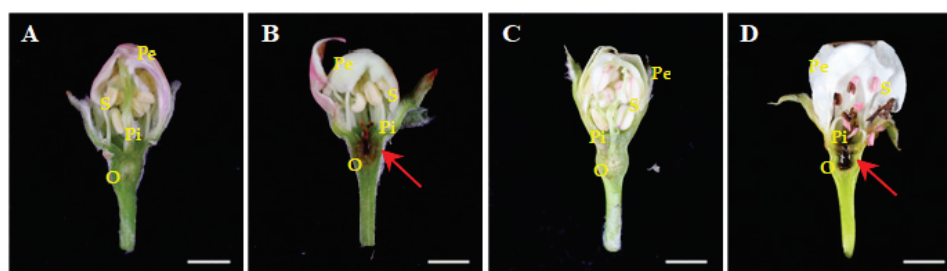


Figure 3. Comparison of cross-sections of normal (A,C) and damaged (B,D) flowers in apple (A,B) and pear (C,D) at the flowering stage. The white solid scale bars indicate 0.5 cm. The red arrows indicate the injured pistil tissues, including the ovule, of apple and pear flowers. Pe: petal; Pi: pistil; S: stamens; O: ovary.

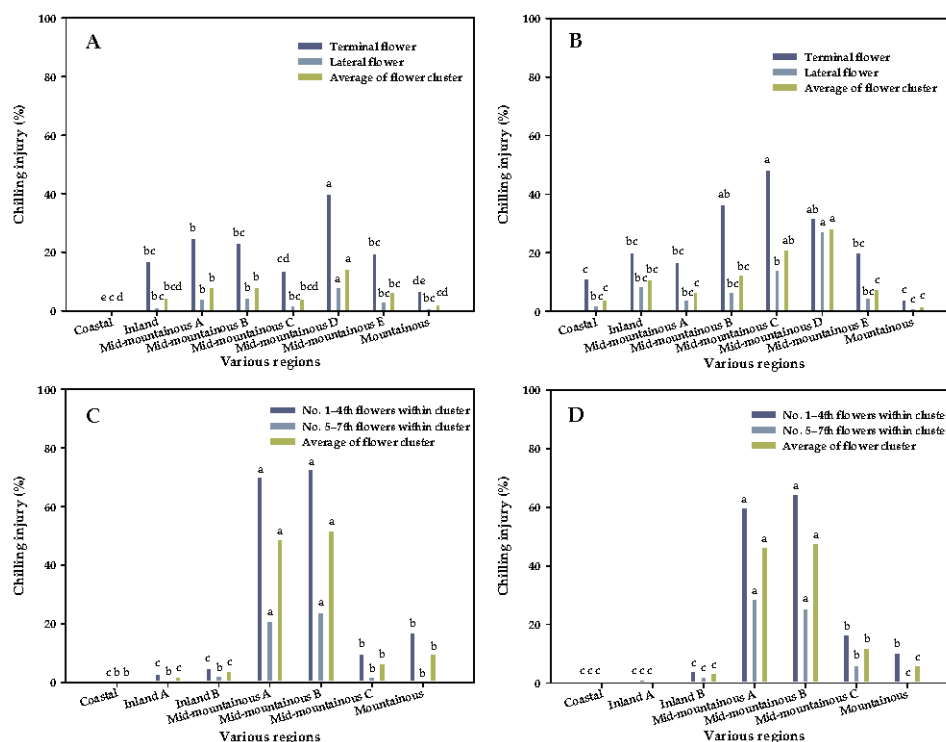


Figure 4. Chilling injury at various regions of ‘Hongro’ (A) and ‘Fuji’ (B) apples and ‘Wonwhang’ (C) and ‘Niitaka’ (D) pears in 2013. Coastal: altitude of less than 100 m ASL; inland: altitude of less than 100 m; mid-mountainous: altitude of 100–300 m; mountainous: altitude above 300 m. Lowercase letters indicate the separation of means among various regions according to Duncan’s multiple range test at $p < 0.05$ ($n = 5$).

3.3. Analysis of Injury Severity During the Flowering Period of Pears by Cultivation Region

For ‘Wonwhang’ (Figure 4C) and ‘Niitaka’ (Figure 4D), injury trends and extents were similar across regions. Notably, in mid-mountainous orchards A and B, injury to the first to fourth flowers in ‘Wonwhang’ reached 70.0% and 72.0%, respectively, significantly higher than in other regions, with a similar pattern in average injury per inflorescence. The severity of frost injury in different cultivation regions followed the order coastal < plain < mountainous < mid-mountainous areas. For ‘Niitaka’, the injury was similar to ‘Wonwhang’, with the highest rate in mid-mountainous orchards A and B, at 59.8% and 64.8%, respectively, for the first four flowers. In particular, the rate of frost injury decreased progressively from the first to the seventh flower within inflorescences in mid-mountainous orchards, suggesting that significant frost injury occurred during an advanced stage of flowering around April 19 to 22. Additionally, damaged flowers of apples and pears either fail to fertilize or, even if fertilization occurs, result in a high incidence of malformed fruits, leading to a significant decline in quality [22,23]. Despite similar flowering periods among varieties within the same species, the variance in injury rates likely stemmed more from differences in cold resistance between varieties than from climate factors.

3.4. Analysis of Climatic Characteristics by Cultivation Region

Based on meteorological data from April to May, the research detailed in Figure 5 investigated temperatures including average, maximum, and minimum, diurnal range, daily sunlight hours, and average wind speed during the flowering period. For the average temperature (Figure 5A), Jeonju (plain area) exhibited the highest values, while Jangsu (mountainous area) recorded the lowest; however, the regional patterns were similar. In contrast, the highest daily temperatures (Figure 5C) showed that Imsil and Namwon (mid-mountainous areas), which generally have cooler average temperatures,

experienced maximum temperatures that were 2–7 °C higher than Jeonju, as well as the coastal and other mountainous areas. Gunsan and Buan (coastal area), however, recorded the lowest maximum temperatures. The minimum daily temperatures (Figure 5E) in Jeonju, Buan, and Gunsan were approximately 2–4 °C higher than those in Imsil, Namwon, and Jangsu. During the flowering period from 16 April to 12 May, the mid-mountainous areas experienced 2–3 days with temperatures below 0 °C, while the coastal and plain areas had none. Additionally, during April and May, the mid-mountainous areas recorded 11–13 days below 0 °C, in contrast to only 1–2 days in the coastal and plain areas, with the majority occurring in April. Diurnal range variability also followed this pattern, with the largest fluctuations observed in Namwon, Imsil, and Jangsu. Despite lower minimum temperatures in mountainous orchards, apple and pear trees suffered less frost injury compared to mid-mountainous orchards, where earlier flowering periods are accelerated by relatively higher average and maximum temperatures, as indicated in Figure 5A,B [21]. Conversely, daily sunlight hours (Figure 5D) and average wind speed (Figure 5F) showed similar patterns across areas and were thought not to significantly influence injury levels.

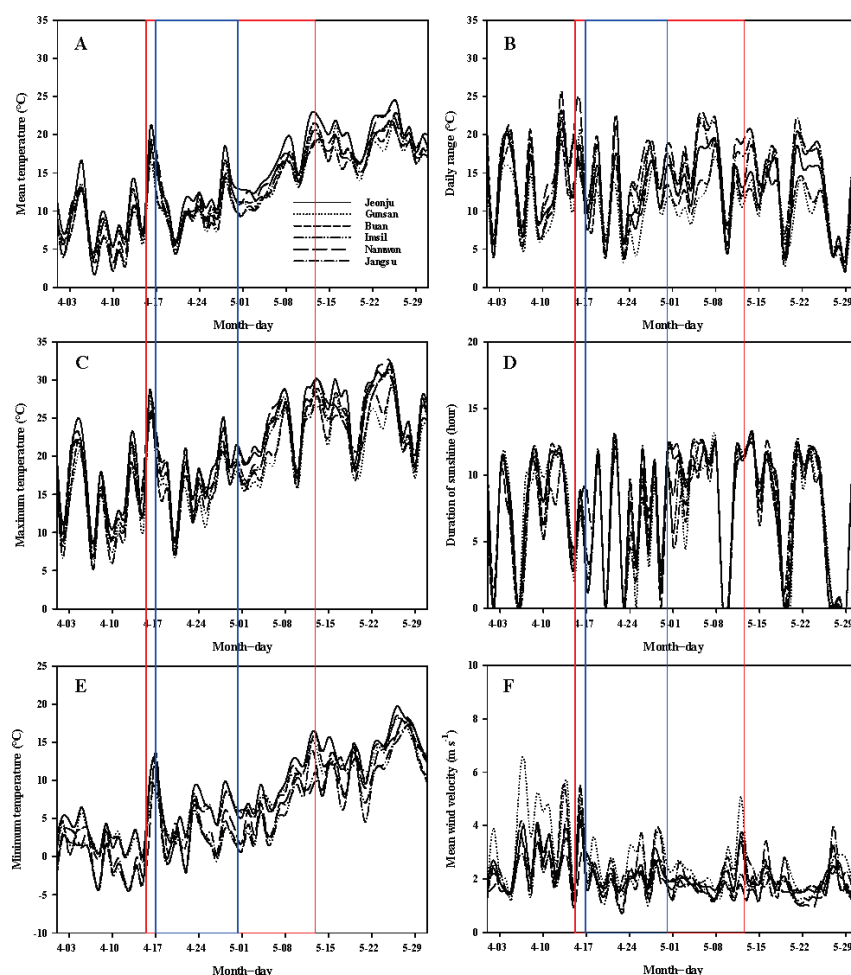


Figure 5. Characteristics of meteorological factors at various regions based on AWS (Gunsan: an altitude of 23 m; Buan: an altitude of 12 m; Jeonju: an altitude of 60 m; Imsil: an altitude of 247 m; Namwon: an altitude of 133 m; Jangsu: an altitude of 407 m). Red and blue boxes represent the blooming period (first flowering to flower dropping) of apple and pear trees, respectively. Mean temperature (A); daily range (B); Maximum temperature (C); duration of sunshine (D); minimum temperature (E); mean wind velocity (F).

3.5. Analysis of Climatic Characteristics by Cultivation Region for the Past 12 Years and the Long-Term Average

An analysis of the average minimum temperature range for April and May from 1981 to 2010 (Figure 6A,B) showed that the plain area (Jeonju) had the highest minimum temperatures, approximately 2 to 5 °C higher than other areas, followed by the mid-mountainous areas (Namwon and Imsil) and the mountainous area (Jangsu), indicating a trend of decreasing temperatures with increasing altitude. Meanwhile, the diurnal range showed the lowest fluctuations in the plain area (Jeonju), with increasing range observed in the mountainous (Jangsu) and mid-mountainous (Namwon and Imsil) areas. During the flowering period in mid-to-late April 2013, for ‘Wonhwang’ and ‘Niitaka’, there was a notable difference of over 4 °C between Namwon and Jeonju. This pattern persisted through April and May, leading to varied frost injury across the cultivation regions. Similarly, the data for the past 12 years (2011 to 2022) on minimum temperatures and diurnal ranges across the cultivation regions (Figure 6C,D), compared to the long-term average (1981 to 2010), showed that minimum temperatures were highest in the plain area, followed by the mid-mountainous and mountainous areas. Conversely, diurnal ranges were greatest in the mid-mountainous areas, followed by the mountainous area and then the plain area.

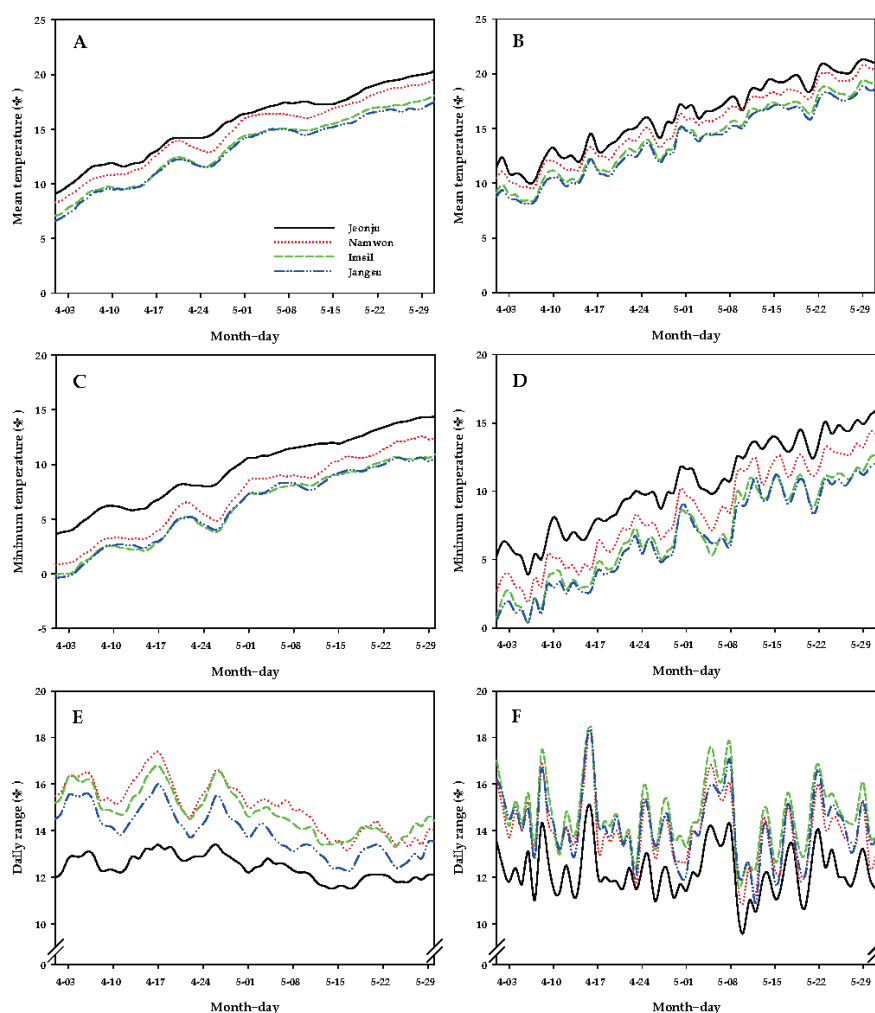


Figure 6. Mean (A,B) and minimum (C,D) temperature, and daily temperature range (E,F) at various regions (inland: an altitude of 61 m; mid-mountainous: an altitude of 133 and 247 m; mountainous: an altitude of 407 m) of the past 30 years (1981–2010, (A,C,E)) and the past 12 years (2011–2022, (B,D,F)).

During the flowering period (April and May) for apples and pears, the weather data of the past 12 years showed an increasing trend in average minimum temperatures compared to the long-term average, with the mid-mountainous areas experiencing a more significant rise than other cultivation regions. Conversely, while the average diurnal range has shown a decreasing trend over the past 12 years, the range itself expanded by 1.3 to 2.7 times. In May, the average diurnal range varied across different cultivation regions, as detailed in Table 3.

Table 3. Comparison of minimum temperature and daily range at various regions based on AWS (Jeonju: an altitude of 60 m; Imsil: an altitude of 247 m; Namwon: an altitude of 133 m; Jangsu: an altitude of 407 m) of the past 30 years (1981–2010) and the past 12 years (2011–2022).

Month	Period	Classification	Minimum Temperature (°C)				Daily Range (°C)			
			Inland (Jeonju)	Mid-Mountainous (Namwon)	Imsil	Mountainous (Jangsu)	Inland (Jeonju)	Mid-Mountainous (Namwon)	Imsil	Mountainous (Jangsu)
April	1981–2010	Mean	6.7	4.0	3.0	3.1	12.8	15.9	15.6	14.8
		Range	6.6	6.9	6.8	7.1	1.4	2.8	2.3	2.3
	2011–2022	Mean	7.6	5.4	4.1	3.7	12.2	14.2	14.8	14.4
		Range	7.8	8.2	8.0	8.2	3.8	6.6	5.6	6.1
May	1981–2010	Mean	12.4	10.3	9.1	9.1	12.0	14.2	14.1	13.1
		Range	3.8	4.1	3.6	3.3	1.3	2.1	1.7	2.0
	2011–2022	Mean	13.1	11.3	9.7	9.4	12.2	13.8	14.6	14.0
		Range	6.1	7.2	7.4	6.1	4.6	5.9	5.7	6.0

While reports on frost injury to fruits due to early leafing and blooming prompted by mild winter temperatures are prevalent [24,25], studies on the risk of spring frosts are scarce [26]. Crop frost hardiness decreases progressively after dormancy [27,28], varying with the growth stage of the flower buds and weakening notably just before blooming [29, 30]. Meteorologically, plants are more vulnerable in fluctuating temperatures than in steadily declining cold, with temperature descent speed playing a role [14]. Not just minimum temperatures, but average temperatures and diurnal ranges are also critical factors [31]. Despite a rise in average temperature, a variability under 5% could increase frost injury risk by offsetting up to 5.5 °C rises [32]. Therefore, as seen in Figures 4 and 5, frost injury in mid-mountainous apple and pear orchards was higher than in mountainous areas due to significant impacts from extreme cold and large temperature ranges during advanced flowering stages. Weather analysis over the past 12 years compared to long-term average norms indicates increased weather variability [33], including unusual weather events and region-based differences [21]. This variability, differing from typical patterns, is expected to heighten concerns about potential frost injury due to unpredictable meteorological phenomena in the future [26].

To avoid frost injury during the flowering period, it is recommended to analyze terrain and microclimate to identify high-risk areas, choose cold-resistant varieties [34], and increase resistance through robust plant health [35]. However, these methods are challenging to implement swiftly in urgent situations [11]. Global warming is expected to increase the adaptability of major apple-producing areas to higher temperatures, potentially leading to greater frost injury when it occurs [36], despite anticipated lower risks of frost exposure. This could adversely affect annual growth cycles [37]. Additionally, weather factors such as average temperature and diurnal range can show significant variability within small orchards, with location-specific differences reaching up to 6 °C [38]. The temperature differences observed between the sun-exposed and shaded areas of the apple canopy are consistent with findings in a similar context [39]. This highlights the need

for further research into how microclimate, topographical characteristics [40], and the physiological state of the plants relate to frost injury [41].

4. Conclusions

This study analyzed the relationship between frost injury during the flowering period and meteorological factors for apples ('Hongro' and 'Fuji') and pears ('Wonhwang' and 'Niitaka') cultivated at various altitudes in Jeonbuk State in Republic of Korea. The theoretical foundation of our study is based on reports of recurrent low-temperature injuries during the flowering period in regions where such damage occurs persistently, despite the low cultivation altitudes. It was revealed that the timing of flowering stages across various cultivation regions was delayed by approximately 1–3 days at higher altitudes, with this effect being more pronounced in apples than in pears. All four varieties of both fruit types experienced a 4–6 days period from full bloom to flower dropping. Specifically, apples required an average of about 15 days from the first flowering to full bloom, which is 3–5 times longer than pears, and the overall flowering period was also about 1.5–2.0 times longer. Frost injury was most severe in the mid-mountainous areas for both apples and pears. Apples showed more significant injury in the central flowers, while pears exhibited higher injury rates in the earliest blooming flowers within the inflorescences. Weather analysis showed that during the flowering period in mid-mountainous areas, the lowest temperatures and the largest diurnal ranges were recorded, both of which significantly contributed to the occurrence of frost injury. In mountainous areas, despite lower minimum temperatures than mid-mountainous areas, slower blooming progression and lower average temperatures resulted in relatively less frost injury. Our study findings indicate that mid-mountainous regions, where flowering stages are accompanied by complex conditions such as significant diurnal temperature variations and low temperatures, are more prone to chronic low-temperature damage compared to higher-altitude mountainous areas with consistently lower temperatures.

Furthermore, weather data comparison between the long-term average (1981 to 2010) and the past 12 years (2011 to 2022) indicated a decrease in April's average diurnal range. However, the variability in the diurnal range expanded by 1.3 to 2.7 times, signaling increasing meteorological variability. This trend suggests that frost injury during flowering periods may become more frequent in the future. These results underscore the necessity of integrated strategies to prevent frost injury that consider cultivation regions, weather conditions, variety's frost resistance, and blooming physiology. Particularly in mid-mountainous areas, selecting cold-sensitive varieties and implementing preventative and emergency measures to minimize frost injury are crucial. Additionally, further research should continue to explore the relationships between microclimate, topographical characteristics, and the physiological states of plants in relation to frost injury. Our study highlights that factors beyond low temperatures can influence injury during the flowering period, emphasizing the importance of diverse environmental conditions. These findings are expected to provide valuable insights for future research in this area.

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