Artificial chilling for floral induction in strawberries

Suplementação artificial de frio para indução floral em morangueiro

Luiz Vitor Barbosa de Oliveira[1]*; Laura Souza Santos[1]; Heder Asdrubal Montanez Valencia[1]; Juliano Tadeu Vilela de Resende[2]

Highlights

Vernalization increased productivity, improved physiology, and enhanced fruit quality.
There was genotypic variation in response to vernalization.
Vernalization reduces the need to import seedlings and lowers production costs.

Abstract

Strawberry cultivation holds significant socioeconomic importance throughout the world, particularly because it is largely family based. Recently, strawberry production has faced constraints, as the main cultivars grown globally require a certain number of chilling hours for floral induction; thus, growers in tropical and subtropical areas are required to import nursery plants that provide these conditions. This importation has significantly increased the cost of strawberry production. The chilling requirement can be supplemented artificially via growth chambers. This study evaluated the production, post-harvest, and physiological aspects of three strawberry cultivars (Albion, Monterey, and San Andreas) subjected to different vernalization periods (10, 20, or 30 days). The research was conducted in a low tunnel system, where agronomic parameters (the number of commercial and non-commercial fruit, the total fruit weight, and the commercial fruit weight), fruit quality (titratable acidity, vitamin C, reducing sugars, anthocyanins, phenolic compounds, total soluble solids, and the titratable acidity to total soluble solids ratio), and physiological parameters (stomatal conductance, net photosynthesis, transpiration rate, internal cellular carbon, and total chlorophyll) were evaluated. The results revealed a genotype × environment interaction, with the cultivars responding variably depending on the duration of cold exposure. Vernalization in a controlled environment during seedling formation generally promoted gains in productive yield and physiological performance, and slight improvements in fruit quality. In conclusion, vernalization in a cold chamber offers benefits that can help reduce the importation of seedlings and thus lower costs for farmers and increase their profitability.

Key words: *Fragaria × ananassa.* Adaptability. Floral induction. Chilling periods. Domestic seedlings.

[1] Doctoral Students of the Postgraduate Program in Agronomy, Universidade Estadual de Londrina, UEL, Londrina, PR, Brazil. E-mail: luizvitor.barbosa@uel.br; laura.souza.santos@uel.br; heder.montanez1@uel.br

[2] Prof. Dr., UEL, Londrina, PR, Brazil. E-mail: jvresende@uel.br

* Author for correspondence

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1567

Resumo

O morangueiro apresenta grande importância socioeconômica no mundo, principalmente por ser de base familiar. Ultimamente, a produção de morangos tem passado por restrições, tendo em vista que as principais cultivares plantadas no mundo são exigentes em horas de frio para indução floral, necessitando de importações de viveiros que proporcionam essas condições. Essa importação de mudas tem onerado significativamente a produção de morangos. Assim, entendemos que a suplementação da necessidade de frio pode ocorrer de forma artificial, em câmaras de crescimento. Dessa forma, a pesquisa avaliou aspectos de produção, de pós-colheita e fisiológicos de cultivares de morangueiro submetidas a diferentes períodos de vernalização (10, 20 e 30 dias). Os tratamentos consistiram em três cultivares de morangueiro de dia neutro (Albion, Monterey e San Andreas) aos três períodos de vernalização. A pesquisa foi realizada em sistema de túnel baixo em que foram avaliados parâmetros agronômicos (número de frutos comerciais e não comerciais, massa de frutos totais, massa de frutos comerciais), qualidade de frutos (acidez titulável, vitamina C, açúcares redutores, antocianinas, compostos fenólicos, sólidos solúveis totais e a relação acidez titulável/sólidos solúveis totais), e parâmetros fisiológicos (condutância estomática, fotossíntese líquida, taxa transpiratória, carbono interno celular) e clorofilas totais. Mediante resultados obtidos, observou-se que houve interação genótipo vs ambiente em que as cultivares responderam de forma variada em função do tempo de exposição ao frio. De forma geral, o fornecimento de horas de frio artificial, durante a formação das mudas promoveu ganhos para rendimento produtivo, fisiológico e discreto ganho na qualidade de frutos. Com base no disposto acima, pode-se afirmar que a técnica de fornecimento de frio em câmara fria traz benefícios que podem contribuir para reduzir a importação de mudas, onerando menos o agricultor e proporcionando maior lucratividade.

Palavras-chave: *Fragaria × ananassa*. Adaptabilidade. Indução floral. Períodos de frio. Mudas nacionais.

Introduction

The availability of strawberry (*Fragaria × ananassa* Duch.) cultivars can be limited by several factors, particularly agro-environmental adaptation and types of cultivars (López-Aranda et al., 2011). Strawberry cultivars are highly sensitive to various environmental conditions, making it difficult to establish a clear geographic boundary to separate agroclimatic areas. Strawberry plants are temperate-climate species that require the accumulation of chilling hours for floral induction. Typically, in temperate climates, the accumulation of cold is sufficient for strawberries to produce fruit without any supplementation; however, in warmer climates, strawberry plants do not naturally accumulate enough chilling hours (R. C. Costa et al., 2014). As a result, producers buy seedlings from nurseries that fulfill the cold requirement (chilling hours) (Diel et al., 2017; J. T. V. Resende et al., 2020a; Wrege et al., 2007).

The number of chilling hours between 2.0 and 7.2°C that is required for floral induction varies among cultivars (Ronque, 1998). In general, cultivars grown in tropical and subtropical regions come from breeding programs developed in countries with a temperate climate, such as the United

1568

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

States, Italy, and Spain (Zeist & Resende, 2019). As a result, these plants have a higher chilling requirement for flowering and fruit production. For floral induction to occur, the temperature and photoperiod must remain favorable for a sufficient duration, referred to as the inductive cycle. Studies have shown that the minimum number of short-day cycles needed to induce flowering in octoploid cultivars mainly ranges from 7 to 14 days, but it can sometimes be as long as 23 days (Guttridge, 1985). However, there are limited data available for day-neutral (everbearing) cultivars.

In tropical and subtropical areas, the production of seedlings of high physiological quality is constrained due to the lack of suitable climatic conditions. Consequently, strawberry producers seeking high yields are forced to rely on imported seedlings from temperate regions. This approach not only increases production expenses but also brings phytosanitary hazards (Moreira et al., 2022). Producing seedlings within the country of cultivation is the most effective strategy to lower costs and to minimize phytosanitary risks (Cocco et al., 2020). The use of climate-controlled chambers can serve as an alternative to produce these high-quality seedlings that enable high crop yields in the country of cultivation. This technique is based on the acquisition or acceleration of flowering ability through cooling (vernalization) (Chouard, 1960; Taiz et al., 2017). Research has shown that vernalization can supplement insufficient chilling to promote floral induction in strawberry plants (Martins & Polo, 2009; Oliveira & Scivittaro, 2009), especially in tropical and subtropical climates, where the chilling requirements are not met (Oviedo

et al., 2020). Brandão et al. (1989) examined the impact of vernalization at 10°C for 10, 15, and 20 days on the photoperiod-sensitive strawberry cultivar AGF-80 strawberry cultivar. They found that vernalization had a significant effect and noted that extending the vernalization period led to the earlier development of differentiated floral buds, visible buds, and open flowers.

The chilling requirement varies among strawberry cultivars, ranging from 100 to 700 h at 2–7.2°C (L. E. C. Antunes & Peres, 2013; Darnell et al., 2003; Lavín & Maureira, 2019). Understanding how cultivars respond physiologically to vernalization can assist farmers in selecting the most suitable cultivar, to ensure that cultivation is cost-effective and that the financial yield is maximized. Thus, this study determined the optimal vernalization period in a climate chamber for the primary everbearing strawberry cultivars cultivated in tropical and subtropical regions throughout the world: Albion, Monterey, and San Andreas. The optimal vernalization period was selected based on productivity, stolon emission, and photosynthetic parameters.

Materials and Methods

Experimental site

The experiments were carried out at the State University of Londrina, in the State of Paraná (23°19′42″S, 51°12′11″W, altitude 594 m) and in a commercial farming area (23°10′48″S, 51°13′23″W, altitude 543 m). Based on the Köppen climate classification, the region is characterized by the Cfa climate type, featuring hot and rainy summers, with average annual precipitation of 1600 mm.

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1569

Seedling production and vernalization

The experiments utilized the everbearing cultivars Monterey, Albion, and San Andreas, developed by the University of California (United States) (Shaw & Larson, 2009). The Monterey cultivar demonstrates strong genetic performance in floral differentiation, leading to higher yields while preserving the fruit's quality traits. It produces fruit with a good soluble solids content, a uniform flat-conical shape, and a large size (averaging 32.4 g fruit^{-1}). The Albion cultivar has a more open plant structure, which simplifies harvesting. It offers consistent production with fewer peaks and superior flavor compared with other day-neutral varieties. The San Andreas cultivar is well suited to the central and southern coasts of California. It produces red fruit, slightly heavier than the Albion and Aromas cultivars but darker than the Diamante cultivar. The fruits are large and elongated, with a mean weight of 31.6 g. They exhibit firmness and flavor similar to the Albion cultivar, with darker and redder flesh. In addition, their production timing and pattern are comparable to the Albion cultivar (Lucchi et al., 2011; Shaw & Larson, 2009).

The strawberry seedlings utilized in the study were grown in a greenhouse under controlled conditions, with a mean temperature of 27 ± 3°C, relative humidity of 75% ± 5%, and a 14:10-h photoperiod. The seedlings were propagated from stolons and rooted in 64-cell polystyrene trays filled with the commercial substrate Carolina Soil®. Production of the seedlings took place between August and October 2022. Four trays of each cultivar were prepared for each vernalization period.

Following the rooting process, the seedlings were subjected to phytosanitary measures, including the preventive application of mancozeb for fungal control (Unizeb Gold®, applied at a dose of 2 kg ha^{-1}) and abamectin (Abamex, applied at a dose of 30 mL ha^{-1}) to manage mites and aphids. Afterward, the seedlings were transferred to a growth chamber equipped with an environmental control system, set to maintain a relative humidity of 85% ± 5%, a temperature range of 2–7.2°C, and a 16:8-h photoperiod (adapted from Oliveira et al., 2009). Irrigation was provided via a drip system, and the trays were placed on benches over a layer of washed sand.

The plants were exposed to three different vernalization periods (10, 20, and 30 days). The transfer of the seedlings to a phytotron-type growth chamber occurred on November 1 (for the 30-day vernalization treatment), November 10 (for the 20-day vernalization treatment), and November 20 (for the 10-day vernalization treatment). The control group, which did not undergo vernalization, was kept in the greenhouse until the date of transplantation.

Throughout the vernalization process, phytosanitary measures for fungal disease control were implemented every 10 days, with the initial application occurring at the beginning of the cold treatment phase. Fungicides containing mancozeb, such as Unizeb Gold®, were applied at a dose of 2 kg ha^{-1}. To manage pests, especially the two-spotted spider mite, abamectin-based products such as Abamex® were applied weekly at a dose of 50 mL ha^{-1}. The chemicals were applied using an electric backpack sprayer (Lynus brand, 18-L capacity) fitted with a fan-type nozzle and a flow rate of

1570

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

757 mL min^{-1}. Following the completion of the vernalization period, the seedlings were acclimatized in the greenhouse for 3 days before transplanting (at a temperature of 27 ± 3°C and relative humidity of 75% ± 5%).

Field experiment

The soil at the experimental site is classified as Eutroferric Red Latosol (Bhering, 2020). The soil was prepared by plowing and harrowing and adjusted based on chemical analysis, with dolomitic limestone applied at a dose of 1,200 kg ha^{-1}. Raised beds were formed using a bed shaper, measuring 1.20 m in width and 0.25 m in height, with limestone incorporated during the process. Base fertilization was performed according to soil chemical analysis, using NPK fertilizer (formulation 04-14-08) at a dose of 1,300 kg ha^{-1}. After transplanting at a spacing of 0.30 × 0.30 m, the seedlings were heavily irrigated to ensure successful establishment. Thirty days after transplanting, the beds were covered with double-faced mulch (25-µm thick) and enclosed with a 120-µm plastic tunnel. Drip tubes with holes spaced 0.10 cm apart were used for irrigation; according to the manufacturer, they provide an approximate flow rate of 1.5 L h^{-1}.

The experiment was carried out using a randomized block design with split plots, with nine plants per plot and four replications, resulting in a total of 12 treatments. The treatments included three everbearing strawberry cultivars (Albion, Monterey, and San Andreas), three vernalization periods (10, 20, or 30 days), and a control treatment where the cultivars were not subjected to vernalization.

The plants were provided mineral nutrition via fertigation, with the frequency adjusted according to the electrical conductivity of the drainage. During the vegetative phase, the electrical conductivity of the tank was maintained at 0.8–1.2 mS cm^{-1}, and during the production phase, it was maintained at 1.4–1.6 mS cm^{-1}. The composition of the nutrient solution varied according to the plant's developmental stage. Pests and diseases were controlled using biological and natural products such as neem oil (1.5 L ha^{-1}) and sulfur (2 kg ha^{-1}). When necessary, chemical insecticides or fungicides were applied, such as Pirate® and Amstar Top®, at a dose of 50 and 300 mL ha^{-1}, respectively.

Measurement of gas exchange and net photosynthesis

The physiological parameters were analyzed using an Infrared Gas Analyzer (IRGA) (model LCpro-SD, ADC Bioscientific, UK). The parameters assessed were stomatal conductance (Gs), the net photosynthetic rate (A), the leaf transpiration rate (E), and the intercellular CO_2 concentration (Ci).

Physiological measurements were taken four times throughout the experiment: before transplanting the seedlings, 30 days after transplanting (vegetative stage), 60 days after transplanting (onset of the reproductive stage), and 90 days after transplanting (during the peak reproductive stage). The measurements were performed between 8:00 and 10:00 AM on sunny days with no cloud cover. From every plot, three plants were selected randomly for analysis, focusing on the apical leaflet of the youngest

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1571

fully expanded trifoliate leaf. The final value was determined by calculating the weighted mean of the four measurements.

Yield and biometric characteristics

Harvests were conducted twice a week over 6 months. For assessment, the fruit was weighed using a semi-analytical balance (AUW220D, Shimadzu, Philippines) and categorized with the aid of a digital caliper into non-commercial (≤ 35 mm and 8 g) and commercial (> 35 mm and 8 g) (PBMH & PIMo, 2009). From this classification, the following parameters were obtained: the total number of fruit (TNF), the number of commercial fruit (NCF), and the number of non-commercial fruit (NNCF). Furthermore, the total fruit weight (TFW, g plant^{-1}), commercial fruit weight (CFW, g plant^{-1}), average fruit weight (AFW, g fruit^{-1}), non-commercial fruit weight (NCFW, g plant^{-1}), and average weight of commercial fruit (AWCF) were calculated.

Weekly counts were conducted to record the number of stolons produced and the number of trifoliate leaves per plant in each plot, continuing until the onset of the reproductive stage.

Physicochemical analyses

The physicochemical analyses were carried out in the post-harvest laboratory at the State University of Londrina. For each harvest, two pieces of commercially mature fruit were collected from each plot during the first three months (May, June, and July), representing the peak production phase. The fruit was packed in plastic containers, labeled, and stored in a freezer for subsequent chemical analysis.

The internal and external color of the fruit was determined using a colorimeter (Minolta CR-410, USA) with illuminant C as the reference standard. Each piece of fruit was cut transversely, and four measurement points were recorded, encompassing both the inner and outer regions. These measurements were performed on the fruit harvested and reserved for chemical analysis. The results are expressed in the LCh color system, where L represents lightness (0–100, from or darker to lighter colors); C indicates chroma (0–60, from less saturated to more saturated colors), and h denotes the hue angle (0°–360°, where 0° is red, 90° is yellow, 180° is green, and 270° is blue).

Flesh firmness was assessed on one piece of commercially ripe fruit per harvest from each plot. Measurements were conducted at two points equidistant from the fruit's center using a digital penetrometer (DD-200, Instrutherm, Brazil) equipped with a 6-mm tip. The mean firmness across harvests was calculated, and the results were reported in Newtons (N).

The fruit stored during the harvest period was thawed, processed in a blender, and homogenized to prepare aliquots for further analysis. The soluble solids content was measured using 2-3 drops of juice extracted from the homogenized sample. Analyses were conducted in triplicate using a benchtop refractometer (MA871, Milwaukee Instruments, USA), and the results are reported in °Brix. Titratable acidity (g of citric acid per 100 g) was determined using the titrimetric method. A 10-mL juice sample was titrated with 0.1 N NaOH, using phenolphthalein as the acid-base indicator. This procedure was conducted according to the methodology outlined by the Adolfo

Lutz Institute [ALI] (2008). The ratio of soluble solids to titratable acidity was also calculated.

Phenolic compounds were quantified using the Folin–Ciocalteu method. It relies on redox reactions between the Folin Ciocalteu reagent and phenolic compounds, resulting in the formation of blue molybdenum and tungsten oxides. The development of these oxides was measured with a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific) at a wavelength of 765 nm.

Vitamin C (mg 100 g^{-1}) was determined using the Tillmans method, which is based on the reduction of the dye sodium 2,6-dichlorophenolindophenol by an acidic solution of vitamin C (ascorbic acid). This determination was carried out following the method of the ALI (2008).

Reducing sugars were quantified based on the 3,5-dinitrosalicylic acid method, as described by Miller (1959). This procedure involves the reaction of the acid in an alkaline medium, converting it into 3-amino-5-nitrosalicylic acid through the action of reducing sugars. The mixture was incubated in a water bath at 100 °C for 10 min, and then the absorbance at 570 nm was read using a spectrophotometer (Genesys 10S UV-VIS, Thermo Scientific).

The total anthocyanin content was quantified based on the spectrophotometric method proposed by Association of Official Analytical Chemists [AOAC] (1995), with modifications. The absorbance at 535 nm was read with a spectrophotometer, and the results are expressed as milligrams pelargonidin-3-glucoside per 100 g.

Statistical analyses

The data were subjected to tests for normality and homogeneity of variances. When the assumptions were met, they were analyzed using joint analysis of variance (P < 0.05). When significant, the treatment means were compared using Tukey's test (P < 0.05). The data related to the vernalization periods were subjected to regression analysis, and the significance was assessed using the t-test (P < 0.05). All data were analyzed using the Sisvar software (Ferreira, 2016).

Results and Discussions

Gas exchange and net photosynthesis

Higher photosynthetic activity generally leads to greater plant growth and development, resulting in increased productivity and economic gains. Evaluation of the effects of vernalization periods on everbearing strawberry cultivars revealed significant interaction effects on gas exchange and photosynthetic activity. The cultivars responded differently when exposed to varying vernalization periods. Vernalization stimulates the apical meristem, triggering hormonal responses that activate genes related to chemical and physical traits, such as the development of reproductive structures (McDaniel, 1994; Verdial et al., 2009).

When subjected to 10 and 20 days of vernalization, the Albion and Monterey cultivars exhibited the highest Ci in the substomal chamber. This parameter decreased as the duration of cold exposure increased (Figure 1a), likely because of the

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1573

enhanced production of abscisic acid, which promotes stomatal closure. For the San Andreas cultivar, Ci showed a slight increase after 20 days of vernalization, but this cultivar consistently displayed the lowest Ci values among the three tested cultivars.
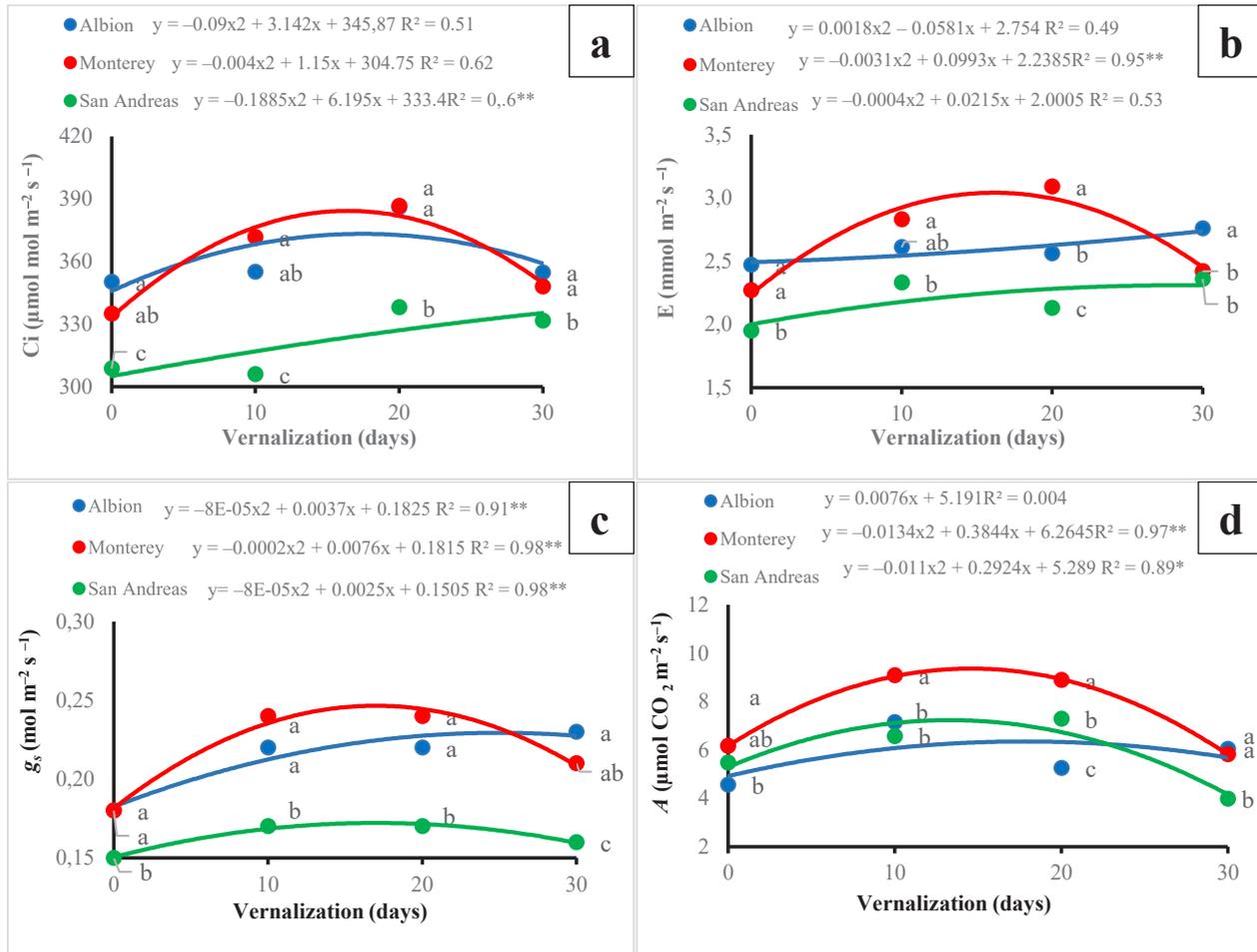

Figure 1. The changes in the physiological parameters measured before transplanting the seedlings and after exposure to different vernalization periods: (a) the intercellular CO_2 concentration (- Ci); (b) the transpiration rate (E; (c) stomatal conductance (Gs); and (d) the photosynthetic rate (A).

For each figure, points with the same letter do not differ significantly according to Tukey's test ($p > 0.05$). The asterisks indicate the significance of the regression: * $p < 0.05$ and ** $p < 0.01$.

1574

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

The Monterey cultivar exhibited a higher E when subjected to 10 and 20 days of vernalization, but it decreased after 30 days of vernalization (Figure 1b). This result explains the reduction in Ci at 30 days of vernalization because on average, for every 600 water molecules transpired, one CO_2 molecule diffuses into the plant. The Albion and San Andreas cultivars presented a lower E compared with the Monterey cultivar, but it remained stable as the vernalization period increased.

The Albion and Monterey cultivars showed an increase in Gs, particularly after 10 and 20 days of vernalization (Figure 1c). These findings confirm the relationship between E, Ci, and Gs. Higher Gs results in higher E and, consequently, higher Ci in the plant. The San Andreas cultivar showed the lowest Gs, with a slight increase as the vernalization period was extended.

It is important to note that the lower Ci in the substomatal chamber is due to reduced stomatal conductance, which limits transpiration and, as a result, CO_2 uptake. Stomata are highly responsive to changes in environmental conditions (Barbosa et al., 2018). The reduction in gas exchange observed in the plants subjected to longer cold exposure (denoted by lower Ci, E, and Gs) may have been caused by a marked increase in abscisic acid, a hormone known to induce stomatal closure (Pridgeon & Hetherington, 2021; Qu et al., 2019; Ye et al., 2017; T. Y. Zhang et al., 2017). On the other hand, when present in lower concentrations, abscisic acid maintains a balance with other hormones and regulates the activity of stomatal gas exchange (Taiz et al., 2017).

This accounts for the improved Ci, E, and Gs values seen in the Monterey and Albion cultivars during intermediate vernalization periods (10 and 20 days).

Vernalization had a positive impact on A of the cultivars. The Monterey cultivar exhibited the highest A, especially after 10 and 20 days of vernalization. The Albion cultivar reached its maximum photosynthetic potential after 10 days of vernalization, while San Andreas reached its peak after 10 and 20 days (Figure 1d). All cultivars showed a decline in photosynthetic efficiency when exposed to longer periods of cold. The elevated A is likely associated with the low E, indicating that the plant focuses on optimizing photosynthesis while conserving water (Montillet et al., 2021; Roux & Leonhardt, 2018). The limited diffusion of CO_2 into the cells reduced the amount of carbon available for the Calvin cycle, leading to a decrease in net photosynthesis.

Gas exchange parameters in plants are interconnected, as higher Gs enhances gas exchange efficiency, enabling the release of water molecules (transpiration) and the uptake of CO_2 molecules. This results in greater CO_2 accumulation in the substomatal chamber, promoting higher photosynthetic activity (Kluge et al., 2015; Taiz et al., 2017) and, ultimately, better productive performance (Kamperidou & Vasilakakis, 2006). More efficient gas exchange directly affects the amount of CO_2 a plant can fix and convert into glyceraldehyde per unit of time in the Calvin cycle. Under ideal conditions, increased gas exchange and internal cellular carbon can lead to various benefits for the plants.

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1575

Number of stolons and trifoliate leaves

Stolons are important for the vegetative propagation of strawberry plants, but in commercial cultivation, they act as strong sinks that can compromise fruit production. In strawberry plants, the growth and development of leaves, flowers, and runners depend on the interactions between temperature, photoperiod, and thermal amplitude (Husaini & Xu, 2016). The results revealed significant cultivar × vernalization period interactions for the number of trifoliate leaves and runners emitted. The Albion and San Andreas cultivars were not influenced by the duration of vernalization, with no significant adjustment of the regression equation, maintaining low stolon emission regardless of the cold exposure duration (Figure 2a). When subjected to longer vernalization (20 and 30 days), the Monterey cultivar produced more stolons, which partly explains the decline in agronomic parameters related to fruit production under these conditions. Verdial et al. (2009) and L. D. A. Resende et al. (1999) reported that in photoperiod-sensitive (short-day) cultivars exposed to vernalization, there is greater early stolon emission within the first 15 days after transplanting, and it remains stable until 60 days. They also noted that cultivars varied in their runner emission, with the Campinas cultivar producing stolons regardless of vernalization, indicating that this cultivar has a low chilling requirement. Guevara-Matus et al. (2023) found that the Albion, Festival, and Oso Grande cultivars, subjected to four vernalization treatments (0, 250, 500, and 750 h of cold at 6°C), generated a higher number of stolons when vernalized, a result that is generally unexpected under such conditions.
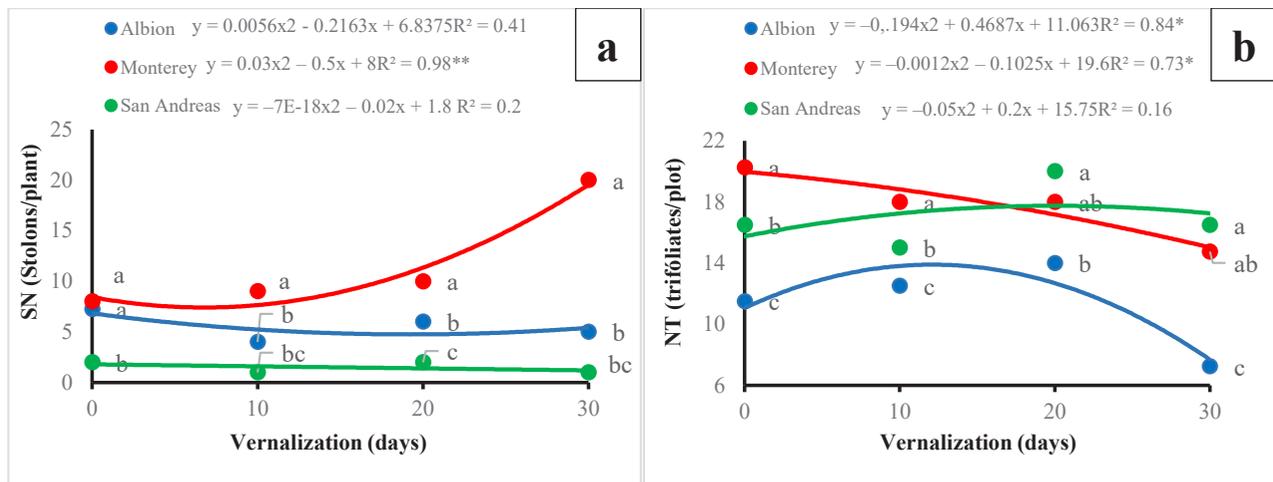

Figure 2. The (a) number of stolons and (b) trifoliate leaves as a function of different vernalization periods.
For each figure, points with the same letter do not differ significantly according to Tukey's test (p > 0.05). The asterisks indicate the significance of the regression: * p < 0.05 and ** p < 0.01.

1576

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

The Albion and San Andreas cultivars showed a higher number of trifoliate leaves emitted when subjected to 10 and 20 days of vernalization (Figure 2b). In contrast, the Monterey cultivar produced the highest number of trifoliate leaves when the plants were not vernalized, with a decrease as the cold exposure time increased. We observed an antagonistic relationship between runner emission and the number of trifoliate leaves, particularly in the Monterey cultivar. These results can be explained by the source-sink balance within the plant

Diel et al. (2017) found that the Albion cultivar requires a larger accumulated thermal sum for the production of trifoliate leaves on the main crown, whereas the Camarosa cultivar requires a smaller thermal sum. The Albion cultivar also demonstrated a slower leaf emission rate compared with other cultivars (Mendonça et al., 2012), aligning with our research results. A. F. Costa et al. (2015) reported differences in the phyllochron, with non-vernalized seedlings needing 93.30°C day leaf^{-1} and vernalized seedlings requiring 117.80°C day leaf^{-1}. A reduction in the leaf emission rate leads to a longer phyllochron, indicating that plants likely need a higher thermal sum for leaf production (Rosa et al., 2011). The fact that Monterey cultivar showed increased runner emission when not vernalized which is consistent with the findings reported by Rosa et al. (2011) suggests it has a lower thermal sum requirement for floral induction. This outcome supports our hypothesis that the Monterey cultivar requires fewer chilling hours for floral induction.

The source-sink relationship can be adjusted by either enhancing or reducing the strength of the source (the crop's photosynthetic rate) or the sink (the demand for assimilates). The appearance of flowers, fruits, and budding structures such as stolons disrupts the balance of vegetative growth (leaves and stolons) in strawberry plants, changing the distribution of photoassimilates among vegetative organs (Dal Picio et al., 2013; Y. C. Zhang et al., 2009). Plant growth modulation is regulated by the balanced interplay of various plant hormones, which work together to either promote or inhibit development throughout the growth cycle. Runner production in strawberry plants is linked to the auxin-cytokinin balance. Extended cold exposure stimulates cytokinin production, creating an imbalance with auxin, which encourages stolon growth and reduces the production of trifoliate leaves from the plant crown (Kerbauy, 2017).

Yield parameters

The Monterey cultivar exhibited the highest fruit production per plant (based on TFW), irrespective of the vernalization period (Figure 3a). The optimal yields were observed when plants underwent 20 days of vernalization. For the San Andreas cultivar, fruit production increased linearly, with the highest yield achieved after 30 days of vernalization. In contrast, the Albion cultivar showed a decline in fruit production after 30 days of vernalization, suggesting that this cultivar requires a shorter vernalization time for floral induction. The fruit yields recorded in this study are consistent with those documented in the literature for both vernalized and non-vernalized cultivars (L. E. C. Antunes et al., 2010; Oviedo et al., 2020).
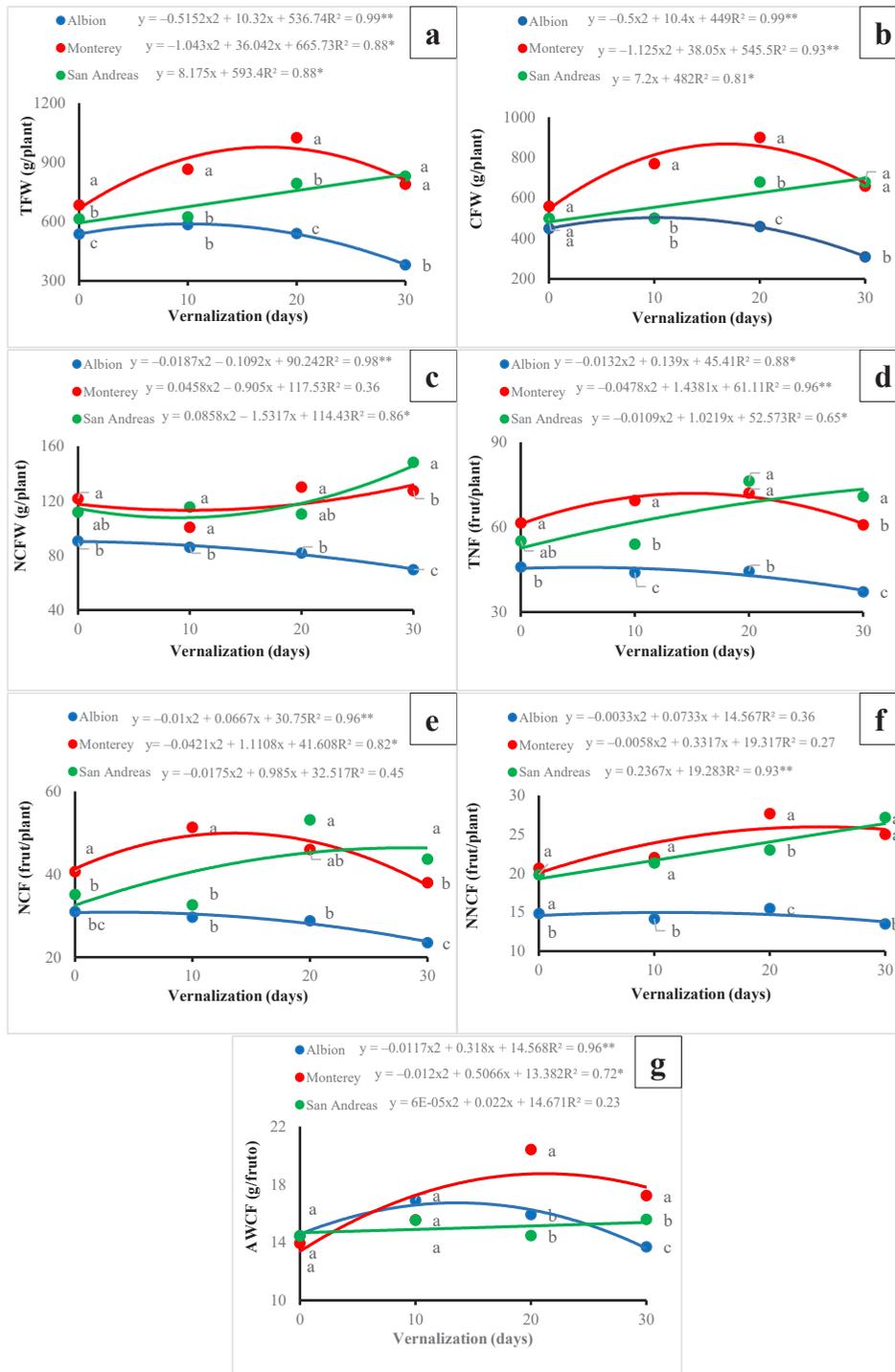
Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1577

Figure 3. The effect of different vernalization periods on yield parameters: (a) total fruit weight (TFW); (b) commercial fruit weight (CFW); (c) total non-commercial fruit weight (NCFW); (d) total number of fruit (TNF); (e) number of commercial fruit (NCF); (f) number of non-commercial fruit (NNCF); (g) average weight of commercial fruit (AWCF).

For each figure, points with the same letter do not differ significantly according to Tukey's test (p > 0.05). The asterisks indicate the significance of the regression: * p < 0.05 and ** p < 0.01

1578

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

The Monterey cultivar subjected to 20 days of vernalization presented the highest CFW (Figure 3b). The Albion and San Andreas cultivars presented their highest CFW after 10 and 30 days of vernalization, respectively. For the San Andreas cultivar, the equation adjustment followed a linear trend, indicating that the production of commercial fruit could further increase when vernalization is extended.

NNCF was highest for the San Andreas and Monterey cultivars (Figure 3c), although this variable was not significantly influenced by the vernalization period, especially for the Monterey cultivar. Extending vernalization to 30 days decreased NNCF in the Albion cultivar but increased this variable in the San Andreas cultivar.

Environmental shifts, such as exposure to low temperatures, induce hormonal changes that regulate genes potentially involved in cell division, influencing fruit growth in ways that differ depending on the genotype. Groppo et al. (1997) studied multiple strawberry cultivars and observed that the timing of fruit production and ripening varies across cultivars. Therefore, this behavior is a physiological trait unique to each cultivar.

TNF was higher in the Monterey and San Andreas cultivars (Figure 3d) across all vernalization periods compared with the Albion cultivar. For the Monterey and San Andreas cultivars, the highest TNF occurred after 20 days of vernalization, with a slight reduction when this period was extended to 30 days. The Albion cultivar maintained a stable TNF up to 20 days of vernalization, with a decrease observed at 30 days.

The Monterey and San Andreas cultivars also achieved the highest NCF (Figure 3e), specifically after 10 and 20 days of vernalization, respectively. Oliveira and Scivittaro (2009) reported that vernalization for 21 days increased strawberry fruit production. Exposure to 30 days of vernalization reduced NCF for all cultivars.

NNCF increased with the vernalization period for the Monterey and San Andreas cultivars (Figure 3f), with a linear equation adjustment for the San Andreas cultivar. Our results align with those obtained by Ledesma and Kawabata (2016), who observed that vernalized strawberry plants showed an increase in the number of fruit produced per plant, albeit of medium and small sizes. Verdial et al. (2009) reported that vernalized plants of the Campinas, Cartuno, and Oso Grande cultivars produced a large NNCF with low average weight, even though seedling vernalization increased the percentage of flowering and fruiting in the plants.

The Monterey cultivar subjected to 20 days of vernalization had the highest CFW (Figure 3g). The San Andreas cultivar did not show a significant equation adjustment for this variable, indicating that vernalization did not influence AWCF. The Albion cultivar showed an increase in AWCF at 10 days of vernalization, with a gradual reduction after 20 and 30 days of vernalization.

The results for the production components (TFW, CFW, MFNC, TNF, NCF, NNCF, and AWCF) revealed that the cultivars respond differently depending on the vernalization period, showing a marked genotype × environment interaction. The cold requirement for floral induction in the Albion, San Andreas, and Monterey cultivars ranges from 250 to 450 h (Shaw & Larson, 2009), but the response to this accumulation is conditioned by interactions with the environment. Ledesma and Kawabata (2016) reported different responses from certain

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1579

cultivars to cold exposure; they only reported an increase in fruit weight and size due to vernalization in one of the four evaluated cultivars. This result highlights the genotype × environment interaction, and such studies are necessary for cultivar selection and, more importantly, to determine the number of cold hours required for floral induction.

Albion was the least productive cultivar and showed the lowest response to vernalization, demonstrating a lower cold requirement for flowering. This finding is consistent with the studies by Diel et al. (2017) and Reekie et al. (2003) when evaluating the Albion cultivar under artificial cold supplementation. San Andreas was the most cold-demanding cultivar of the three, showing the best production when exposed to longer vernalization periods. Monterey was the most productive cultivar and responded best to vernalization. Intermediate vernalization periods (10 or 20 days) were the most suitable to achieve higher total and commercial fruit productivity, with a greater AWCF, the factor most preferred by consumers.

The results also demonstrated that exposure to cold for more than 20 days does not bring productive benefits to these cultivars, especially for the variables MCF and AWCF. In this regard, there are discrepancies regarding the effectiveness of the vernalization of plants before field transplanting. Several researchers obtained higher yields when the seedlings were vernalized in cold chambers (Bish et al., 2002; Li et al., 2021), while others found that natural vernalization in nurseries is more effective in increasing productivity (Cantliffe et al., 2007). However, the response varies due to the interaction between the cultivar and the number of chilling hours, regardless of how the plants are exposed to cold. It is clear that the accumulation of chilling hours is essential for the physiological processes of strawberry plant flowering and fruit production. Furthermore, flower initiation varies by cultivar but generally increases as the temperature decreases and, for some varieties, depends on a specific number of chilling hours (Hernández-Martínez et al., 2023; Verdial et al., 2009).

Considering the agronomic parameters together, it can be inferred that the Monterey cultivar is superior for most of the evaluated traits compared with the others, especially when subjected to 20 days of vernalization. The San Andreas cultivar responded linearly for some traits, highlighting the need to extend the cold exposure period to reach its maximum potential. This extension could have a positive effect, considering that NNCF and AWCF increased after 30 days of vernalization.

Fruit color characteristics

Figure 4 shows the internal and external luminosity, chroma, and hue angle of the strawberries produced by the plants. The equations for the external fruit luminosity of the cultivars subjected to vernalization were not significantly adjusted (Figure 4a), although 30 days of vernalization reduced luminosity in the fruit produced by the Albion and Monterey cultivars, but not the fruit produced by the San Andreas cultivar. Internal fruit luminosity was affected by both the cultivar and the vernalization period. The Albion cultivar subjected to vernalization produced fruit with the highest internal luminosity (i.e., the lightest fruit) (Figure 4b). After 10 days of vernalization, the Monterey cultivar produced fruit with higher luminosity, whereas, after 30 days of vernalization, the San Andreas cultivar produced fruit with the highest luminosity.

The fruit produced by the Albion cultivar showed a decrease in internal luminosity as the vernalization period increased, while the fruit produced by the San Andreas cultivar exhibited increased luminosity as the vernalization period increased.
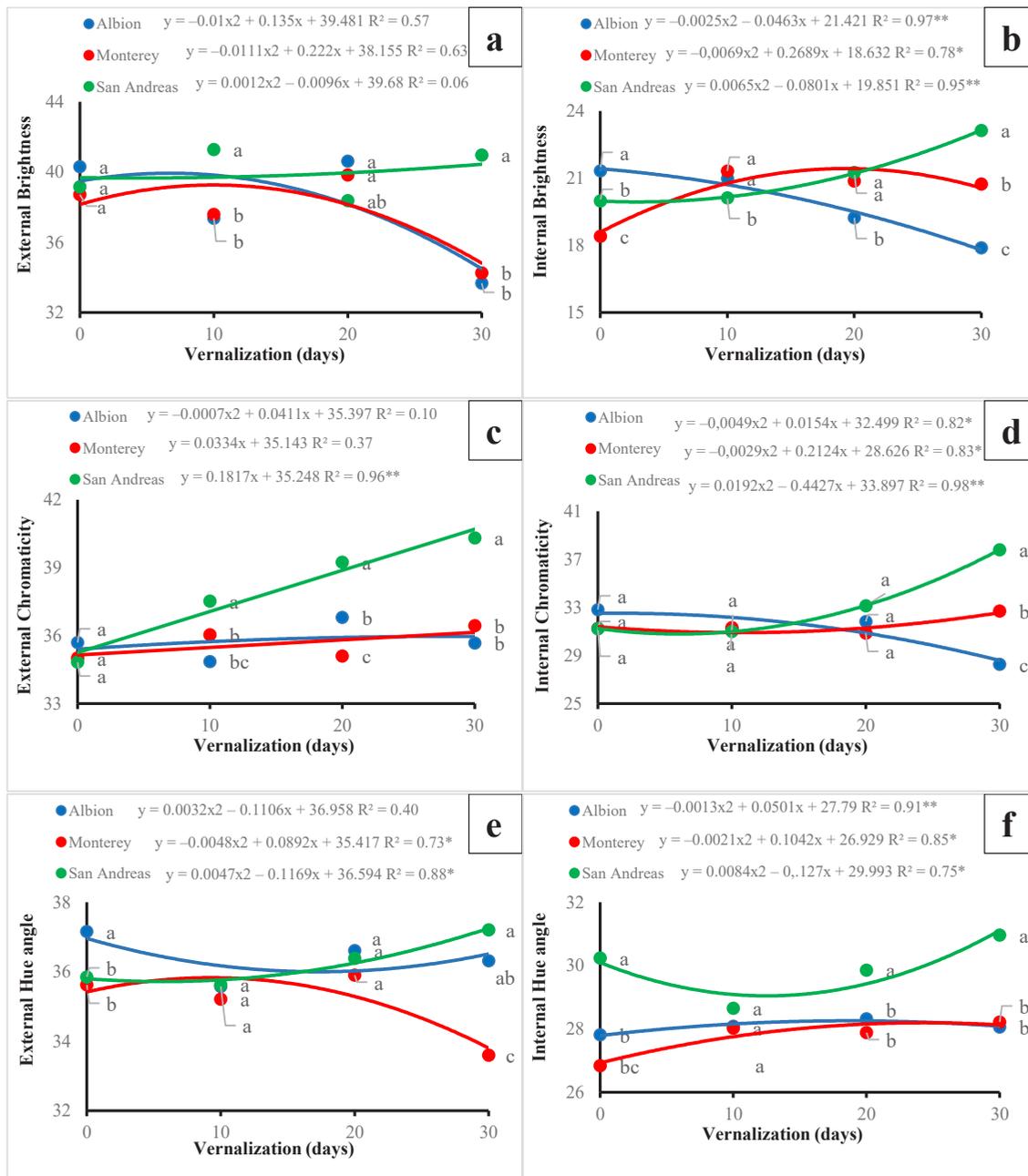

Figure 4. The effects of different vernalization periods on the color parameters of strawberries: (a) external lightness; (b) internal lightness; (c) external chroma; (d) internal chroma; (e) external hue angle; and (f) internal hue angle.

For each figure, points with the same letter do not differ significantly according to Tukey's test (p > 0.05). The asterisks indicate the significance of the regression: * p < 0.05 and ** p < 0.01.

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1581

For the external chroma of the fruit (Figure 4c), only the regression equation for the fruit produced by the San Andreas cultivar showed a significant adjustment, with longer vernalization periods increasing this parameter. The fruit produced by Albion and Monterey cultivars displayed no significant changes in chroma as a result of the vernalization periods. For the internal chroma of the fruit (Figure 4d), there was minimal variation among the cultivars up to 20 days of vernalization. After 30 days of vernalization, the fruit produced by the San Andreas cultivar showed an increase in internal chroma, while the fruit produced by the Albion cultivar showed a decrease. Internal chroma remained stable for the fruit produced by the Monterey cultivar, even after 30 days of vernalization.

There was not a significant equation adjustment for the external and internal hue angle (Figure 4e and f, respectively) of the fruit produced by the cultivars relative to the vernalization period. The fruit produced by the Monterey cultivar subjected to 30 days of vernalization showed the best external hue angle. The fruit produced by the San Andreas cultivar exhibited the lowest hue angle, irrespective of the vernalization period. There was minimal variation in the hue angle of the fruit produced by the Albion and Monterey cultivars at the different vernalization periods, but this parameter was superior compared with the fruit produced by the San Andreas cultivar.

Chitarra and Chitarra (2005) suggested that vernalization slightly interferes with the color parameters of cultivated strawberries. This is an important consideration due to the importance of these parameters for consumer acceptance at the time of purchase. Our findings indicate that vernalization has some effect on the expression of genes related to strawberry color characteristics. Genes involved in the anthocyanin biosynthetic pathway and other color-related compounds may be regulated during vernalization (Aharoni et al., 2001; Castillejo et al., 2020; Cocco, 2014; Cocco et al., 2020; Medina-Puche et al., 2014). It is important to emphasize that vernalization is not an isolated or static process and can impact the plant's reaction to later environmental conditions, such as light and temperature, during fruit growth and ripening. These factors can affect the development and stability of the pigments that determine color (Jaakola, 2013).
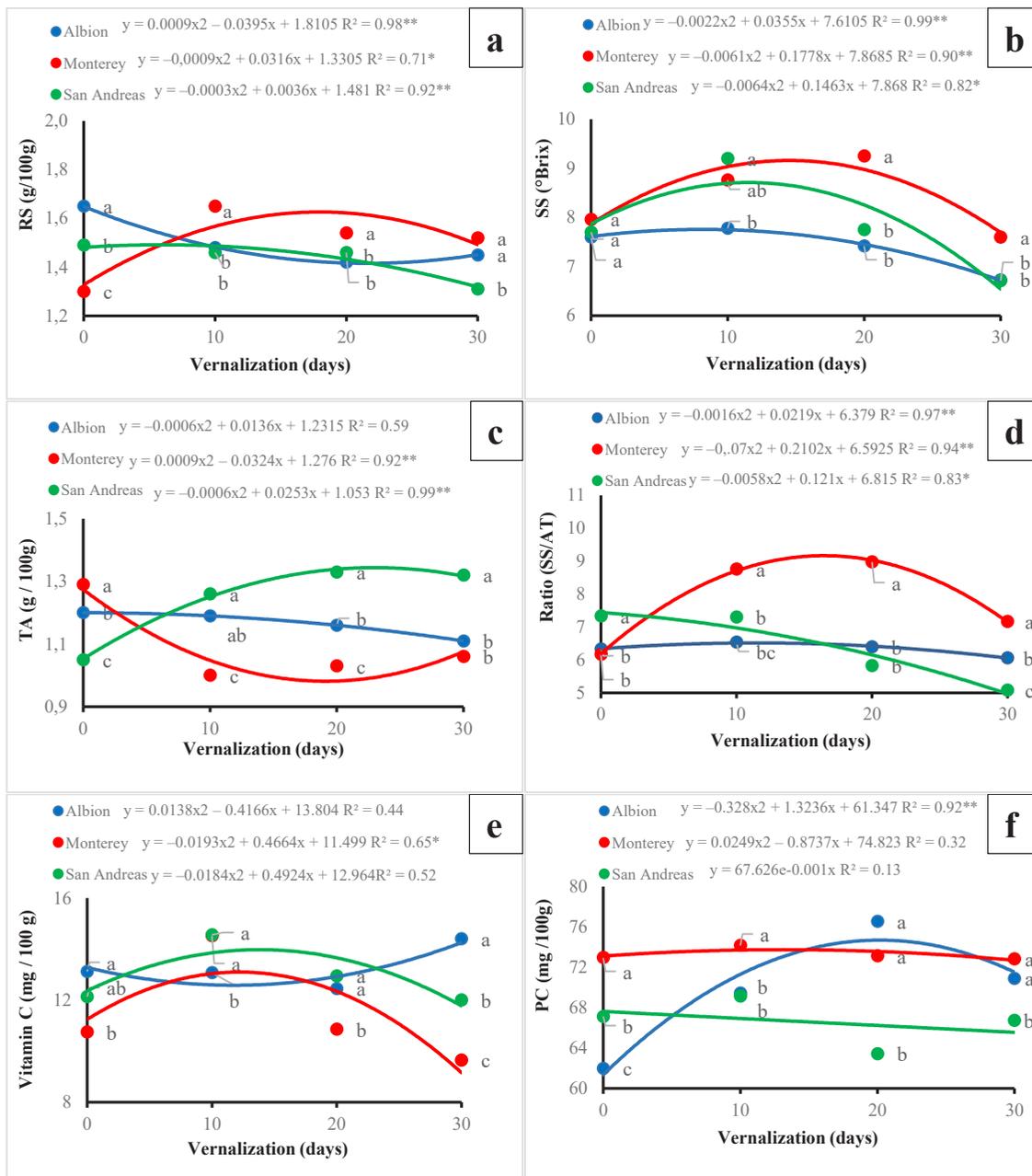
Physicochemical and antioxidant characteristics

The chemical and nutritional properties of strawberries are crucial for consumer appeal. These properties can vary depending on environmental factors (light, temperature, irrigation, and fertilization), the ripening process, the cultivation system, and the genetic makeup of the cultivars (Coelho, 2016).

The primary sugars glucose and fructose play a key role in sweetness and, together with organic acids, influence the flavor and aroma of the fruits (Ma et al., 2015). These monosaccharides can undergo oxidation in the presence of oxidizing agents in alkaline solutions, earning them the classification of reducing sugars (R. D. N. Silva et al., 2003). There were significant adjustments to the regression equations regarding the reducing sugar content in the fruit produced by all cultivars relative to the

1582

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

vernalization periods (Figure 5a). The fruit produced by the Monterey cultivar had a lower reducing sugar content when not subjected to vernalization, but this concentration increased as the vernalization period increased. In contrast, the fruit produced by the San Andreas and Albion cultivars showed a decrease in the reducing sugar concentration as a result of vernalization. Many fruit and plant traits are influenced by the accumulation of cold hours, largely due to hormonal synthesis and balance. Therefore, the sugar content in the fruit was likely determined during vernalization, accounting for the observed results.
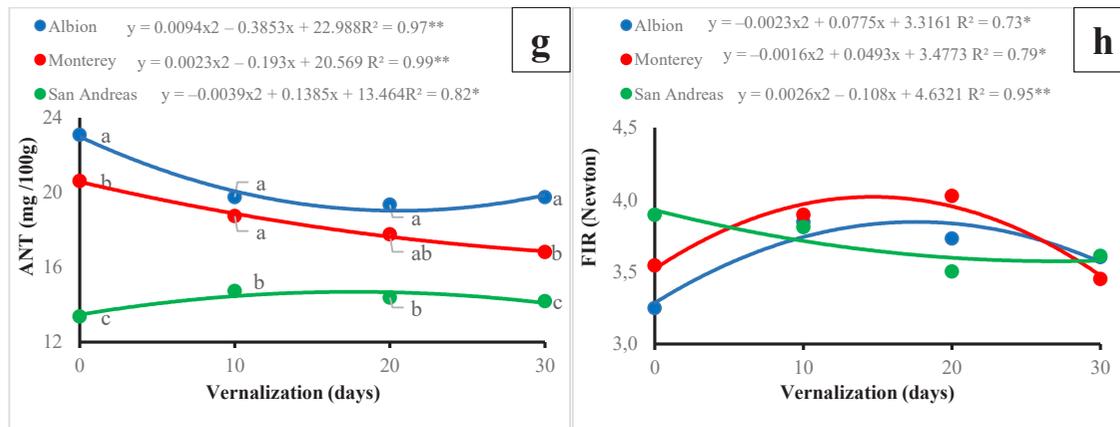

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1583

Figure 5. The effects of different vernalization periods on the physiochemical and antioxidant characteristics of strawberries: (a) reducing sugars; (b) soluble solids; (c) titratable acidity; (d) ratio; (e) vitamin C; (f) phenolic compounds; (g) anthocyanins; and (h) firmness.
For each figure, points with the same letter do not differ significantly according to Tukey's test (p > 0.05). The asterisks indicate the significance of the regression: * p < 0.05 and ** p < 0.01.

The soluble solids content was higher in the fruit produced by cultivars exposed to vernalization for 10 or 20 days, particularly the Monterey and San Andreas cultivars, likely due to a favorable hormonal balance that modulates the expression of genes associated with this trait. The soluble solids content was stable in the fruit produced by the Albion cultivar up to 10 days of vernalization, with a significant reduction after 20 and 30 days. This reduction also occurred in the fruit produced by the San Andreas and Monterey cultivars (Figure 5b). Oviedo et al. (2020) did not find significant changes in soluble solids when comparing vernalized and non-vernalized strawberry genotypes. However, they observed a significant effect on titratable acidity, with vernalized genotypes showing higher values.

Titratable acidity reflects the total acid content in fruit. It is high during the initial stages of fruit development and declines as the fruit ripens (Rahman et al., 2016). In fresh strawberries, titratable acidity can vary from 0.52% (Cecatto et al., 2013) to 1.51% (M. C. Antunes et al., 2014). In this study, titratable acidity was estimated by measuring the citric acid content per 100 grams of fruit pulp. Figure 5c highlights a strong cultivar × vernalization period interaction on titratable acidity. There was lower titratable acidity in fruit produced by the vernalized Monterey cultivar, whereas the fruit produced by the San Andreas cultivar showed a gradual increase in titratable acidity with vernalization. The regression equation for the titratable acidity in the fruit produced by the Albion cultivar did not exhibit a significant adjustment, suggesting that vernalization had no impact on its titratable acidity.

The total soluble solids to titratable acidity ratio showed variations based on the cultivar and vernalization period (Figure 5d). The fruit produced by the Albion cultivar maintained a stable ratio, showing no significant influence from the vernalization

1584

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

period. The fruit produced by the Monterey cultivar had the highest ratio, especially when the plants were exposed to 10 or 20 days of vernalization. In contrast, the fruit of the San Andreas cultivar showed a decline in the ratio as the vernalization period was extended.

Oviedo et al. (2018) reported that sugar levels, soluble solids, total acidity, and the ratio of total soluble solids to titratable acidity are influenced by the vernalization period and cultivar. Scott et al. (2021) stated that the sugar, acid, and volatile compound contents in strawberries decrease as temperature rises, with increased respiration during warm nights and faster fruit development in warm days and nights being the contributing factors (Menzel, 2022).

Only the regression equation for the fruit produced by the Monterey cultivar demonstrated a significant adjustment for the vitamin C content (Figure 5e), with the highest content recorded after 10 days of vernalization. The vitamin C content was also highest in the fruit produced by the San Andreas cultivar, even though the regression equation was not significantly adjusted. Interestingly, the Albion cultivar yielded fruit with a higher vitamin C content when the plants were not vernalized and when exposed to the maximum cold period (30 days), likely as a result of hormonal responses.

Phenolic compounds exhibit important antioxidant properties (H. Zhang & Tsao, 2016). The fruit produced by the Albion cultivar exposed to vernalization for 20 days had the highest phenolic compound content. The fruit produced by the Monterey cultivar had a high phenolic compound content that remained stable regardless of the length of vernalization. On the other hand, when the San Andreas cultivar was exposed to vernalization for periods, the fruit had a reduced phenolic compound content.

In strawberries, pelargonidin 3-glucoside is the most abundant anthocyanin, making up 90% of the total anthocyanins (Barth et al., 2020; Ruiz et al., 2019). The Albion and Monterey cultivars that were not vernalized produced fruit with the highest anthocyanin concentrations (Figure 5g). These results suggest that vernalization can decrease the anthocyanin content. The San Andreas cultivar yielded fruit with lower pigment levels compared with the Monterey and Albion fruit. Consistently, previous studies found that low temperatures negatively impact the accumulation of anthocyanins and vitamin C but positively affect soluble sugars, while the flavonol and total phenolic compound contents remain unchanged (Barth et al., 2020; J. T. V. Resende et al., 2020b).

Pulp firmness (Figure 5h) is regarded as one of the most critical attributes assessed in strawberries, as it enhances handling and transportation conditions while preserving organoleptic qualities for an extended period, thereby increasing the shelf life and marketability (Nasrin et al., 2017). The San Andreas cultivar yielded strawberries that progressively lost their firmness as the vernalization period was extended. On the other hand, the Albion and Monterey cultivars produced strawberries that were firmer when the plants underwent vernalization for up to 20 days. Fruit firmness is a key attribute that impacts the quality and consumer appeal of the product, and it is influenced by vernalization. The reallocation of plant resources to flower and fruit development can alter fruit texture over the production

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1585

cycle. Hormones such as gibberellins are crucial for fruit development and can be regulated by vernalization. This hormonal control can influence fruit formation and firmness (J. F. Silva et al., 2014).

Conclusions

The positive effects of vernalization on strawberry seedlings were most noticeable for the physiological, biometric, and production-related characteristics, whereas the physicochemical properties showed only slight changes. The cultivars exhibited varying responses to cold exposure, demonstrating a pronounced genotype × environment interaction. The Monterey cultivar achieved the best results in terms of productivity and physiological parameters at intermediate vernalization periods, with an exposure duration of 10 to 20 days. In contrast, the San Andreas cultivar required longer vernalization periods to yield positive outcomes, while Albion was the least responsive to changes in the vernalization period.

Acknowledgements

Declaration of Conflict of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Authors' Contributions

All authors contributed to data collection and analysis, as well as to writing and translating the manuscript.

References

Aharoni, A., De Vos, C. R., Wein, M., Sun, Z., Greco, R., Kroon, A., Mol, J. N., & O'Connell, A. P. (2001). The strawberry FaMYB1 transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. *The Plant Journal, 28*(3), 319-332. doi:10.1046/j.1365-313X.2001.01154.x

ALI. ADOLFO LUTZ INSTITUTE. *Physico-chemical methods for food analysis. Digital, São Paulo, (2008). 102p. Physicochemical methods for food analysis.* Ministry of Health, National Health Surveillance Agency.

Antunes, L. E. C., & Peres, N. A. (2013). Strawberry production in brazil and south america. *International Journal of Fruit Science, 13*(1-2), 156-161. doi:10.1080/15538362.2012.698147

Antunes, L. E. C., Ristow, N. C., Krolow, A. C. R., Carpenedo, S., & Reisser, C., Jr. (2010). Yield and quality of strawberry cultivars. *Horticultura Brasileira, 28*(2), 222-226. doi: 10.1590/S0102-0536 2010000200015

Antunes, M. C., Cuquel, F. L., Zawadneak, M. A., Mogor, Á. F., & Resende, J. T. (2014). Postharvest quality of strawberry produced during two consecutive seasons. *Horticultura Brasileira, 32*(2), 168-173. doi: 10.1590/S0102-05362014000200008

Association of Official Analytical Chemists (1995). *Official method 43.064. Official methods of analysis of AOAC International.* AOAC.

Barbosa, L. C., Porto, S. M., & Bertolde, F. Z. (2018). Stomatic analysis of two native tree species from the atlantic forest. *Revista Pindorama, 8*(8), 1-9. doi: 10.55847/pindorama.v8i8.589

Barth, E., Resende, J. T. V., Moreira, A. F. P., Mariguele, K. H., Zeist, A. R., Silva, M. B., Stulzer, G. C. G., Mafra, J. C. M., Gonçalves, L. S. A., Roberto, S. R., & Youssef, K. (2020). Selection of experimental hybrids of strawberry using multivariate analysis. *Agronomy, 10*(4), 598-616. doi: 10.3390/agronomy10040598

Bhering, S. B. (2020). *Mapa de solos do estado do Paraná.* https://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/339505

Bish, E. B., Cantliffe, D. J., & Chandler, C. K. (2002). Temperature conditioning and container size affect early season fruit yield of strawberry plug plants in a winter, annual hill production system. *HortScience, 37*(5), 762-764. doi: 10.21273/HORTSCI.37.5.762

Brandão, J. U. T., Fº., Ikuta, J., Ishimura, I., Izioja, H., Narita, N., Oda, N., & Kimoto, T. (1989). Effect of vernalization time on strawberry flowering. *Horticultura Brasileira, 7*(45), 11.

Cantliffe, D. J., Paranjpe, A. V., Stoffella, P. J., Lamb, E. M., & Powell, C. A. (2007). Influence of soilless media, growing containers, and plug transplants on vegetative growth and fruit yield of 'Sweet Charlie' strawberry grown under protected culture. *Proceedings of the Florida State Horticultural Society, 120,* 142-150.

Castillejo, C., Waurich, V., Wagner, H., Ramos, R., Oiza, N., Muñoz, P., Triviño, J. C., Caruana, J., Liu, Z., Cobo, N., Hardigan, M. A., Knapp, S. J., Vallarino, J. G., Osorio, S., Martín-Pizarro, C., Posé, D., Toivainen, T., Hytönen, T., Oh, Y., Barbey, C. R., ... Amaya, I. (2020). Allelic variation of MYB10 is the major force controlling natural variation in skin and flesh color in strawberry (Fragaria spp.) fruit. *The Plant Cell, 32*(12), 3723-3749. doi: 10.1105/tpc.20.00474

Cecatto, A. P., Calvete, E. O., Nienow, A. A., Costa, R. C. D., Mendonça, H. F. C., & Pazzinato, A. C. (2013). Culture systems in the production and quality of strawberry cultivars. *Acta Scientiarum. Agronomy, 35*(4), 471-478. doi: 10.4025/actasciagron.v35i4.16552

Chitarra, M. I. F., Chitarra, A.B. (2005). *Post-harvest of fruits and vegetables: physiology and handling.* Universidade Federal de Lavras.

Chouard, P. (1960). Vernalization and its relations to dormancy. *Annual Review of Plant Physiology, 11*(1), 191-238. doi: 10.1146/annurev.pp.11.060160.001203

Cocco, C. (2014). *Produção e qualidade de mudas e frutas de morangueiro no Brasil e na Itália.* Tese de doutorado em Fruticultura de Clima Temperado,

Universidade Federal de Pelotas, Pelotas, RS, Brasil.

Cocco, C., Schildt, G. W., Giacomel, F., Fagherazzi, A. F., Zanin, D. S., & Kretzschmar, A. A. (2020). Productive performance of day-neutral strawberry genotypes in Serra Gaúcha. *Revista Eletrônica Científica da UERGS, 6*(2), 155-163. doi: 10.21674/2448-0479.62.155-163

Coelho, J. M., Jr. (2016). Strawberry cultivation in Brazil. *GEMA Journal, 6*(1), 122-130.

Costa, A. F., Leal, N. R., Ventura, J. A., Gonçalves, L. S. A., Amaral, A. T. D., Jr., & Costa, H. (2015). Adaptability and stability of strawberry cultivars using a mixed model. *Acta Scientiarum. Agronomy, 37*(4), 435-440. doi: 10.4025/actasciagron.v37i4.18251

Costa, R. C., Calvete, E. O., Mendonça, H. F. C., & Cecatto, A. P. (2014). Phenology, phyllochron, and gas exchanges in frigo and fresh strawberry (*Fragaria x ananassa* Duch.) plants of cv. Albion. *Australian Journal of Crop Science, 8*(6), 901-908.

Dal Picio, M., Andriolo, J. L., Jänisch, D. I., Schmitt, O. J., & Lerner, M. A. (2013). Fruit yield of strawberry stock plants after runner tip production by different cultivars. *Horticultura Brasileira, 31*(3), 375-379. doi:10.1590/S0102-05362013000300006

Darnell, R. L., Cantliffe, D. J., Kirschbaum, D. S., & Chandler, C. K. (2003). The physiology of flowering in strawberry. In J. Janick (Ed.), *Horticultural reviews* (vol. 28, pp. 325-349). New York. doi: 10.1002/9780470650851.ch6

Diel, M. I., Pinheiro, M. V. M., Cocco, C., Thiesen, L. A., Altíssimo, B. S., Fontana, D. C., & Schmidt, D. (2017). Artificial vernalization in strawberry plants: phyllochron, production and quality. *Australian Journal of Crop Science, 11*(10), 1315-1319. doi: 10.21475/ajcs.17.11.10.pne603

Ferreira, D. (2016). SISVAR: a program for analyzing and teaching statistics. *Ciência e Agrotecnologia, 6*(2), 36-41.

Groppo, G. A., Tessarioli, J., Neto, & Blanco, M. C. S. G. (1997). Strawberry culture. *Boletim Técnico. Coordenadoria de Assistência Técnica Integral,* CATI. (201), 1-27.

Guevara-Matus, K., Loría-Quirós, C. L., & Granados-Montero, M. (2023). Effect of vernalization on the production of strawberry runners. *Revista Mexicana de Ciências Agrícolas, 14*(1), 129-134.

Guttridge, C. G. (1985). *Fragaria × ananassa.* In A. Halevy (Ed.), *CRC handbook of flowering* (vol. 3, pp. 16-33). Boca Raton.

Hernández-Martínez, N. R., Blanchard, C., Wells, D., & Salazar-Gutiérrez, M. R. (2023). Current status and future prospects of commercial morango production: a review. *Scientia Horticulturae, 312*(10), 111893. doi: 10.1016/j.scienta.2023.111893

Husaini, A. M., & Xu, Y. W. (2016). Challenges of climatic changes for strawberry cultivation: uncertainty and truth. In Amjad M. Husaini and Davide Neri, *Strawberry: growth, development and achievements* (pp. 262-287). CABI.

Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends in Plant Science, 18*(9), 477-483. doi: 10.1016/j.tplants.2013.05.003

Kamperidou, I., & Vasilakakis, M. (2006). Effect of propagation material on some quality attributes of strawberry fruit (*Fragaria x ananassa*, var. Selva). *Scientia Horticulturae, 107*(4), 337-343. doi: 10.1016/j.scienta.2005.06.009

Kerbauy, G. B. (2017). *Plant physiology.* Guanabara Koogan.

Kluge, R. A., Tezotto-Uliana, J. V., & Silva, P. P. M. da. (2015). Physiological and environmental aspects of photosynthesis. *Revista Virtual de Química, 7*(1), 56-73. doi:10.5935/1984-6835.20150004

Lavín, A., & Maureira, M. (2019). *The Chilean white-fruited strawberry: a development project for poor communities in dryland areas* (PRODECOP-SECANO) (2nd ed., n. 39). INIA.

Ledesma, N. A., & Kawabata, S. (2016). Responses of two strawberry cultivars to severe high temperature stress at different flower development stages. *Scientia Horticulturae, 211*(1), 319-327. doi: 10.1016/j.scienta.2016.09.007

Li, X., Jing, T., Zhou, D., Zhang, M., Qi, D., Zang, X., Zhao, Y., Li, K.,Tang, W., Chen,Y., Qi, C., Wang, W., & Xie, J. (2021). Biocontrol efficacy and possible mechanism of Streptomyces sp. H4 against postharvest anthracnose caused by Colletotrichum fragariae on strawberry fruit. *Postharvest Biology and Technology, 175,* 111401. doi:10.1016/j.postharvbio.2020.111401

López-Aranda, J. M., Soria, C., Santos, B. M., Miranda, L., Domínguez, P., & Medina-Mínguez, J. J. (2011). Strawberry production in mild climates of the world: a review of current cultivar use. *International Journal of Fruit Science, 11*(3), 232-244. doi: 10.1080/15538362.2011.608294

Lucchi, C., Mosconi, F., Stefanini, S., & Battelli, T. (2011). La fragola rifiorente nel Cesenate: apofruit Italia prova a rilanciare. *Rivista di Frutticoltura e di Ortofloricoltura, 73*(5), 16-23.

Ma, B., Chen, J., Zheng, H., Fang, T., Ogutu, C., Li, S., Han, Y., & Wu, B. (2015). Comparative assessment of sugar and malic acid composition in cultivated and wild apples. *Food Chemistry, 172*(1), 86-91. doi: 10.1016/j.foodchem.2014.09.032

Martins, F. T., & Polo, M. (2009). Reproductive development of Hyptis suaveolens (L.) Poit.: relationship between photoperiod, meristematic cell density and expression pattern of a putative orthologue of the arabidopsis LEAFY gene. *Brazilian Journal of Botany, 32*(1), 131-142. doi: 10.1590/S0100-84042009000100013

McDaniel, C. N. (1994). Photoperiodic induction, evocation and floral initiation. In R. L. Greyson (Ed.), *The development of flowers* (pp. 25-43). Oxford.

Medina-Puche, L., Cumplido-Laso, G., Amil-Ruiz, F., Hoffmann, T., Ring, L., Rodríguez-Franco, A., Caballero, J. L., Schwab, W., Muñoz-Blanco, J., & Blanco-Portales, R. (2014). MYB10 plays a major role in the regulation of flavonoid/phenylpropanoid metabolism during ripening of Fragaria× ananassa fruits. *Journal of Experimental Botany, 65*(2), 401-417. doi: 10.1093/jxb/ert377

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1589

Mendonça, H. F. C., Calvete, E. O., Nienow, A. A., Costa, R. C. D., Zerbielli, L., & Bonafé, M. (2012). Phyllochron estimation in intercropped strawberry and monocrop systems in a protected environment. *Revista Brasileira de Fruticultura, 34*(1), 15-23. doi: 10.1590/S0100-29452012000100005

Menzel, M. C. (2022). A productivity review in morango: Do plants need larger canopies, more flowers or better CO_2 assimilation for higher yields? *The Journal of Horticultural Science and Biotechnology, 97*(6), 674-696. doi: 10.1080/14620316.2022.2077240

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry, 31*(3), 426-428.

Montillet, J. L., Rondet, D., Brugière, S., Henri, P., Rumeau, D., Reichheld, J. P., Couté, Y., Leonhardt, N., & Rey, P. (2021). Plastidial and cytosolic thiol reductases participate in the control of stomatal functioning. *Plant, Cell & Environment, 44*(5), 1417-1435. doi: 10.1111/pce.14013

Moreira, A. F. P., Resende, J. T. V. de, Shimizu, G. D., Hata, F. T., Nascimento, D. do, Oliveira, L. V. B., Zanin D. S., & Mariguele, K. H. (2022). Characterization of strawberry genotypes with low chilling requirement for cultivation in tropical regions. *Scientia Horticulturae, 292*(27), 110629. doi: 10.1016/j.scienta.2021.110629

Nasrin, T. A. A., Rahman, M. A., Hossain, M. A., Islam, M. N., & Arfin, M. S. (2017). Postharvest quality response of strawberries with aloe vera coating during refrigerated storage. *The Journal of Horticultural Science and Biotechnology, 92*(6), 598-605. doi: 10.1080/14620316.2017.1324326

Oliveira, R. P., & Scivittaro, W. B. (2009). Production of morango fruits based on different periods of vernalization of molts. *Horticultura Brasileira, 27,* 91-95. doi: 10.1590/S0102-05362009000100018

Oviedo, V. R. S., Cabral, M., Garay, C. R. E., & Arredondo, G. A. J. C. (2018). Postharvest quality of strawberry (Fragaria χ ananassa (Duchesne ex Weston) Duchesne ex Rozier) genotypes according to vernalization. *Acta Agronómica, 67*(2), 208-214. doi: 10.1s446/acag.v67n2.60866

Oviedo, V. R. S., Enciso-Garay, C. R., & Figueredo, E. I. G. (2020). Vernalizing pre-transplants improved the agronomic characteristics of strawberry genotypes under tropical conditions. *Revista Caatinga, 33*(3), 653-659. doi: 10.1590/1983-21252020v33n308rc

PBMH & PIMo (2009). *Brazilian program for the modernization of horticulture and integrated strawberry production. Strawberry grading standards.* CEAGESP.

Pridgeon, A. J., & Hetherington, A. M. (2021). ABA signalling and metabolism are not essential for dark-induced stomatal closure but affect response speed. *Scientific Reports, 11*, 5751. doi: 10.1038/s41598-021-84911-5

Qu, X., Cao, B., Kang, J., Wang, X., Han, X., Jiang, W., Shi, X., Zhang, L., Cui, L., Hu, Z., Zhang, Y., & Wang, G. (2019). Fine-tuning stomatal movement through small signaling peptides. *Frontiers in Plant Science, 10*, 1664-462. doi: 10.3389/fpls.2019.00069

Rahman, M. M., Moniruzzaman, M., Ahmad, M. R., Sarker, B. C., & Alam, M. K. (2016). Maturity stages affect the postharvest quality and shelf-life of fruits of strawberry genotypes growing in subtropical regions. *Journal of the Saudi Society of Agricultural Sciences, 15*(1), 28-37. doi: 10.1016/j.jssas.2014.05.002

Reekie, J. Y., Hicklenton, P. R., Duval, J., Chandler, C., & Struik, P. C. (2003). Manipulating transplant morphology to advance and enhance fruit yield in strawberry. *Proceeding of the International Horticultural Congress: Berry Crop Breeding, Production and Utilization for a New Century. ISHS Acta Horticulturae, 26.*

Resende, J. T. V., Lima, R. B. D., Fº., Ribeiro, L. K., Corrêa, J. V. W., Maciel, C. D. D. G., & Youssef, K. (2020a). Strawberry genotypes with resistance to Tetranychus urticae mediated by leaf trichomes. *Ciência e Agrotecnologia, 44,* e006920. doi: 10.1590/1413-7054 202044006920

Resende, J. T. V., Silva, T., Novelo, D., Resende, N. C. V., Santos, L. S., Lima, D. P. de, & Schwarz, K. (2020b). Chemical characterization of frozen organic strawberries packaged in aluminum. *Research, Society and Development, 9*(5), e73953115. doi: 10.33448/rsd-v9i5.3115

Resende, L. D. A., Mascarenhas, M. H. T., & Paiva, B. D. (1999). Overview of strawberry production and marketing. *Informe Agropecuário, 20*(198), 5-19.

Ronque, E. R. V. (1998). *Strawberry culture.* Emater.

Rosa, H. T., Walter, L. C., Streck, N. A., Andriolo, J. L., Silva, M. R. D., & Langner, J. A. (2011). Temperatura-base de emissão de folhas e filocrono de algumas cultivares de morangueiro em ambiente subtropical. *Bragantia, 70*(4), 939-945. doi: 10.1590/S0006-87052011000400029

Roux, B., & Leonhardt, N. (2018). The regulation of ion channels and transporters in the guard cell. In C. Maurel (Ed.), *Advances in botanical research* (vol. 87, pp. 171-214). London, United Kingdom. doi: 10.1016/bs.abr.2018.09.013

Ruiz, A., Sanhueza, M., Gómez, F., Tereucán, G., Valenzuela, T., García, S., Cornejo, P., & Hermosín-Gutiérrez, I. (2019). Changes in the content of anthocyanins, flavonols, and antioxidant activity in Fragaria ananassa var. Camarosa fruits under traditional and organic fertilization. *Journal of the Science of Food and Agriculture, 99*(5), 2404-2410. doi: 10.1002/jsfa.9447

Scott, G., Williams, C., Wallace, R. W., & Du, X. (2021). Exploring plant performance, fruit physicochemical characteristics, volatile profiles, and sensory properties of day-neutral and short-day strawberry cultivars grown in Texas. *Journal of Agricultural and Food Chemistry, 69*(45), 13299-13314. doi: 10.1021/acs.jafc.1c00915.

Shaw, D. V., & Larson, K. D. (2009). *Strawberry plant named 'Monterey'* (U.S. Patent No. PP19,767). U.S. Patent and Trademark Office.

Silva, J. F., Pinheiro, R. F., Amaro, A. L., Pereira, M. J., Roriz, M., Aguiar, A., Pintado, M., Vasconcelos, M., & Carvalho, S. M. (2014). Optimization of the application

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025

1591

of a biostimulant to increase strawberry productivity and quality. *Anais do Simpósio Nacional de Fruticultura. Associação Portuguesa de Horticultura,* Vila Real, Douro, Portugal, 3.

Silva, R. D. N., Monteiro, V. N., Alcanfor, J. D., Assis, E. M., & Asquieri, E. R. (2003). Comparison of methods for the determination of reducing and total sugars in honey. *Food Science and Technology, 23*(3), 337-341. doi: 10.1590/S0101-20612003000300007

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2017). *Plant physiology and development.* Artmed Editora.

Verdial, M. F., Tessarioli, J., Neto, Minami, K., Scarpare, J. A., Fº., Christoffoleti, P. J., Scarpare, F. V., Barela, J. F., Aguila, J. S., & Kluge, R. A. (2009). Strawberry runners physiology produced in conventional and suspended pots systems. *Revista Brasileira de Fruticultura, 31*(2), 524-531. doi: 10.1590/S0100-29452009000200029

Wrege, M. S., Reisser, C., Jr., Antunes, L. E. C., Oliveira, R. P. de, Herter, F. G., Steinmetz, S., Garrastazu, M. C., Matzenauer, R., João, P. L., & Santos, A. M. dos. (2007). *Agroclimatic zoning for strawberry seedling production in Rio Grande do Sul.* (Document, 187). EMBRAPA Clima Temperado.

Ye, Y., Zhou, L., Liu, X., Liu, H., Li, D., Cao, M., Chen, H., Xu, L., Zhu, J. K., & Zhao, Y. (2017). A novel chemical inhibitor of ABA signaling targets all ABA receptors. *Plant Physiology, 173*(4), 2356-2369. doi: 10.1104/pp.16.01862

Zeist, A. R., & Resende, J. T. V. D. (2019). Strawberry breeding in Brazil: current momentum and perspectives. *Horticultura Brasileira, 37*(1), 7-16. doi:10.1590/S0102-053620190101

Zhang, H., & Tsao, R. (2016). Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Current Opinion in Food Science, 8,* 33-42. doi: 10.1016/j.cofs.2016.02.002

Zhang, T. Y., Li, F. C., Fan, C. M., Li, X., Zhang, F. F., & He, J. M. (2017). Role and interrelationship of MEK1-MPK6 cascade, hydrogen peroxide and nitric oxide in darkness-induced stomatal closure. *Plant Science, 262*, 190-199. doi: 10.1016/j.plantsci.2017.06.010

Zhang, Y. C., Zhang, Q. Y., Luo, P., & Wu, N. (2009). Photosynthetic response of Fragaria orientalis in different water contrast clonal integration. *Ecological Research, 24*(3), 617-625. doi: 10.1007/s11284-008-0533-x

1592

Semina: Ciênc. Agrár. Londrina, v. 46, n. 5, p. 1567-1592, set./out. 2025