

## Article

# Analysis of Olive Tree Flowering Behavior Based on Thermal Requirements: A Case Study from the Northern Mediterranean Region

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## Abstract

In recent years, early olive fruit drop has been observed in the northern Mediterranean regions, causing significant economic losses, although the exact cause remains unknown. Recent studies have identified several possible causes; however, our understanding of how olive trees respond to these environmental stresses remains limited. This study includes an analysis of selected meteorological and flowering data for *Olea europaea* L. “Istrska belica” to evaluate the use of a chilling and forcing model for a better understanding of flowering time dynamics under a changing climate. The flowering process is influenced by high diurnal temperature ranges (DTRs) during the pre-flowering period, resulting in earlier flowering. Despite annual fluctuations due to various climatic factors, an increase in DTRs has been observed in recent decades, although the mechanisms by which olive trees respond to high DTRs remain unclear. The chilling requirements are still well met in the region ( $1500 \pm 250$  chilling units), although their total has declined over the years. According to the Chilling Hours Model, chilling units—referred to as chilling hours—represent the number of hours with temperatures between 0 and 7.2 °C, accumulated throughout the winter season. Growing degree hours (GDHs) are strongly correlated with the onset of flowering. These results suggest that global warming is already affecting the synchrony between olive tree phenology and environmental conditions in the northern Mediterranean and may be one of the reasons for the green drop.

**Keywords:** *Olea europaea* L.; phenology; chilling hours; diurnal temperature range; climate change



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## 1. Introduction

Climatic conditions generally play a critical role in tree phenology and adaptation. In the Northern Hemisphere, the seasonal climate of temperate regions has led deciduous trees to adapt by going dormant in winter, during which there is no active growth, low metabolic activity, and high frost tolerance [1]. This dormancy allows fruit trees to survive the cold winter and undergo synchronized bud break when cold accumulation removes dormancy and spring temperatures favor bud growth and development [2]. As an evergreen species, olive trees do not have a true dormancy, i.e., they do not completely stop physiological activity during the winter. A major difference between olive trees and deciduous trees, where reproductive differentiation takes place within the bud before the onset of dormancy,

is that the morphology of olive tree buds remains undifferentiated, i.e., neither vegetative nor reproductive, until the release of dormancy the following year, when bud reproductive induction and initiation are evident [3–5].

Defining dormancy is difficult due to its complexity; however, a nomenclature has been established: endo-dormancy (internal physiological signals such as chilling and photoperiod), para-dormancy (physiological signals from other parts of the plant such as apical dominance), and eco-dormancy (environmental factors such as temperature and water stress) [6]. Temperature is the main factor determining the phenology of the olive tree. It regulates the release from endo-dormancy once sufficient cold units have accumulated during winter (measured as the number of hours with an air temperature below 7.2 °C according to the Chilling Hours Model) and the release from the eco-dormancy period, the duration of which is based on the accumulated forcing units between the end of endo-dormancy and flowering [7].

Current and future global warming could disrupt this complex synchronization between endo-dormancy, eco-dormancy, and environmental conditions, leading to changes in phenological timing, especially flowering [7–9]. An increase in temperature can deteriorate the chilling conditions [10]. According to some authors, the period (from October to February) is when the process is reversible and the buds induced to flower can return to the vegetative state if they are not cold enough or if they lack sugars or substances produced by the leaves [11–13]. Depending on the variety, the cold requirement of flower buds varies, in contrast to vegetative buds, which do not require chilling to sprout [12]. Accordingly, olive trees planted under tropical conditions usually do not produce fruit, which is usually due to the lack of sufficient chilling accumulation [10,14]. Inadequate chilling reduces the number of inflorescences, increases flower abortion, deforms flower buds, and leads to asynchronous bud burst, which ultimately results in a low fruit set and a lower final yield [15–17].

Unsuitable environmental conditions (moisture stress, low or high temperatures, dry winds, fog, or precipitation) during flowering can severely affect pollen quality (pollen viability, embryo sac viability, rapid growth of pollen tubes, and pollen germination), and consequently, fruit set and yield [10]. Previous studies have shown that drought stress during inflorescence development can lead to incomplete flower development [18]. Water deficit during inflorescence development reduced many different flowering parameters, including inflorescence number, flower number, imperfect flower number and percentage, and ovule development. When a water deficit occurs during flowering and initial fruit set, the petals of many flowers dry and abscise as a unit, exposing a senescent stigma that is no longer receptive to pollination. Fruit formation closely follows the parameters of flowering, with a low fruit number when the flower number is reduced or fertilization is inhibited [18]. In addition, flowering and fertilization in non-irrigated olives are highly sensitive to water deficit during the winter months [19]. However, adding water during inflorescence and flower formation, as well as during fertilization and the initial stages of fruit development, can increase the number of fruits per inflorescence and fruit density per shoot [20]. In this context, it should also be noted that olive fruits do not only develop from the normally fertilized ovaries of olive flowers. Olive fruits often appear normal, but the endocarp lacks a seed because the embryo was aborted in the early stages after fertilization [21]. If olive trees are exposed to environmental stress during the early stages of fruit development, fruit drop can occur. Fruit drop is a self-defense mechanism that allows trees to adapt their fruit load to the environmental conditions of a given year [22].

Olives are one of the most important fruit crops in Slovenia. Olive growing is of great socio-economic importance in the southwestern part of Slovenia (Slovenian Istria), which is the main olive-growing area in the country. According to the Slovenian Ministry of Agriculture, Forestry, and Food, this crop is grown in an area of about 2600 ha.

In recent years, a new phenomenon has emerged in the northern Mediterranean growing areas, including Slovenia, northern Croatia, and northern Italy: premature fruit drop in olives, which leads to significant production losses [23,24]. The real cause of the premature drop of green olives in the first stage (BBCH 71–75—fruit size is about 10–50% of the final size) of fruit development is still unknown. Recent studies have identified several possible causes, including infestation by the invasive brown stink beetle (*Halyomorpha halys*) [24], fungal diseases, and climate change [23,25]. However, knowledge of the reactions of olive trees to these environmental stresses remains limited.

Therefore, our research aims to determine whether recent climatic changes in the northern Mediterranean region, particularly reduced winter chilling, influence important phenological processes by analyzing long-term meteorological and flowering data.

## 2. Materials and Methods

### 2.1. Experimental Site

This study was conducted in Slovenian Istria, which, in a geographical sense, is the only indisputably Mediterranean part of Slovenia [26] and covers just over 300 km<sup>2</sup> [27] (Figure 1). The Mediterranean is a highly heterogeneous region with a highly diverse climate, leading to diverse climate types throughout the region due to its unique geographical location [28]. Therefore, the northern Mediterranean regions tend to be temperate and humid, while the southern Mediterranean regions are relatively warm and dry [29,30]. This study was conducted in the coastal area of Slovenian Istria, focusing on the general agri-environmental conditions that characterize olive-growing areas in this Mediterranean region. No specific experimental plantations were selected, but an area analysis of olive groves in the vicinity of the Portorož meteorological station was conducted. In this area, the olive groves share similar cultivation characteristics, including a planting density of approximately 240 trees ha<sup>−1</sup> (tree spacing 7 m × 6 m). Similar agronomic practices have been applied throughout the region, especially in terms of pruning and plant protection, which correspond to the standard practices in Slovenian Istria.



**Figure 1.** Sampling site in Slovenian Istria (dark green) next to Friuli Venezia Giulia (Italy) in the Adriatic region, the northernmost part of the Mediterranean.

Slovenian Istria belongs to the northern Mediterranean region, and its relief and climate are its most pronounced natural features. The relief results from the close alternation of flysch and limestone bedrocks and the low altitude (up to 400 m a.s.l.) near the sea [31]. According to climate data, the area has a sub-Mediterranean climate. It is characterized by high but irregular rainfall, with high precipitation in autumn, at the end of spring, and at the beginning of summer, and low precipitation in winter and summer [32]. The region has a semi-arid climate, characterized by low rainfall and high summer temperatures, which have contributed to increasingly frequent droughts in recent years [33].

During the 1991–2020 period (ARSO—Slovenian Environment Agency, 2023a [34]: data for Portorož station), it was characterized by sunny days (up to 2700 h year<sup>−1</sup>) with monthly mean air temperatures ranging from 4.9 °C in January to 23.4 °C in July. The mean annual precipitation for the period was 958 mm, and the mean annual reference evapotranspiration was 1086 mm, representing the loss of water to the atmosphere through evaporation and the transpiration of a reference plant, usually a well-watered grass.

## 2.2. Olive Variety

The leading olive variety in Slovenia is “Istrska belica”, which currently accounts for 70 % of all olive trees in productive orchards, according to the Register of Agricultural Holdings. “Istrska belica” is also the most widespread olive variety in the northern part of the Adriatic region (Slovenian Istria and Italian Venezia Giulia) [35,36]. Since the 1956 frost, this variety has been known for many positive characteristics (excellent adaptability to pedoclimatic conditions, resistance to low temperatures, very good and regular fertility, and high oil content), which have contributed to its rapid spread [35]. “Istrska belica” is the main variety for two protected designations of origin: “Extra virgin olive oil from Slovenian Istria” (Slovenia) and “Extra virgin olive oil Tergeste P.D.O.” (Italy). The most notable characteristic of this olive oil is its high biophenol content, which gives it a specific taste characterized by bitterness and pungency [33].

Owing to its wide distribution and importance in the northern Adriatic region, the analyses in this study were performed using this variety.

## 2.3. Meteorological Data

The meteorological data for the last thirty-four years (1989–2022), covering the period from January to December of each year, are available for the Portorož meteorological station and were obtained from the Slovenian Environment Agency [37]. The daily and hourly average, minimum (Tmin), and maximum (Tmax) air temperatures at 2 m above the surface were used. The diurnal temperature range (DTR) was calculated as the difference between Tmax and Tmin. Daily precipitation, average temperature, maximum temperature, minimum temperature, average wind speed, relative air humidity, and sun hours were used to evaluate the degree of drought using the standardized precipitation evapotranspiration index calculated with the help of the R package SPEI [38], which uses precipitation and potential evapotranspiration. In this study, we used the SPEI at a 12-month timescale (SPEI-12), which reflects long-term drought conditions by accumulating monthly climatic data over the preceding 12 months. Values were classified into different categories, namely, extremely wet, severely wet, moderately wet, normal climate, moderately dry, severely dry, and extremely dry, according to the literature [39]. It is important to use SPEI-12, as bud differentiation starts in summer and affects the occurrence of inflorescences in the following year. The correlation between the diurnal temperature range (DTR) and the onset of flowering was assessed using the Pearson correlation method.

To assess the conditions that cause frost damage to plants, the average daily maximum (Tmax) and minimum (Tmin) temperatures from 1 November to 1 April were analyzed for all years between 1989 and 2022. For each year, the temperatures in the month before the onset of frost were analyzed because early spring thaws can influence the initiation of physiological processes in plants. In addition, the duration of consecutive days with Tmax below 0 °C was quantified.

## 2.4. Phenological Data

Phenological data (BBCH 60—First flowers open) [40] covering the last 34 years (1989–2022) were used in this work and were obtained from the publicly available database of the Pan European Phenology Project PEP725, which contains data on the main pheno-

logical phases of olive trees for the Portorož meteorological station since 1960. PEP725 is a project funded by the Zentralanstalt für Meteorologie und Geodynamik, the Austrian Federal Ministry for Education, Science, and Research, and EUMETNET (the network of European meteorological services), with the aim of creating a publicly accessible database of phenological datasets for science, research, and education.

## 2.5. Calculations of Chill Units and Growing Degree Hours

Different olive cultivars show variations in their chilling requirements, which must be satisfied to promote bud break, and exhibit different phenological patterns. Geographical features, management practices, and biotic factors also impact physiological development and determine changes in thermal requirements [41]. Given this phenotypic plasticity, the numerical equations used in thermal models must be calibrated for each specific location, and thermal accumulation periods must be optimized for different olive-growing areas [41,42]. To determine the accumulation periods for the chilling and forcing units, we applied a methodology based on the available thermal models and meteorological data.

The first step was to determine the appropriate chilling accumulation periods, taking into account the temperature thresholds and phenological stages of the olive trees, using historical temperature data to determine when the temperatures fell within the required ranges for the chilling and forcing units. Following the statistical approach proposed by Luedeling et al. [42] and refined by Rojo et al. [41], we estimated the chilling and heat accumulation periods for Portorož (Slovenia) using partial least squares (PLS) regression.

Meteorological and phenological data were used as inputs in four different models from the R library. The models were selected based on their suitability. The first was the Chilling Hours Model [43], which accumulates all hours with temperatures between 0 and 7.2 °C [44,45]; the model interval chosen was the most suitable for olives. The second is the Utah Model, developed by Richardson et al. (1974) [43], which calculates chilling accumulation based on hourly temperature thresholds and assigns different weights to the temperature ranges. It assigns an accumulation of 0.5 for temperatures between 1.4 and 2.4 °C and between 9.1 and 12.4 °C, and an accumulation of 1 for temperatures between 2.4 and 9.1 °C. This model assigns a reduction in chilling effectiveness based on a negative accumulation of −0.5 for temperatures between 15.9 and 18 °C and −1 for temperatures above 18 °C [43]. The Dynamic/Chill Portions Model [46] assumes that winter chill accumulation results from a two-step process. First, an intermediate chilling product is generated at lower temperatures, which can be converted into permanent chill accumulation at moderate temperatures or removed at higher temperatures. The model sums the “chill portions” over the course of winter [47]. The last is the Heat Accumulation or growing degree hours (GDHs) model [48], which is based on three temperature thresholds for physiological effects. The critical temperature was set at 36 °C, and the lower and upper thresholds were optimized for olive trees at the study site. The lower threshold was evaluated in the range of 0 to 10 °C, and the upper threshold in the range of 0 to 35 °C. The chilling and heat accumulation periods and models used were described by Rojo et al. [41].

The equations for these models were described in [49] and implemented for R (v1.4.1717; R Core Team), a free software environment for statistical computing and graphics, using the chillR package [41,42]. Reference evapotranspiration was determined using the FAO Penman–Monteith method, which is the standard method recommended by the Food and Agriculture Organization (FAO) for such calculations. The computations were performed using the FAO56 R package version 0.1.0 [50].

### 3. Results and Discussion

#### 3.1. Late Spring Frost

Extraordinary cold air intrusions are an increasingly frequent phenomenon in the northern Mediterranean regions, causing frosts during olive cultivation (1929, 1956, 1985, 1996, 2012, 2018, and 2021) (Table 1). The frequency of winter frost is apparently increasing with global warming, as shown by Žnidaršič et al. [51] for various grapevine, apple, and sweet cherry varieties at locations across Slovenia. In addition to the occasional cold waves, frost in olives is also influenced by warm weather or, better, high temperatures before the frost, which have a favorable effect on the onset of physiological processes in the plant. Analysis of meteorological data ( $T_{min}$  and  $T_{max}$ ) between November and March in years with cold winters in the period from 1989 to 2022 for Slovenian Istria showed that olives were not affected by winter frost in cold winters when  $T_{min}$  was below 0 °C and  $T_{max}$  was above 0 °C. Catastrophic effects on olive leaves, branches, and trunks were mainly observed in years (1985, 1996, 2012, and 2018) when  $T_{max}$  fell below 0 °C for at least two consecutive days after being above 6 °C on average for the previous 14 days.

As highlighted by Petruccelli et al. [52] in a review article, temperatures below 4 °C or around 0 °C markedly compromise plant growth and productivity and cause a delay in flowering. Furthermore, the reproductive organs, flower buds, flowers, and fruit are seriously damaged.

In most regions where olives are cultivated, trees cease vegetative growth in autumn and enter a period of winter dormancy that lasts until the return of favorable temperature conditions in early spring. This dormancy period appears to be essential for increasing frost tolerance, as is the case for many perennial species [53,54], and it coincides with the period when the chilling requirement for flower differentiation in axillary buds is fulfilled [55,56]. Thus, the onset of spring growth often coincides with the mean long-term probability of frost risk in a given region, such that perennial plants are rarely damaged by frost [57]. This synchronization is the result of the complex response of plants to environmental cues, such as chilling duration in winter, which is responsible for triggering dormancy [58] as well as the increasing photoperiod and warmer spring temperatures, which promote bud break (reviewed in [1,59]). Current and future global warming could disrupt this complex synchrony in natural vegetation [45]. The disrupted synchrony between olive phenology and environmental conditions was clearly demonstrated in early April 2021, when the lowest minimum air temperature fell below −2 °C and Central Europe experienced four days of severe frost.

**Table 1.** Frost damage on olives in Slovenian Istria over the last 100 years, with information on  $T_{min}$  and  $T_{max}$ .

Year	Timeframe of Frost	Lowest $T_{max}$ [°C]	Number of Days with the $T_{max}$ Below 0 °C	Lowest $T_{min}$ [°C]	14-Day Average $T_{max}$ [°C] Before the Occurrence of $T_{max}$ Below 0	Damage	Reference
1929	11 February–13 February 1929 Winter frost	/	/	−14.3	/	90% of trees affected by frost	[60]
1956	2 February–20 February 1956 Winter frost	/	/	−12.8	/	30% of trees affected by frost	[60]

Table 1. Cont.

Year	Timeframe of Frost	Lowest $T_{\max}$ [ $^{\circ}\text{C}$ ]	Number of Days with the $T_{\max}$ Below $0^{\circ}\text{C}$	Lowest $T_{\min}$ [ $^{\circ}\text{C}$ ]	14-Day Average $T_{\max}$ [ $^{\circ}\text{C}$ ] Before the Occurrence of $T_{\max}$ Below 0	Damage	Reference
1985	1 January–18 January 1985 Winter frost	−5.6	6	−9.3	7.64	60% of trees affected by frost	[60]
1996	27 December–29 December 1996 Winter frost	−4.6	3	−8.5	10.52	Young trees completely damaged	[61]
2012	1 February–12 February 2012 Winter frost	−2.4	4	−5.9	6.79	10% of trees affected by frost	[62]
2018	25 February–1 March 2018 Winter frost	−3.2	2	−5.7	9.2	30% of trees affected by frost	[62]
2021	4 April–9 April 2021 Spring frost	10.8	0	−2.3	/	More than 50% reduced yield	[30]

A sudden cold snap in April 2021 damaged numerous crops at a crucial time in their development, including olive trees, especially in the northern Mediterranean, where an anomalously warm March shortened winter dormancy and hastened the vegetative growing season. Our research showed that the damaging frost in April 2021 was unprecedented after 1929, when 90% of olive trees in the Gulf of Trieste, Slovenian Istria, and eastern Friuli were destroyed by the winter frost. The damaging April 2021 frost was unprecedented in the northern part of the Mediterranean in terms of the calendar date (late spring frosts) and the type of damage to olive trees over the last 93 years. Since 1929, destructive frosts affecting olive trees have been recorded in December (1996), January (1985), February (1929, 1956, and 2012), and March (2018); however, 2021 was the first time in which a frost was recorded in April. Therefore, the typical symptoms used to assess the extent of frost damage, such as burns on shoot tips, complete defoliation, or cracks in the bark of branches or the trunk, as reported by Sanzani et al. [63], were not observed. In 2021, an abnormally premature fall was observed in green olives. This is one of the main reasons for the drastic decline in olive production in the northern Mediterranean region. Similar consequences occurred in California after the cold spring of 1967, which delayed flowering by several weeks and resulted in blossom abnormalities and a crop yield of only 14,000 tons, the smallest in California's modern history. The prolonged, unusually cold weather in April and May, when olive tree buds develop rapidly, negatively affected the later stages of flowering, pollination, and fruit set [64].

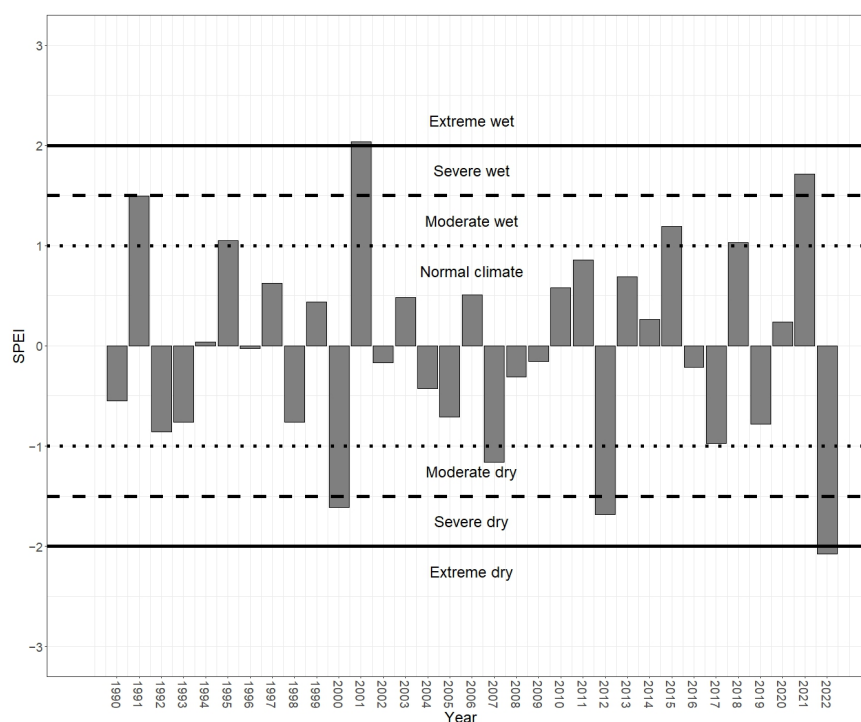
### 3.2. Spring Drought

Studies from the 1980s indicated that drought was not a significant threat to olive production in the northern parts of the Mediterranean [65], but SPEI-12 of Vicente-Serrano et al. [39] showed that severe droughts occur in the region approximately once per decade (Figure 2).

Notably, the damage caused by drought stress cannot be attributed solely to a lack of rainfall, as several factors must be considered, such as the growth stage of the plants,

soil properties, resistance of the variety, and other variables. Owing to the interactions of various factors, olive growers in the Slovenian Mediterranean region have faced nine highly severe droughts over the last decade (2011, 2012, 2013, 2015, 2016, 2017, 2019, 2021, and 2022). According to the Joint Research Center of the European Commission (EC-JRC), the extreme drought of 2022 was the most severe in the last 30 years and unprecedented in the last 500 years in this region [66]. This was most likely the result of a precipitation deficit between winter and spring, which was exacerbated by early heat waves. For example, the experimental site received only 138 mm of precipitation between January and May 2022, which is a significant deviation from the long-term average of 310 mm (1991–2020). The decrease in soil water availability caused by this precipitation deficit and concurrent high temperatures resulted in widespread vegetation stress during olive flowering, which persisted until the stone fruits were fully hardened.

Olive-growing areas in the northern Mediterranean are expanding due to climate change, especially in the flat and mountainous areas of northern Italy and western Slovenia [67,68]. Further research on premature fruit drop will provide vital information for adapting to sustainable and economically viable olive production under changing climatic conditions.

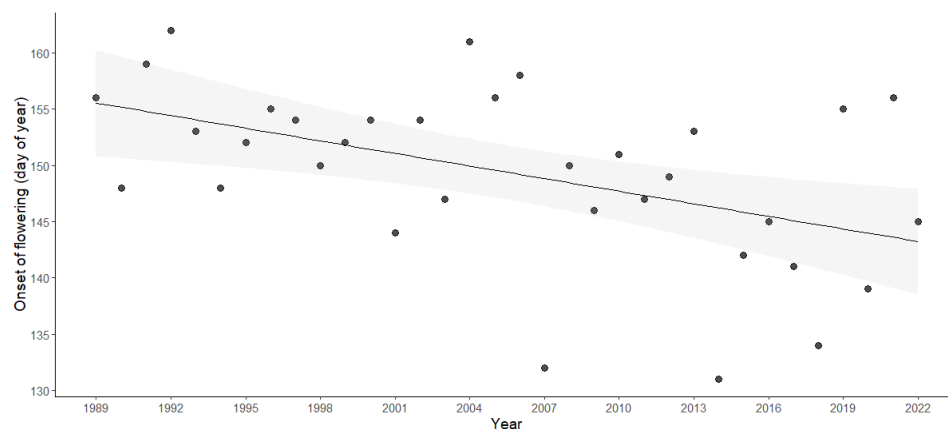


**Figure 2.** The standardized precipitation evapotranspiration index at a 12-month scale (SPEI-12) was evaluated for Portorož between 1990 and 2022.

### 3.3. Changes in the Flowering of Olive Trees and Diurnal Temperature Range

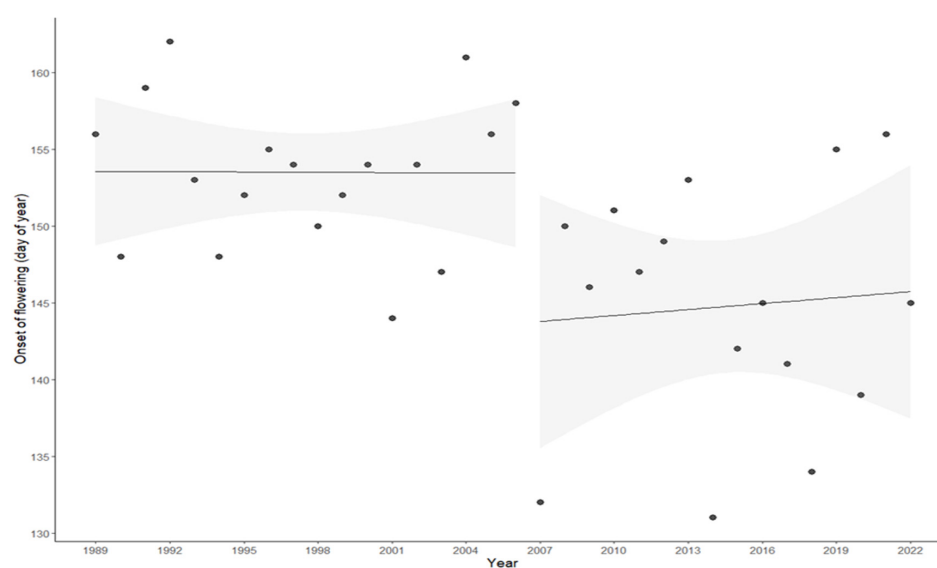
Analysis of the phenological data shows that the onset of flowering in Slovenian Istria occurs on average on May 29 (DOY  $150 \pm 8$ , mean  $\pm$  standard deviation), which is later than in warmer climates such as Tunisia (Chaal) (April 18—DOY  $108 \pm 7$ ), southern Italy (Lecce) (May 14—DOY  $134 \pm 5$ ), and central Spain (Toledo) (May 15—DOY  $135 \pm 8$ ), as reported by [31]. However, the Slovenian Environment Agency has reported that warmer-than-average years have become highly frequent since the mid-1980s, and most of the warmest years since the middle of the last century have occurred recently. In Slovenia, the mean annual temperature increased significantly by about  $0.36^\circ\text{C}$  per decade from 1961 to 2019 [69]. As temperature is one of the most important factors in the flowering phenology of olive trees, we investigated how the timing of flowering initiation

has changed over the last three decades (Figure 3). The results emphasize a trend that has persisted throughout the study period, according to which the onset of flowering (i.e., the timing of blooming) has occurred earlier over time.



**Figure 3.** The occurrence of the onset of olive flowering and its trend in Portorož in the period 1989 to 2022.

To detect changes in trends during the study period, we applied three complementary statistical methods: breakpoint analysis using linear regression with structural change detection (strucchange package) [70,71], Pettitt's test for identifying a single change-point in the time series (trend package) [72], and the Standard Normal Homogeneity Test (SNHT) for detecting shifts in the mean (trend package) [72]. All three methods consistently identified 2006 as the primary breakpoint, indicating a significant change in the trend that began in that year (Figure 4). In the first period (1989–2005), flowering began on average on DOY 153.2, 7.7 days later than in the second period (2006–2022), in which flowering began on DOY 145.5. In addition, the second period showed much greater variability, as demonstrated by the increase in the standard deviation from 4.89 in the first period to 8.21 in the second. This increased variability contributes to greater uncertainty and instability in olive cultivation. This is supported by other studies that have found that the timing of flowering onset has shifted in recent decades because of rising air temperatures [73–75].

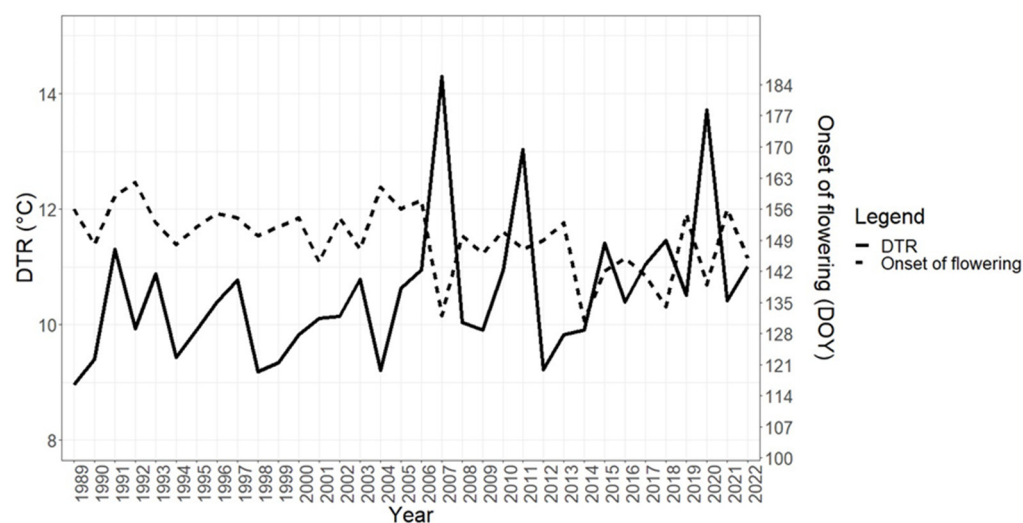


**Figure 4.** Olive flowering onset trend in Portorož in the most recent decade of measurements (2006–2022) compared with the previous decade (1989–2005). The black dots represent each data point, the trend is represented with a regression line and the grey area represent the 95 % confidence interval.

However, the effects of climate change on shifts in flowering times are complex. DTRs have detrimental effects on many morphological and physiological characteristics of plants. They exponentially increase forward and reverse biochemical reactions, resulting in enzyme denaturation. Plants evolve resilient mechanisms to survive under temperature stress by limiting the adverse effects on metabolic and physiological processes [76]. Considering that DTRs play a key role in aspects of plant development and that little information is available on their effects on olive trees, although a 28-year study has revealed remarkable climatic trends affecting phenological phases [77], we analyzed the meteorological data to determine whether olive groves in the northern Mediterranean region are exposed to high DTRs.

The analysis of meteorological data for all months showed that the highest DTRs occurred in April between 1989 and 2022. Despite year-to-year variations due to various climatic factors, a steady increase in the DTR values with higher variability has been observed since 2006. Mann-Whitney U test revealed a statistically significant DTR divergence ( $p < 0.001$ ) in April when we compared the data from 1989 to 2005 with the data from 2006 to 2022. The April DTR averages for the first and second periods were 10.1 °C and 11.1 °C, respectively.

The influence of the average monthly DTRs on the onset was also observed. A moderate to strong statistically significant negative correlation was found between the average monthly DTR values during the pre-flowering period and the onset of flowering ( $p < 0.001$ ) (Figure 5). Lower DTRs in the month before flowering may result in a delayed onset of flowering. However, the higher April DTRs observed after 2006 may have resulted in earlier flowering.



**Figure 5.** The onset of olive flowering and average monthly diurnal temperature range in April in Portorož from 1989 to 2022.

These findings align with those of a similar study in central Italy [78], which showed that temperature changes significantly affect the spring phenology of olive trees, which respond to rising temperatures with earlier flowering. Olive trees adjusted the Growing Degree Days (GDDs) required for flowering once a threshold of maximum advancement was reached. Before 2004, the trees accumulated similar GDD values, but as temperatures increased, earlier flowering occurred at fixed GDD values of 650 and 750. After this point, higher GDD values accumulated, which stabilized the flowering date and prevented further advancement. This phenological plasticity enables olive trees to adjust their life cycle and reproductive stages in response to environmental changes. This helps them survive under changing conditions and prevents excessively early flowering [78].

However, other factors may influence the onset of flowering, such as the amount of light, soil water availability [33,79], altitude, exposition [79], nutrients, and the genetic makeup of the plant [80]. DTR plays a decisive role in the onset of flowering. In most parts of Slovenia, the strongest warming can be observed in the spring and summer, at about 0.4 and 0.5 °C per decade, respectively, with warm climate regions warming more than cold ones [81]. The increasingly frequent phenomenon of shorter winter dormancy caused by anomalously warm conditions could lead to an earlier start of the vegetative growth phase. In addition, changes in atmospheric circulation patterns also contribute to extreme temperature trends. Cold air masses with temperatures below freezing can move from northern Europe to the northern Mediterranean region in spring [81]. The combination of warmer winters and earlier springs, interrupted by cold air intrusions and sudden cold snaps in late spring in the northern Mediterranean, leads to high DTR values in late spring, which can delay the start of flowering. These temperature fluctuations can influence the onset of flowering. Therefore, it is important to consider the DTR along with other factors, such as water availability, to gain a comprehensive understanding of its effects on the onset of olive flowering.

### 3.4. Cold (Chilling) and Heat (Forcing) Requirements

#### 3.4.1. Estimation of the Thermal Accumulation Period

The PLS regression analysis revealed the effect of daily temperature on the onset of the flowering period, and the most influential days were highlighted using a variable importance in projection (VIP) value of 0.7. VIP quantifies the importance of independent variables in explaining the variation in dependent variables. In this study, we examined the importance of temperature on flowering date.

According to the data presented in Appendix A (Figure A1), the critical period for chilling accumulation was between October 4 and November 15, about, one month earlier than in the southern (Lecce, Italy, and Chaal, Tunisia) and central Mediterranean (Toledo, Spain) (between late autumn and early winter) [41]. The heat accumulation period was between November 15 and May 10, which is longer than that in the southern Mediterranean (Lecce, Italy, and Chaal, Tunisia) and similar to that in the central Mediterranean (Toledo, Spain).

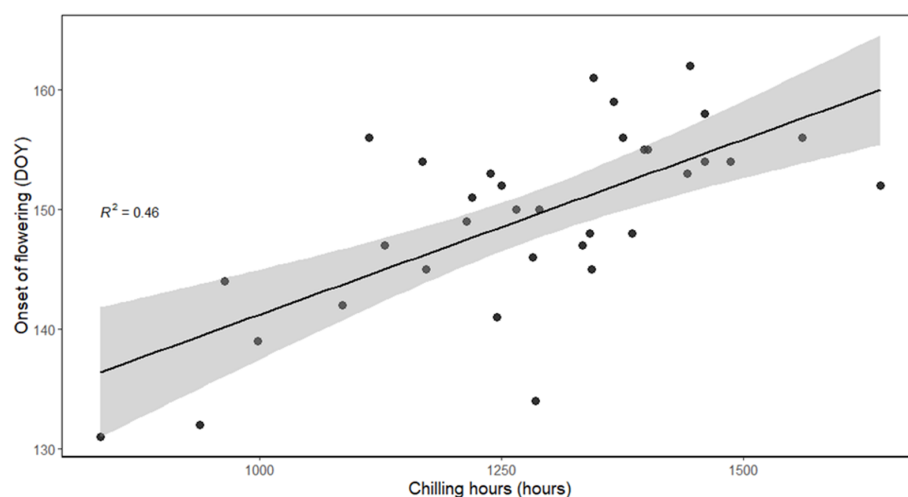
This might be explained by the impact of the continental climate, which, according to Rojo et al. [41], establishes the future conditions necessary for inducing ecodormancy.

These findings are also supported by data from El Yaacoubi et al. [82] and Gabaldón-Leal et al. [45], suggesting that heat accumulation is more important than chilling requirements in olive flowering, and that the timing of flowering is closely related to heat accumulation. Further analysis of the data obtained using the chilling and forcing models revealed a strong statistically significant negative correlation between the growing degree hours and chilling (Appendix B, Figure A2). Although it is not known whether the negative correlation between chilling and heat accumulation is correctly explained by these ecosystem models, Wang et al. [83] showed that more than half (seven out of 12) of the current chilling models are invalid, as they have a positive relationship between chilling and heat accumulation because they do not account for the effects of freezing temperatures on the release of dormancy. Freezing temperatures (<0 °C) can slow down or interrupt the release of dormancy. If this is not taken into account, the models may incorrectly record the chilling during ineffective periods, leading to falsified results.

#### 3.4.2. Models for Calculating Chilling Requirements Using Different Chilling Models

The difference between the northern and southern and central Mediterranean regions was also noted in terms of chilling requirements. Figure 6 and Appendices C

(Figure A3) and D (Figure A4) illustrate the data from the three models used to calculate chilling requirements. Although Omran et al. [84] reported that the Chilling Hours Model for conditions in Egypt does not reflect the number of actual chilling units and requires more complicated mathematical methods, the results of our study showed that this model was the most appropriate for the conditions in this study compared with the others tested (the Utah Model and Chill Portions Model). A medium statistically significant positive correlation was found between chilling hours and the onset of flowering (Pearson's  $r = 0.680$ ,  $p < 0.001$ ).

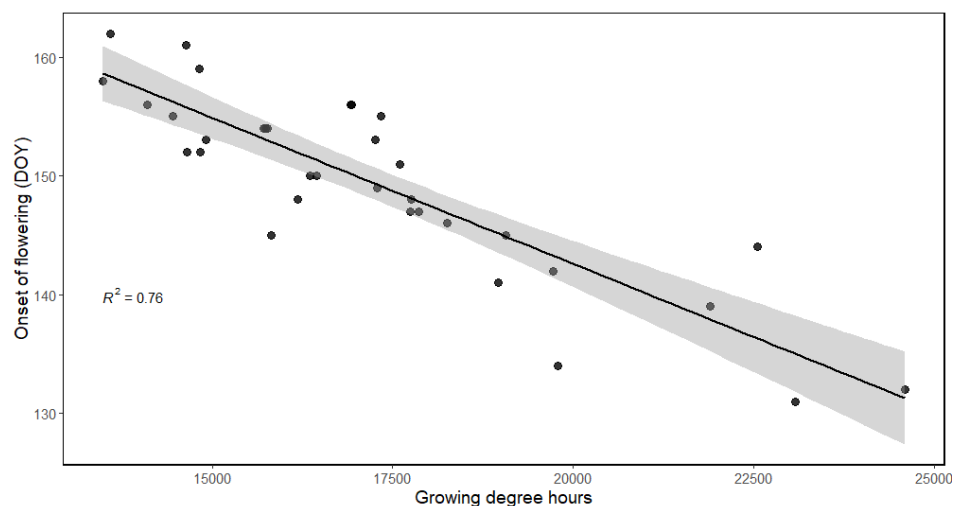


**Figure 6.** The onset of olive flowering vs. chilling hours calculated using the Chilling Hours Model for the 1989–2022 period in Portorož. The black dots represent the each data point, the trend is represented with a regression line and the grey area represent the 95 % confidence interval.

The average chilling accumulation at the Portorož station was  $1588 \pm 235$  chilling units, calculated according to the Chilling Hours Model. As most olive cultivars have a chilling requirement of 200–600 chilling units [85–87], global warming will not lead to losses in olive production in the northern Mediterranean region. However, given this trend, the replacement of cultivars with high chilling requirements must be considered. These results are partially consistent with those of De Melo-Abreu et al. [85], who reported that global warming could lead to significant losses in olive production in Córdoba and similar areas and/or force the replacement of some varieties with high chilling requirements. Strong warming in the winter slows the fulfillment of chilling requirements, which may delay spring phenology [88]. In this context, it is necessary to conduct studies to predict the spread of olive cultivation in new regions using models that consider topographic and climatic factors such as slope, soil type, solar radiation, temperature, and precipitation. By better understanding how these factors interact with olives, we can develop effective strategies to mitigate the negative effects of climate change on olive production and ensure the long-term sustainability of the olive industry.

### 3.4.3. Model for Calculating Growing Degree Hours (GDHs)

The Heat Accumulation Model results showed a strong statistically significant negative correlation ( $p < 0.001$ ) between the growing degree hours (GDHs) and day of onset of flowering (Figure 7). This indicates that higher heat accumulation (higher GDHs) is associated with an earlier onset of flowering. These data are consistent with those of a study in which chilling and heat accumulation times were estimated based on the timing of olive pollination [41]. The average heat accumulation in Portorož was  $17.132 \pm 2.760$  growing degree hours.



**Figure 7.** The onset of olive flowering vs. heat accumulation (growing degree hours), calculated using the optimum threshold (15 Nov–10 May) for the 1989–2022 period in Portorož. The black dots represent each data point, the trend is represented with a regression line and the grey area represent the 95 % confidence interval.

Although the general trend indicates an increase in the growing degree hours, there is considerable variability in the data. GDH values [41] were lower in recent years, as there was also a late onset of flowering, which could be explained by early high temperatures and cold-air intrusions from the north. Temperatures in previous months cause significant changes in phenological events, and plants are more sensitive to spring temperatures when their heat requirements are fulfilled [89]. These data suggest that the northern Mediterranean regions have problems with occasional cold air intrusions arriving from the north in the spring months before flowering, which affect the course of flowering and further fruit development.

#### 4. Conclusions

The results of this study suggest that global warming is already affecting the synchrony between the phenology of the olive, *Olea europaea* L., and environmental conditions in the northern Mediterranean.

A trend toward earlier flowering and intermittent cold air intrusions during the spring months disrupts the normal flowering process, which is furthermore affected by high diurnal temperature ranges (DTRs) during the period before flowering. An increase in DTRs has been observed in recent decades, and further research is needed to understand the stress response mechanisms of olives to high-temperature fluctuations.

The chilling requirements are still being met in the region ( $1500 \pm 250$  chilling units), although the total number of chilling units has decreased over the years. There is also a strong correlation between the growing degree hours (GDHs) and the onset of flowering, with the results showing that an increase in growing degree hours (GDH) is associated with the earlier onset of olive flowering.

We also determined that winter frost occurred mainly when the  $T_{max}$  fell below  $0^{\circ}\text{C}$  for at least two consecutive days after it had been, on average, above  $6^{\circ}\text{C}$  for 14 days. In addition, the occurrence of spring frosts when  $T_{max}$  does not fall below  $0^{\circ}\text{C}$  can lead to incomplete fertilization, resulting in seed damage and subsequent fruit drop.

The climate change effects described here are interrelated, and several factors may amplify their effects on olive grove ecosystems in the northern Mediterranean. These results highlight the urgent need for further research on other factors that influence flowering dynamics, such as heat waves, warm winds, air mass circulations, and others in combination with climate

change scenarios to prepare projections for the future. Sustainable management practices, conservation measures, and adaptation strategies are essential for mitigating the effects of climate change and ensuring the long-term resilience of olive grove ecosystems.

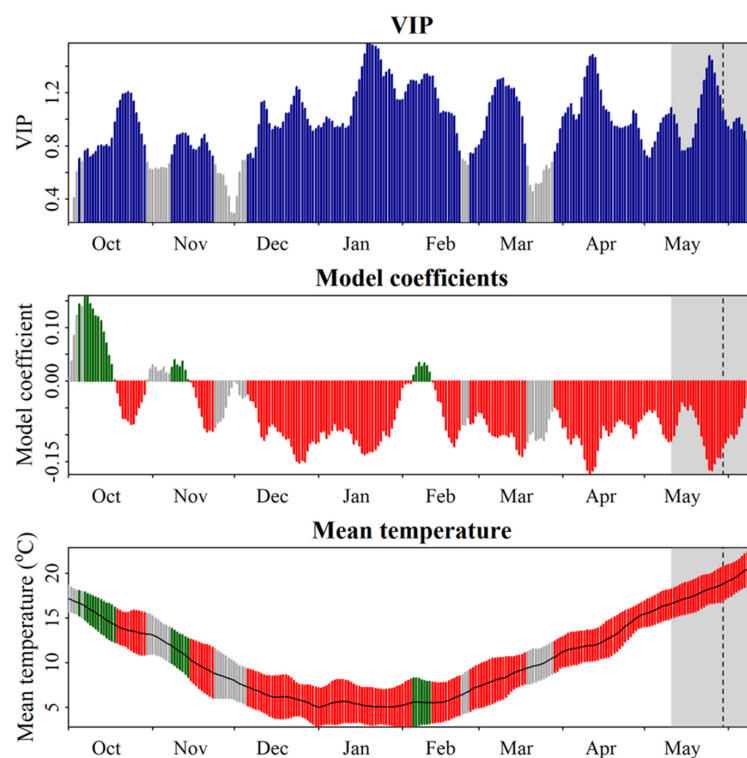
**Author Contributions:** Conceptualization, M.P. and J.F.; Methodology, M.P.; Software, J.F.; Validation, T.P., V.Z., M.P. and J.F.; Formal Analysis, J.F. and M.P.; Investigation, T.P., V.Z., M.P. and J.F.; Resources, T.P., V.Z., M.P. and J.F.; Data Curation, T.P., V.Z., M.P. and J.F.; Writing—Original Draft Preparation, M.P. and J.F.; Writing—Review and Editing, T.P. and V.Z.; Visualization, T.P., V.Z., M.P. and J.F.; Supervision, M.P.; Project Administration, M.P.; Funding Acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Phenological data were obtained from the publicly available database of the Pan European Phenology Project PEP725. The meteorological data for the last thirty-four years (1989–2022), covering the period from January to December of each year, are available for the Portorož meteorological station and were obtained from the Slovenian Environment Agency.

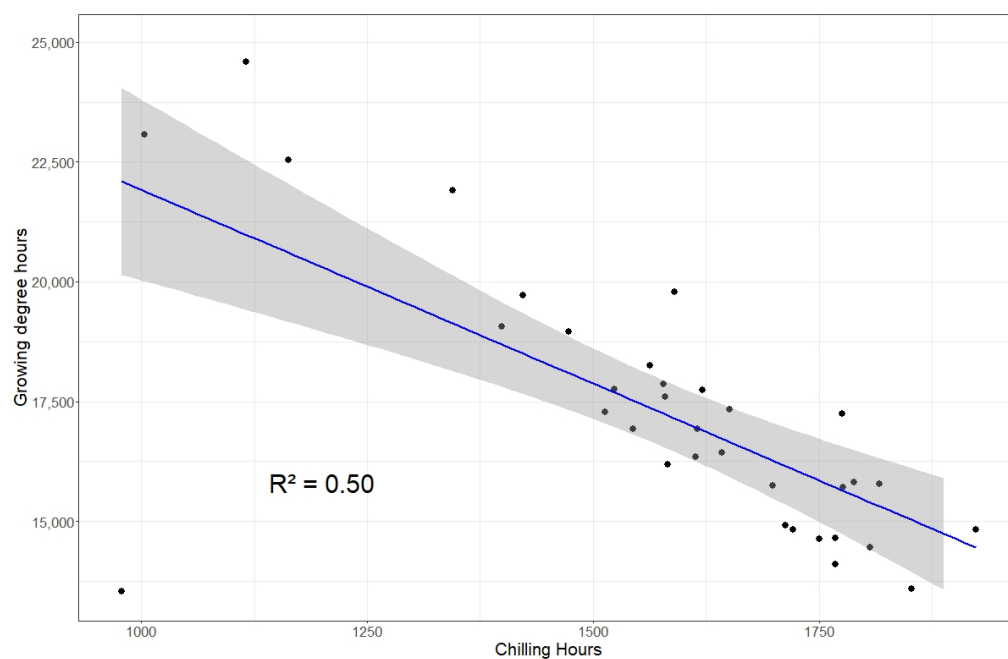
**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of this study; in the collection, analysis, or interpretation of the data; in writing the manuscript; or in the decision to publish the results.

## Appendix A



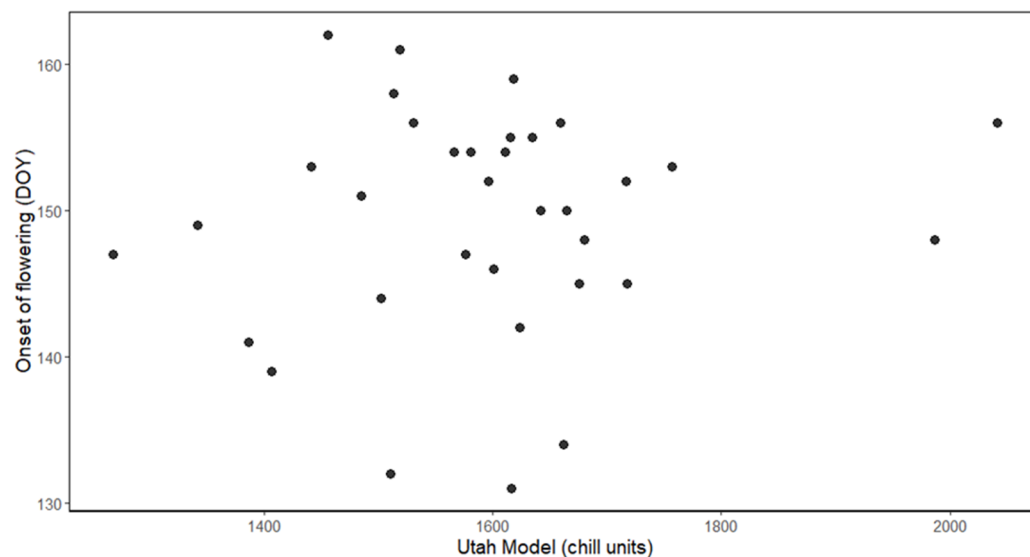
**Figure A1.** Estimation of the chilling and heat accumulation periods according to thermal requirements in Portorož from 1989 to 2022. Green indicates that daily temperatures were positively correlated with flowering dates. Red indicates when the temperatures were negatively correlated with flowering dates. The gray area represents the range (min–max) of the onset of flowering during the study periods. VIP quantifies the importance of temperature in explaining the variation in flowering date.

## Appendix B



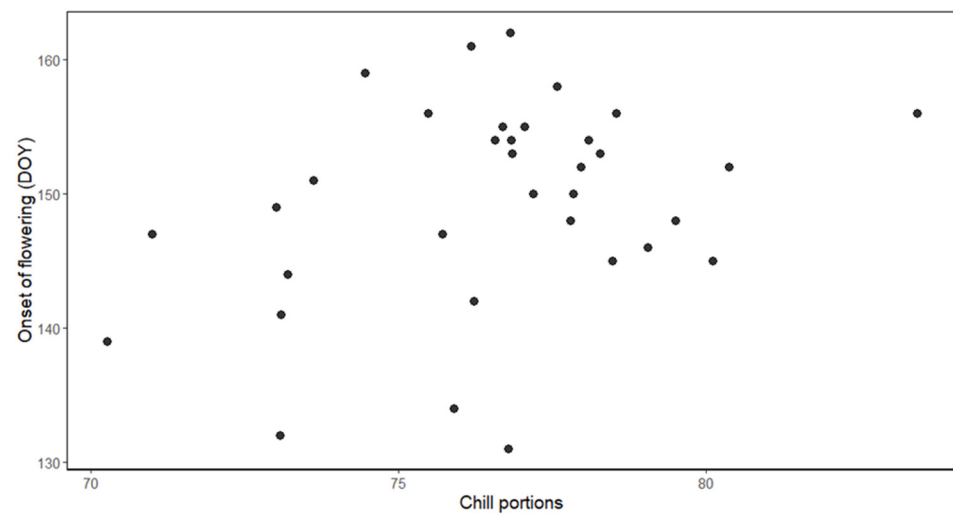
**Figure A2.** Pearson's correlation between chilling (chilling hours) and heat accumulation (growing degree hours). The black dots represent each data point, the trend is represented with a regression line and the grey area represent the 95% confidence interval.

## Appendix C



**Figure A3.** The onset of olive flowering vs. chill units calculated using the Utah Model for the 1989–2022 period in Portorož. The black dots represent each data point, the trend is represented with a regression line and the grey area represent the 95% confidence interval.

## Appendix D



**Figure A4.** The onset of olive flowering vs. chill units calculated using the Chilling Portions Model for the 1989–2022 period in Portorož. The black dots represent each data point.

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