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Natural Sciences

Original article

Prediction of the duration of phenological stages in two varieties of standard carnation (*Dianthus caryophyllus* L.) in terms of thermal time

Predicción de la duración de las etapas fenológicas en dos variedades de clavel estándar (*Dianthus caryophyllus* L.) en términos de tiempo térmico

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Abstract

Although carnation is one of the world's most widely used cut flowers, several basic aspects of its growth and development have yet to be studied. Here, we aimed to determine the phenological behavior and development of a commercial carnation crop relating temperature in terms of thermal time for Mizuky and Zafiro cultivars. The study was conducted in a commercial production farm greenhouse in Tocancipá, Cundinamarca (Colombia). Sampling was done from the plants' pinching to the end of the harvest in four plantings on different dates. We estimated the base temperature (Tb) for the vegetative and reproductive stages using the minimum coefficient of variation method for growing degree-days (GDD or thermal time). Our results showed that Tb and thermal time varied depending on the developmental stage of the crop. The life cycle was 242 days for the Mizuky cultivar and 240 days for the Zafiro. The Mizuky cultivar required Tbs of 0.66°C for the vegetative stage and 0.84°C for the reproductive stage, while the Zafiro required higher Tbs of 1.81 and 2.64°C, respectively. The GDD accumulations from the vegetative stage to cutting were 2606° for the Mizuky and 2624° for the Zafiro; the times from pinching to harvest were 187 days for the Mizuky and 185 days for the Zafiro. We concluded that the Zafiro needs a higher Tb than the Mizuky, but given their close thermal times, we recommend similar crop management for both.

Keywords: Phenology; Phenological model; Base temperature; Growing degree-days.

Resumen

Aunque el clavel es una de las flores de corte más utilizadas en el mundo, varios aspectos básicos de su crecimiento y desarrollo aún están por estudiarse. Nuestro objetivo fue determinar el comportamiento fenológico y el desarrollo de un cultivo comercial de clavel, relacionando la temperatura en términos de tiempo térmico para los cultivares Mizuky y Zafiro. El estudio se hizo en el invernadero de una finca de producción comercial en Tocancipá, Cundinamarca (Colombia). El muestreo se hizo desde el despunte de la planta hasta el final de la cosecha. Se estimó la temperatura base (Tb) para las etapas vegetativa y reproductiva mediante el método de mínimo coeficiente de variación de los grados día de desarrollo (GDD o tiempo térmico). Los resultados mostraron que la Tb y el tiempo térmico varían según el estado de desarrollo del cultivo. Se encontró que el ciclo de vida del cultivar Mizuky fue de 242 días y el de Zafiro de 240 días; el Mizuky requirió una Tb de 0,66 °C en la etapa vegetativa y de 0,84°C en la reproductiva, en tanto que el Zafiro necesitó una Tb mayor, de 1,81 °C y 2,64 °C, respectivamente. La acumulación de los GDD desde la fase vegetativa hasta el corte fue

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de 2606° y de 2624° para Mizuky y Zafiro, respectivamente, por lo que el tiempo trascurrido desde el despunte hasta el corte fue de 187 días para el Mizuky y 185 para el Zafiro. Se concluyó que el cultivar Zafiro exige una Tb mayor que el Mizuky, pero dada la proximidad del tiempo térmico en las dos variedades, se recomienda un manejo similar para las dos.

Palabras clave: Fenología; Modelo fenológico; Temperatura base; Grados día de desarrollo.

Introduction

The carnation (*Dianthus caryophyllus* L.) is one the five most important cut flower species in the world (**Maitra & Roychowdhury**, 2013; **Pace *et al.***, 2022) and after the rose and the chrysanthemum, it is the third most important cut flower in the global market (**Abeliotis *et al.***, 2016; **Boxriker *et al.***, 2017). Colombia is the second largest exporter of flowers worldwide after the Netherlands (**Leguízamo-Medina *et al.***, 2022) and the largest exporter of carnations (**Minagricultura**, 2021), and in the Bogotá savanna, where we conducted our study, carnation cultivation represents the second-largest cut flower export by Colombia (**Baracaldo Argüello *et al.***, 2010), with standard carnations and minicarnations being, after roses, the most important cut flower crops (**López *et al.***, 2014).

Regarding the environmental conditions that most affect carnation production, **Gocan *et al.*** (2022) highlight temperature stress due to its influence on plant growth and development processes, which involve a large number of temperature-dependent biochemical reactions (**Hasanuzzaman *et al.***, 2013). Carnation cultivation does not thrive at extreme temperatures and it is well-adapted to temperatures ranging between 10 and 20°C (**Gobade**, 2024). **Röber & Wohanka** (2014) recommend optimal temperatures between 15 and 20°C in summer conditions. Temperature mainly affects carnations' bud development, which takes markedly longer at low temperatures (**Bunt & Cockshull**, 2017), but minimum temperatures are not known for their different phenological stages.

Passive plastic greenhouses with natural ventilation predominate in the ornamental sector in Colombia, so there may be spatial variations in temperature, vapor pressure deficit, and relative humidity during day and night hours, affecting not only the quality and uniformity of the carnations produced (**Villagran & Bojacá**, 2020) but also their ecophysiology.

Crops' development, behavior, and survival are fundamentally determined by the climatic characteristics of their location (**Bordón & Blasco**, 2018). Several studies have confirmed that temperature, solar radiation, plant age, source-sink relationships, and the response to photoperiod influence plant phenological development (**Salazar-Gutiérrez *et al.***, 2013; **Mayorga *et al.***, 2020).

Phenology plays a fundamental role in the life cycle of plants (**Zapata *et al.***, 2015). As **Salazar *et al.*** (2008) indicate, phenology is the periodic study of biological or natural phenomena determined by stages that characterize the emergence, transformation, or rapid disappearance of plant organs, separated by two successive stages: the emergence of plants and the appearance of nodes, buds, flowers, and fruits. Organ development is controlled by the rate of emergence of nodes and the development of leaves, flowers, and fruits, which are temperature-dependent seasonal functions. The development of various organs responds to heat, cold, or day and night alternation (**Salazar**, 2006). Each species has specific temperature requirements to reach a particular phenological stage (**Sikder**, 2009). Thus, for each species, there is a range of maximum and minimum temperatures that indicate the thresholds of discernable growth (**Hatfield & Prueger**, 2015).

The combination of temperature and time has led to the concept of thermal time (TT), also known as heat summation, degree days, or growing degree days (GDD). This concept is defined as "the number of degree-days needed to finish a certain developmental process or phenological stage" **(Parra-Coronado *et al.***, 2015; **López *et al.***, 2010; **Trudgill *et al.***, 2005). Models for calculating thermal time presume a linear relationship between temperature and plant development (**Zapata *et al.***, 2015).

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Salazar (2006) reiterates that the basic requirement for determining thermal time in degree-days is the critical or reference temperature, also known as base temperature (Tb), below which phenological development or metabolic processes in plants cease (**Parra-Coronado *et al.***, 2015). This lower limit is important because it determines the effect of temperature on the development (including growth) of the organism. It is important to keep in mind that if the average daily temperature is one degree above the Tb during 24 hours, one degree-day accumulates (**Parra-Coronado *et al.***, 2015; **Zapata *et al.***, 2015).

Our study aimed to determine the base temperatures (Tb) for two standard carnation varieties by establishing the thermal time in growing degree-days (GDD) in all phenological stages from pinching (topping) to the end of harvest.

Materials and methods

Location and characterization of the study areas

The study was conducted in the plastic greenhouses of Flores La Mana SAS farm, located in the municipality of Tocancipá (Cundinamarca, Colombia), El Porvenir, at 4°59’’ N and 73°55’’ W. The area is 2,605 MASL, the mean annual rainfall is 857 mm, the mean annual external temperature is 14.1°C, and the external relative humidity is 80%. During the study, inside the greenhouses, the average maximum temperature was 26.5°C, and the minimum was 9.1°C, i.e., an average temperature of 15.4°C and an average relative humidity of 77%.

We planted and evaluated two carnation varieties, Mizuky and Zafiro, in four greenhouses and on four different planting dates. The Mizuky variety, whose hybridization is Breier, has a pink color, and the Zafiro variety, with SB Talee hybridization, has a purple color. They are characterized by having consistent stems, a stem length between 80 and 110 cm, a uniform opening, and a vase life between 12 and 15 days. On each planting date, we planted 20 30×1 m hydroponic beds with a planting density of 29.6 plants/m^2 and a 0.12 m distance between plants and 0.13 m between rows. We used a substrate based on burnt rice husk, the most frequent in hydroponics for cut flowers in Colombia (**Vélez-Carvajal *et al.***, 2022), composed of 55% raw husk, 35% used substrate, and 10% compost for the two varieties studied. Fifty-five days after planting, the stem tip was removed above the eighth leaf node to stimulate the lateral sprouting of the plant.

During the research, conventional agronomic management of the plants was used with phytosanitary control products according to the monitoring. Routine cultural tasks, stem guiding, disbudding, and floral stem harvesting were done according to the phenological stages of the crop.

The climatic information inside the greenhouse was recorded every 15 minutes using the WIGA software, and the data corresponding to the minimum, maximum, and average daily temperatures during the different production periods (four sowings) were extracted. A sensor installed 2 m above the ground next to the experiment was used to monitor the greenhouse climate.

Determination of phenological stages duration in days

Three plant beds were selected for each variety after bud break. In each selected bed, 50 plants were taken randomly discarding those at the beginning and the end of the bed; a shoot from the fifth node was selected in each plant as the sampling unit. The observation unit was the individual plant, and we obtained 150 experimental units per variety. The selected stems were monitored every seven days during the vegetative and reproductive stages; when the floral stems began to be harvested, they were monitored every two days. Development data were recorded for each of the stages to determine the duration period in days for each of them.

Base temperature (Tb) estimation

Three different statistical methods were used to estimate Tb: the smallest variance in GDD, the smallest standard deviation in GDD, and the smallest coefficient of variation in GDD using a quadratic regression model (**Acero-Camelo *et al.***, 2021; **Parra-Coronado**

et al., 2015). When applying these methods, we recorded the duration in days of each phenological stage and carnation variety and we added the mean daily temperatures registered during the study periods (four plantings). Starting from 0°C, which we increased by 0.1°C until reaching 12°C, we used equation 2 to estimate the thermal time (or GDD) for each of these temperatures per carnation variety and phenological stage, considering the four sowings made. The GDD matrix generated was used to apply the aforementioned methods to estimate base temperatures (Tb). The most logical Tb estimation method according to carnation physiology was the lowest coefficient of variation in GDD using a quadratic regression model that consisted in calculating the GDD coefficient of variation (CV) for each temperature from 0 to 12°C with the values generated in the GDD matrix. The Tb for each phenological stage and carnation variety corresponds to the temperature for which the minimum CV is obtained by applying a quadratic regression model or where the first derivative of the regression model is equal to zero.

The quadratic equation is in the form of equation 1: $CV = A\,(Tb)^2 + B\,(Tb) + C$ where CV is the coefficient of variation, Tb is the base temperature, and A, B, and C are parameters of the equation determined with the help of Excel® Solver. The Solver tool performs a non-linear iterative process, which makes a preliminary calculation using the values initially assigned to the coefficients of the quadratic equation and modifies these values until the minimum sum of the squared deviations presented between the CVs calculated by the equation and those calculated by statistics is found. Subsequently, by deriving the obtained equation and equaling the derivative to zero, the value corresponding to Tb is obtained (**Orjuela-Angulo *et al.***, 2022; **Parra-Coronado *et al.***, 2015). To determine whether there were differences between the Tb of the different phenological phases, a Student's t-test was used for two samples assuming unequal variances and comparing between varieties.

Thermal time (TT) estimation

According to **Parra-Coronado *et al.*** (2015) methodology to determine the time of each phase in terms of growing degree-days (GDD), we used the pre-estimated Tb values for each of the two varieties at each developmental stage considered, which was calculated using the daily sum of the difference between the mean temperature of each phase and the reference temperature (Tb) (equation 2).

$$TT = GDD = \sum_{t=1}^{n} Ti - n * Tb\,, \qquad (2)$$

where TT is the thermal time expressed in degree-days (GDD), n is the number of days, Ti is the mean daily temperature, and Tb is the base temperature. The GDDi for TT estimation are calculated following the following indications:

$$Ti = \frac{Tmax + Tmin}{2} \qquad (3)$$

$$Si\ Ti > Tb, GDDi = Ti - Tb \qquad (4)$$

$$Si\ Ti < Tb, GDDi = 0, \qquad (5)$$

where Tmax and Tmin correspond to the maximum and minimum temperatures, respectively, expressed in °C for day i.

To monitor crop development, it is important to know the starting date of each phenological stage or phase for each variety and planting (**Table 1**).

Cross validation

We cross-validated the results obtained from the degree-day model to make sure they were independent using the K folds method where the data is randomly divided into five groups of similar size: four are used to train the model and the other one to validate it; finally, the process is repeated up to 36 times using a different group as validation in each iteration until reaching the ideal result at which point the test is adjusted to the maximum possible. At the end, a root mean square error (RMSE), which quantifies how different a set of values is, is obtained. The smaller the RMSE value, the closer the predicted and observed values are. The Phyton statistical program was used for this validation.

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Table 1. Phenological, vegetative, and reproductive stages' onset dates, including the end of cycle for the two carnation varieties and each planting

Phase	Planting number	Variety	
		Mizuky	Zafiro
Vegetative	1	10/04/2018	10/04/2018
	2	28/05/2018	28/05/2018
	3	14/09/2018	14/09/2018
	4	23/11/2018	23/11/2018
Reproductive	1	20/06/2018	04/07/2018
	2	01/08/2018	16/08/2018
	3	26/11/2018	30/11/2018
	4	12/01/2019	01/02/2019
End of cycle	1	13/10/2018	11/10/2018
	2	11/12/2018	18/11/2018
	3	10/02/2019	01/03/2018
	4	31/03/2019	25/04/2019

Results and discussion

Duration of phenological stages in days

In each variety studied, the period between pinching and harvest was divided into two phenological stages (vegetative and reproductive), and the duration of each was determined. **Table 2** shows the general duration in days of each phenological stage by variety. By comparing the developmental behavior of the two varieties, we determined that Mizuky had a shorter vegetative stage but a longer reproductive stage than Zafiro, i.e., the life cycle of the two varieties (of approximately 34 weeks after planting) was very similar.

Additionally, differences were not only observed in the life cycle time (considered from planting to the beginning of harvest) but also in the time of each stage, despite that the varieties were pinched at the same time and began their vegetative development on the same day. The Mizuky variety had a shorter vegetative stage of 71 days (or approximately 18 weeks) from pinching to the end of the vegetative stage, while Zafiro took two more weeks (85 days). The Zafiro variety's reproductive stage was 100 days, while the Mizuky was longer, 116 days from the end of the vegetative stage to the beginning of the harvesting stage. For both varieties, the period from sowing to the end of the reproductive stage lasted approximately 34 weeks.

The results in terms of time in days for each of the stages of the two varieties are similar to those found by **Baracaldo *et al.*** (2010), who describe in detail the development of the carnation in five stages until the end of the first harvest. The authors mention a first stage consisting of root fixation after transplanting between weeks 0 and 6; the second stage is the development of lateral shoots after pinching between weeks 5 and 15,

Table 2. Duration of carnation phenological stages by variety from planting to the end of harvest

Variety	Duration of phenological stages (d)			
	To pinch	Vegetative	Reproductive	Life cycle
Mizuky	55	71	116	242
Zafiro	55	85	100	240

approximately, and the third is stem elongation between weeks 14 and 25. These three stages can be grouped into one and compared with the vegetative stage. Although the time of pinching differs from that found in this study by two weeks, the time that elapsed between planting and the end of the vegetative stage is consistent with ours, as this stage occurred between weeks 18 and 20. The fourth stage described by **Baracaldo *et al.*** (2010) is the development of the main floral bud and laterals between weeks 16 and 30, which matches the description and timing of the reproductive stage of the varieties in this study, where both reached this stage between weeks 29 and 30. The fifth and last stage is the first harvest, occurring between weeks 23 and 34, which matches the harvesting times of each of the varieties in our study and their respective end of first peak. The stages in the **Baracaldo *et al.*** (2010) study can be integrated into the two stages we defined by grouping the first three stages into one.

Base temperature (Tb) estimation

Thermal models require knowing the Tb at each phenological stage of the crop to estimate the corresponding growth degree-days (GDD) (**Acero-Camelo *et al.***, 2021; **Hou *et al.***, 2014). To estimate the Tb mean values in the two varieties, we considered the data from the plantings carried out on March 3, July 21, and September 29, 2018, as well as the values obtained in the first sowing on February 14, 2018, for result-validation purposes.

Among the methods used to estimate Tb, the most logical according to carnation physiology was the lowest coefficient of variation in GDD using a quadratic regression model. The results obtained using the lowest variance in GDD and the lowest standard deviation were not considered because the Tb presented values between 14 and 22.5°C, which does not correspond to a temperate climate crop such as carnation.

Figure 1 shows the coefficient of variation and the regression equation for the total accumulated heat used to estimate the reference temperatures (Tb) for the two cultivars considered in this study at various seasonal stages. A common trend can be observed.

Table 3 shows the TB values obtained for the phenological stages of the two carnation cultivars studied. The results showed that Tb values depend on the variety and the developmental stage of the crop (**López *et al.***, 2011; **Trudgill *et al.***, 2005). The Mizuky variety registered the lowest Tb in the vegetative and reproductive stages at 0.66°C and 0.84°C, respectively. **Singh *et al.*** (2013) state that carnation varieties can vary according to the growth environment, the genotype, the region, and the season. However, considering the complete cycle (from the vegetative phase to the end of harvest), the two varieties had Tb values close to 0°C (**Table 3**).

The existence of differences between the two varieties' base temperatures in the vegetative and reproductive phenological stages was not determined by t-test because numerically the differences were not very large; therefore, it was not possible to confirm the statement of **López *et al.*** (2011), who indicated that it is not possible to use a single base temperature in all the phenological stages of each of the varieties.

Our results for Tb in the two varieties ranged from 0.29 to 2.64°C, depending on the variety and phenological stage (**Table 3**). This is consistent with the Tb data obtained by **López *et al.*** (2010), who estimated the Tb for Delphi carnation node elongation and found that it was statistically equal to 0°C with a value of 0.12°C, the same value determined for internode elongation in chrysanthemum (**Larsen & Gertsson**, 1992). However, this is lower than the temperatures reported for other phenological processes, for example, that for rose petal emergence is 5.2°C (**Mattson & Lieth**, 2007). This may be because the carnation is native to the temperate climates of southern Europe and western Asia (**Dona *et al.***, 2017).

Filgueira (2011) describes the different types of carnations and how they can be successfully grown in the cooler parts of the plains and at medium to high elevations. Currently, three ecotypes of carnation are produced commercially, the first of Mediterranean origin and the other two developed in the United States. According to this author, "the

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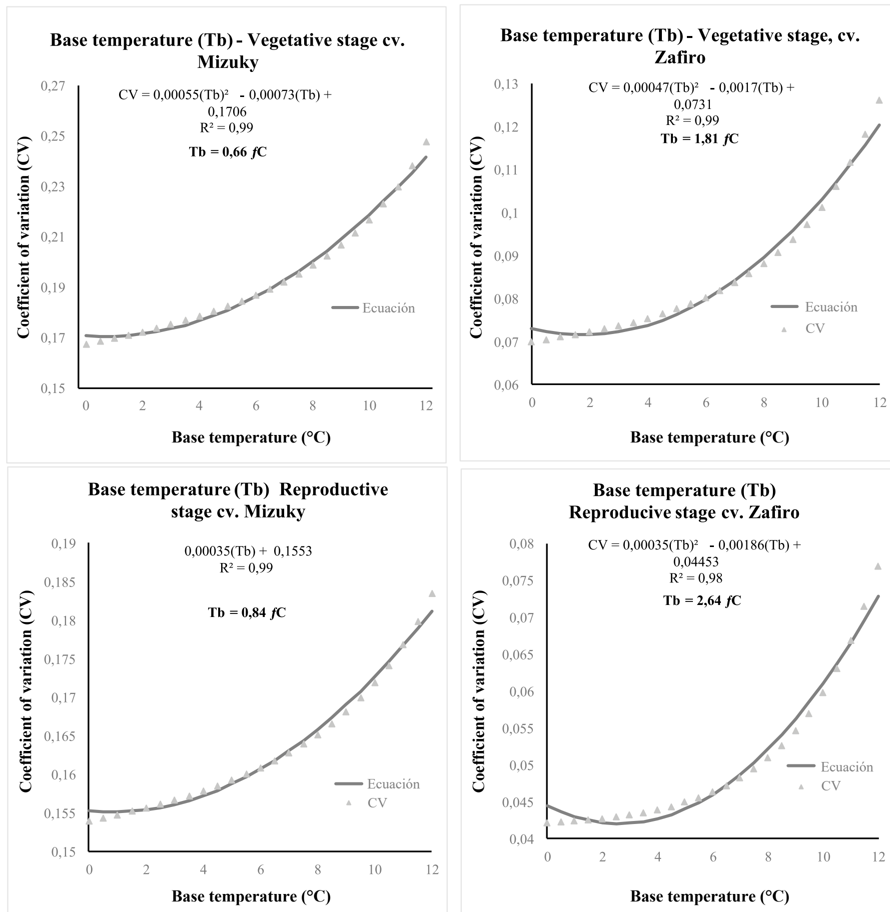

Figure 1. Base temperatures (Tb) for the phenological stages of the two carnation cultivars, estimated using the minimum coefficient of variation (CV) of the calculated thermal time (GDD) or where the slope (first derivative) of the regression model is equal to zero.

Table 3. Base temperature for the different phenological stages in the two carnation varieties

Phenological stage	Variety	
	Mizuky	Zafiro
Vegetative	0,66	1,81
Reproductive	0,84	2,64

genus *Dianthus* would be adapted to cold regions". To obtain standard carnations of high quality, therefore, cooling of night temperatures in greenhouses to 21°C is applied during hot seasons in Japan (**Higashiura *et al.***, 2020).

Regarding cold climate crops other than carnation in Colombia, **Mayorga *et al.*** (2020) found that in the case of banana passion fruit (*Passiflora tripartita* var. *mollissima*), "the base temperatures for growth of primary branches, flower buds, and fruits were 4.3°C, 3.1°C, and 0.01°C, respectively." As for the plum (*Prunus salicina* Lindl.) cultivar Horvin, **Orjuela-Angulo *et al.*** (2022) found a base temperature of 2.9°C for the stage between fruit set and harvest, which shows base temperatures for cold climate crops can vary between 0 and 5°C, as seen for carnations in our research.

Thermal time (GDD) estimation

Thermal time models have been used with many cultivated species to predict their development (**Zapata *et al.***, 2015). Here, we determined the time of each stage in GDDs and in each of the plantings considered based on the estimated Tb values for each of the two phenological stages of the two cultivars under study (**Table 4**).

Table 4. Calendar days and mean values of thermal time (GDD) for the phenological stages of the Mizuky and Zafiro carnation cultivars (mean ± SD)

Phenological stage	Variety			
	Mizuky		Zafiro	
	# Days	GDD (°C)	# Days	GDD (°C)
Vegetative	71	1057.7 ± 178.5	85	1193.1 ± 85.9
Reproductive	116	1506.1 ± 232.9	100	1437.2 ± 61.8

The accumulated thermal time in GDDs for the two varieties (**Table 4**) behaved differently in terms of accumulated degree-days in each phenological stage, which coincides with **Phadnawis & Saini** (1992) and **Qadir *et al*.** (2006) results. Although the accumulation of GDDs in the different phenological stages is relatively stable and independent of sowing time, each hybrid, cultivar, or seed can have specific values in these parameters.

Table 4 shows the mean value of the total accumulated thermal time for each variety, with Mizuky having a lower thermal requirement of 2606 GDD. However, this variety needs more calendar days (187) to finish its life cycle. In any case, the two varieties showed similar behavior in terms of the total accumulated thermal time and the number of calendar days from the topping stage to the end of cutting.

According to our results, we concluded that these two carnation varieties require an average of 1125.4 GDD (ºC d) from bud break to the end of the vegetative phase and 1471.7 GDD from the end of the vegetative phase to the end of flower bud formation. In general, the thermal time required to reach flower cutting from bud break is 2615.1 GDD, varying from 185 to 187 days (mean value, 186 days) depending on the variety (**Table 4**).

Our results are similar to those obtained by **López *et al.*** (2010) for Delphi carnations: 2,226.5 GDDs were accumulated for the peak harvest. However, the thermal time in the vegetative stage was higher, as the authors counted 1363.2 GDDs from the pinching to the visualization of the flower bud, which may be explained by the difference in the varieties studied (**Qadir *et al.***, 2006; **Leguízamo-Medina *et al.***, 2022).

Cross-validation

Cross-validation showed that the model developed and used in this study to obtain the GDD was fit for both carnation varieties, as we obtained low RMSE values (1.439 for Misuky and 1.99 for Zafiro), indicating that the predicted values were very close to those observed.

Conclusions

Misuky and Zafiro carnation varieties had similar phenological behavior in terms of the time required from planting to harvest, with 242 and 240 days of life cycle, respectively. We recommend, therefore, similar crop management for the two varieties.

The Tb depends on the variety and the development stage of the crop. In general, in our study, it varied between 0.29 and 2.64°C, depending on the variety and the phenological stage, with the Mizuky variety presenting a lower Tb in the vegetative (0.66°C) and reproductive (0.84°C) stages. From the vegetative stage to the end of stem harvest, both

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varieties' Tb values were close to 0°C, with the Zafiro variety showing a lower Tb (0.29°C), which means that a single base temperature should not be used in all the phenological stages of these varieties.

Mizuky and Zafiro varieties behaved slightly differently in terms of degree day accumulation for their respective phenological stages. Their thermal requirements were 2606.0 and 2624.2 GDDs, respectively. In general, the average thermal time required to reach flower cut from bud break was 2615.1 GDDs, varying between 185 and 187 days (average of 186 days) depending on the variety.

Author contributions

Study design and conceptualization: LB-R, AP-C, GF. Development of field work: LB-R, AP-C. Provision of data for the study: LB-R. Data analysis: AP-C, LB-R. Writing and correction of preliminary manuscripts: LB-R, AP-C, GF. Reading an editing of final manuscript: LB-R, AP-C, GF.

Conflicts of interest

The authors declare that there is no conflict of interest related to the publication of this article.

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