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Low spring temperature may negatively influence olive yield

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Abstract
The olive tree (*Olea europaea* L.), a species adapted to the Mediterranean climate, has expanded into new climatic regions. Uruguay has a humid temperate climate and highly irregular climatic conditions among years. Environmental factors can be an obstacle to full production, as they affect pollination and fruit set. In this research, the phenology of five olive cultivars (Arbequina, Arbosana, Manzanilla, Picual and Koroneiki) widely cultivated in Uruguay was studied, using the BBCH phenological scale. The pollen grains of five cultivars were submitted to in vitro germination and incubated at temperatures of 0°, 10°, 15°, 20°, 25°, 30° and 40°C for 24 hours, and pollen grains germination and pollen tube length were evaluated. These temperatures were analyzed for the occurrence probability. They were based on the historical series analysis of hourly average temperature data, from September 26 to November 15 (flowering period), for the years of 1998 to 2019. Phenology results show that flowering can occur from day 268 to day 320 of the year, with cultivar 'Manzanilla' being the earliest and 'Arbosana' the latest. It was observed that the temperature of 10°C has no effect on pollen grain germination and on pollen tube growth. Moreover, the temperatures of 15 to 20°C are not very effective and the optimal temperature occurs between 25 and 30°C. In Uruguay, during flowering, temperatures between 10 and 20°C are more likely to occur. These results indicate possible causes that affect fruit set and productivity of olive trees in the field.

Keywords: Arbequina, fruit set, *Olea europaea* L., polinization, pollen germination.

Introduction

Climatic factors act directly on cultivated species development, determining their phenology and production. Most of human diet comes from plants' sexual reproduction, for example flower fertilization. Climate factors, especially temperature, plays an important role in phenological phases development of many species, where a change in environment temperature can negatively affect flower bud development and, consequently, flowering period (Aguilera et al., 2014). The effect of high and low temperatures is more sensitive in the reproductive phase, in female and male flower organs, during and after pollination. Plants' responses at this stage may vary with temperature stress intensity, as well as exposure time and species under study (Hedhly 2011).

The olive tree (*Olea europaea* L.) is a species traditionally cultivated in the Mediterranean, whose climate is a transition between temperate and tropical. This climate is characterized by a wet winter (65% of rains occur between November and December) and very dry summers, with an annual rainfall average of 400 to 900mm. Winter temperatures are mild, ranging from 6° to 13°C in the coldest month. In summer months, high temperatures are seeing with ranging averages between 25° and 28°C. In the Mediterranean region, the increase in extreme climatic events frequency during specific stages of plant development, such as high temperatures during flowering period, reduces fruiting and yields in some crops (Moriondo et al., 2008; Vuletin Selak et al., 2013).

Olive tree cultivation has expanded to new regions with similar Mediterranean climate, such as South Africa, the USA, Australia, Argentina and Chile, and also to other regions with very different climate, such as China, Brazil and Uruguay, which have different temperature and precipitation regimes (Herrera-Cáceres et al., 2017; Torres et al., 2017). Uruguay is a country located in South America, and olive trees cultivation in this country occupies an area of approximately 10.000 hectares (Conde-Innamorato et al., 2019). The climate is characterized as temperate-humid, with rainfall above 1,100 mm per year and an average annual temperature of 17.7 °C. However, historical data shows a wide variability among years, with annual maximum and minimum averages of 22.6°C and 12.9°C, respectively (Castaño et al., 2011).

Previous studies have demonstrated the relationship between temperature and of olive pollen grains germination. The appropriate temperature range for germination occurs between 20 – 30°C. However, this response varies depending on the cultivar (Vuletin Selak et al., 2013). Many studies demonstrate how high temperatures, especially above 40°C, inhibit or negatively affect many aspects of flower pollination and fertilization. Besides thatr, there is little information on pollen germination response under climatic conditions different from those found in the Mediterranean, especially under lower temperatures (Koubouris et al., 2009).

There are limiting factors at the time of fruit set in our conditions leading to problems such as high rates of alternate bearing (Conde-Innamorato et al., 2019) Therefore, the objectives of this research were: 1) to evaluate the phenology of five olive cultivars; 2) to study the effect of temperature on pollen grain germination and pollen tube growth '*in vitro*' of Arbequina, Arbosana, Koroneiki, Picual and Manzanilla cultivars , and; 3) to analyze the probability of temperature ranges from a historical data series during the period 1998 to 2019 registered at INIA Las Brujas, Uruguay.

Results

Phenological stages

The phenological records of Koronieki and Arbosana for 9 years permitted recognizing a flowering period.The difference between the earliest date of flowering onset of and the latest date of the petal drop stage was 35 of 35 for Koroneiki and 34 days for Arbosana. There were variations on the dates of flowering onset (BBCH 61), full flowering (BBCH 65) and petal drop (BBCH 68), as shown in Figure 1. It was verified that Manzanilla started flowering before the other cultivars, and its earliest date was on the 268th day of the year (DOY) eight days before the Arbequina cultivar. However, the phase of full flowering and petal drop is similar to other cultivars. Arbequina has a flowering window that can occur from day 276 (DOY) today 315 (DOY), and the full flowering stage is smaller compared to other cultivars, totaling 21 days. Petals drop from day 307 (DOY), six days before Manzanilla and Koroneiki.

The beginning of the full flowering period in Picual occurred concomitantly with the Arbequina', Arbosana and Koroneiki cultivars; however, this period can extend from day 283(DOY) today 316 (DOY). Cultivar Picual is the last to exceed the period of full flowering, and its occurrence window for this period is 30 days, the same number of days found in Manzanilla, for the same period. Arbosana is the last to start the flowering period, only on day 286 (DOY), while Arbequina and Manzanilla can start on days 276 (DOY) and 268 (DOY), respectively. Its full flowering period coincides with that of other cultivars, but the petal drop period is longer and can occur from day 313(DOY) today 320 (DOY). The Koroneiki is the second last cultivar to enter flowering, starting from day 282 (DOY). However, the period of full flowering occurs concomitantly with the other cultivars, being able to occur from day 286 (DOY) today 313 (DOY).

From the minimum and maximum temperature data, during the whole flowering period, it was observed that there was a variation between 7 - 15ºC for the minimum temperature and 18 - 25ºC for the maximum temperature. During the initial phase of the flower opening and full flowering period, the minimum temperatures were approximately 10ºC. The maximum temperature verified at the full flowering stage was close to 20ºC, not reaching 25ºC for all cultivars under study (Fig 1).

Pollen germination and tube growth

The germination percentage and pollen grain length varied among the analyzed cultivars (Table I). It was found that Picual had a 9% lower percentage of pollen grain germination (20.56%) differing statistically from Koroneiki (29.56%) and Arbequina (29.22%) cultivars, and 8% lower compared to Arbosana (28.44%). For the pollen tube length variable, Koroneiki, Arbosana, Arbequina and Manzanilla cultivars did not present statistically significant differences, with values of 116.93; 117.59; 98.26 and 101.62 µm, respectively. There was a statistical difference for Picual, which differed from Arbosana (83.86 and 117.59 µm respectively) (Table 1).

The temperature interfered in germination and pollen tube growth, corresponding to a quadratic fit of R^2 of 0.8973 and 0.9846, respectively. The maximum point of germination, calculated through the curve equation, was obtained at the temperature of 26.66°C, where germination reached 43.38%. In treatments corresponding to the temperatures of 15°C and 20 °C, 21.69% and 36.29% germination was obtained, respectively. For the treatment at a temperature of 40°C, the germination percentage was only 15% and for a temperature of 10°C, there was no germination (Fig 2A).

The maximum pollen tube growth occurred at the temperature of 28.22 °C reaching 133.44 µm. The pollen tube length was significantly longer at 25 °C and 30 °C when compared totemperatures of 15 °C and 40 °C, which did not exceed 80 µm. At 20 ºC, the values were intermediate, and no statistical difference was verified. At the temperature of 10 ºC, it was not possible to estimate the pollen tube growth, hence there was no germination (Fig 2B).

Probability of temperatures at flowering

When analyzing temperature occurrence probability during flowering period in Uruguay and considering bands of 5 °C ranging from 0 to 40 °C (Fig. 2), there is a greater occurrence probability of temperatures between 10 – 15 °C and 15 - 20° C (approximately 30 and 35%, respectively). However, extreme temperatures below 5 ºC and above 30 ºC are rare in the studied environmental conditions. The range of 5 - 10°C can be verified with a probability of about 10%, and the same is true for the optimal temperature range for germination of 25 - 30°C.

Discussion

Olive growth expansion to new climatic regions such as Uruguay, where climate is classified as humid temperate, creates new challenges for the culture advancement and physiological processes knowledge. Further studies in genetic and physiological responses are required in order to obtain a clearer answer on the adaptation of olive trees to this new condition.

Cross-pollination is the main method of pollination in olive trees, and a careful orchard design plan is necessary for an efficient pollination and a fruit set among olive cultivars (Mookerjee et al., 2005; Selak et al., 2021).A flowering phenology evaluation of the five cultivars showed a blossoming window which permitted advanced strategies for planting combinations using cultivars that have phenological synchronization at the full flowering stage. When evaluating the earliest entry dates during flowering period of Manzanilla cultivar, it showed that there was not a pollinator cultivar during this period (268 at 276). Earlier and later dates of flowering entry may be related to chilling accumulation during winter, and they also determined the

Table 1. Pollen grain germination (%) and pollen tube length (µm) of five olive cultivars observed at INIA. Las Brujas- Uruguay, 2019.

Cultivar	Pollen germination (%)	Pollen tube length (µm)
Koroneiki	29.56 a*	116.93 ab
Arbosana	28.44 ab	117.59 a
Arbequina	29.22 ab	98.26 ab
Manzanilla	22.79 bc	101.62 ab
Picual	20.56 c	83.86 b
p value	< 0.001	0.031

* Means separated by different letters within columns are significantly different by Tukey's Test (p < 0.05).

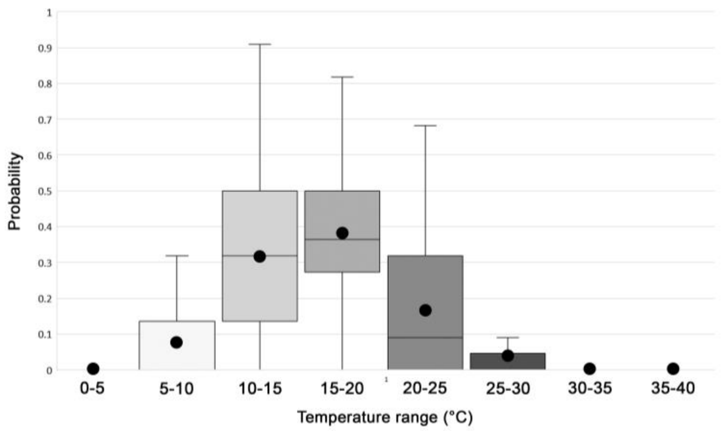

Fig 1. Average of maximum and minimum temperatures (°C) from 1998 - 2019 historical series in Las Brujas- Uruguay and duration of BBCH 61, BBCH 65 and BBCH 68 stages corresponding to the beginning of flowering, full flowering and petal drop, respectively, for each cultivar.

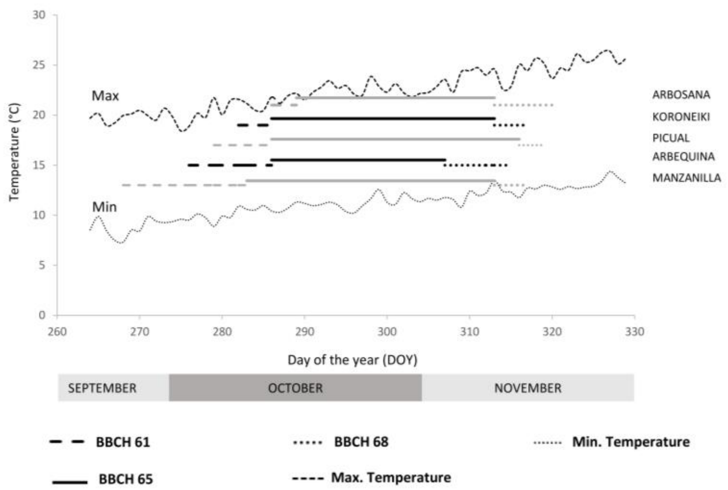

Fig 2. Pollen grain germination in percentage (A) and pollen tube length in µm (B), in response to the increase of temperature. Vertical bars indicate standard error (n = 4).

phenological events (Gordo and Sanz, 2010). For Picual cultivar, which presents the longest period of full flowering, it was identified that, at the end of this period, there was no synchronization with the other cultivars.

For Manzanilla, Arbosana and Picual cultivars, which showed extreme dates in the flowering period, the pollen grains pressure from other cultivars in the atmosphere may not be sufficient to complete flower fertilization. Besides that, a large distance between plant, a small orchard with few cultivars, are factors that contribute to a low fruit set. Studies carried out by Lavee and Datt (1978) indicated that there is an increase in pollen grain germination of olive cultivars when tissue from the stigma of Manzanilla cultivar was added to the culture medium. Selak et al. (2021) concluded that cross-pollination is more often observed preferential in Oblica cultivar, and flower fertilization does not always occur by the cultivar in greater number or the closer one. According to the research, flower large overlap relationship and cross-compatibility represent successful fertilization.

The flowering window of the studied olive cultivars comprises a period of 52 days. This means that during the years of this study, the flowering period occurred on some of these days (268 – 320 DOY). The changes in the flowering date and length of this period is due to the year-to-year variation of temperatures and precipitation that occurs in Uruguay. According to Benlloch-González et al. (2018), a 4°C increase in temperature during the winter is enough to delay the flowering date and extend this period.

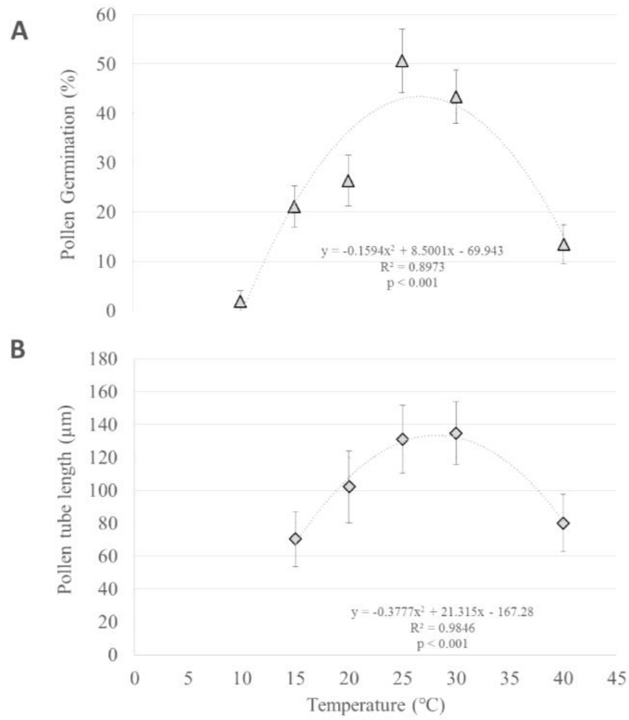

Fig 3: Probability of temperatures occurrence between 0 - 40°C, obtained at INIA- Las Brujas, Uruguay, based on the hourly average temperature for the period of 1998 - 2019, from day 270 to day 320 corresponding to the olive tree's flowering window. The boxes represent the data set for each temperature range, and the mean data point and the bar inside the box show the average. The sprays above and below represent the maximum and minimum values for each temperature range. The points above represent outliers.

The percentage of pollen grain germination of the cultivars ranged from 20.56% to 29.56%, for Picual and Koroneiki, respectively. Thes f values were not close related to those observed bay Reale et al. (2006) in five olive cultivars, where germination percentage was approximately 40% .And they were high related when compared to those reported by Garrido et al. (2021) who observed germination of 12%. The results of Wu et al. (2002) demonstrated a wide variation in pollen germination of olive trees, from 1.6% to 35.5% for the cultivars Pendolino and Frantoio, respectively. The research carried out by Viti et al. (1990), using the same culture medium as this study, showed that germination remained between 17 and 26%. Variations in pollen grain germination may occur due to growing conditions, the cultivars under study and the culture medium used for in vitro germination (Ferri et al., 2008; Giordani et al., 2014; Mazzeo et al., 2014; Silva et al., 2016)

High temperatures affect pollination and pollen grains survival. Studies have shown that the temperature of 40ºC is lethal to pollen grains by inducing heat shock (Koubouris et al., 2009) but there are no research results on the effect of low temperatures on olive trees pollination. This study showed that pollen grain germination is higher at a temperature between 25 and 30 °C, reaching values higher than 40%. The germination percentage decreases at 15 °C, and at 10 °C or less, it does not occur or is drastically lower, as well as at a temperature of 45 °C. When researching in vitro pollen grains germination and high temperature flowers exposure on Leccino and Levantinka cultivars , Vuletin Selak et al. (2014) conclude that high temperatures reduce pollen tube growth in the stylet and the percentage of flower fertilization. As a result, it increases the time needed for the pollen tube to reach the stylet base or the ovaries in the studied cultivars. The temperature of 20 ºC is enough to reduce germination and pollen tube growth in Manzanilla cultivar flowers, negatively impacting fruit set (Cuevas et al., 1994).

Low temperatures during the flowering period have been reported as harmful in other fruit crops such as longan (Pham et al., 2015), hazel (Çetinbaş-Genç et al., 2019) and peach (Hedhly et al., 2005), influencing pollen tube germination and growth. In citrus, Distefano et al. (2012) found that at a temperature of 10°C there was no pollen grains germination for the genotypes under study, and at 15°C only 10% germinated.

The results of Koubouris et al.(2009) showed that pollen grain germination increases at a temperature of 25 °C for Koroneiki (+6%), Mastoidis (52%) Kalamata (+ 10%) and Amigdalolia (+ 10%) cultivars. At the temperature of 30 °C, the germination percentages of Mastoidis, Kalamata and Amigdalolia were 8, 6 and 14% higher, respectively, when compared to the control temperature of 20 °C. According to the authors, the strong and different reactions by the genotype in response to stress at high and low temperatures can be developed for plant improvement, thus resulting in more adapted cultivars. Mild temperatures occurrence in spring was also observed in the olive-growing region of Tenerife (Canary Islands), where the climate is characterized as subtropical (Medina-Alonso et al., 2020). Although there is a greater probability of less efficient temperatures for germination in Uruguay, it is possible to produce olive trees and obtain annual yields of 8 ton/ha (Conde-Innamorato et al., 2019). The year-to-year climatic conditions variation in this country, especially due to changes in precipitation volume and annual average temperature (Vaughan et al., 2017), may be a determining factor for fruit set, as temperature determines germination and pollen grains growth.

Material and Methods

Local and plant material:

The research was performed at the experimental station INIA Las Brujas - Uruguay (34°40' S; 56°20' W; altitude 21 m). Seventeen-year-old olive trees of the cultivars Arbequina, Manzanilla and Picual, and ten-year-old Arbosana and Koroneiki, planted in spacing of 6m between rows and 4m between trees, were used. The local soil has an A horizon with a maximum fine texture of 50 cm. It has 2.5 % organic matter and a pH of 6.5; corresponding to Udifluvent soil in the USDA classification (Durán et al., 2006). The olive trees orchard was fert-irrigated.

Pollen germination

The inflorescences were collected in 59 BBCH phenological stage, characterized by a corolla color change from green to white (Sanz-Cortés et al., 2002). The choice of this phenological stage was made to ensure that evaluated pollen grains were from the cultivar under study; knowing that in our conditions pollen of Arbequina, Picual y Coratina were viable between green corolla and anthesis (Speroni et al., 2021).The inflorescences were transported in Petri dishes to the laboratory, where they were placed above a filter paper moistened with 1000 µL of distilled water and stored in Petri dishes for 48 hours, at room temperature, to allow the flowers' opening and pollen grains' release. Afterwards, the dishes containing the opened inflorescences were carefully shook to release pollen grains from anthers.
The culture medium described by Viti et al. (1990) was used, with the addition of 100 ppm of boric acid, as base medium. After preparation, the culture medium was autoclaved under a pressure of 1.5 atm, for 30 minutes. Then, it was transferred to sterilized Petri dishes (approximately 10 ml of culture medium). Later on, 1000 µL was added to the Petri dish containing pollen grains and, with the aid of a micropipette, 50 µL were removed and placed on culture medium. Next, the Petri dishes were closed with plastic film to prevent gas exchange from the external environment.
To verify the temperature effect, plates containing pollen grains were subjected to the temperatures of 10, 15, 20, 25, 30 and 40°C. Samples for treatments at temperatures of 10 - 30°C were stored in an incubator chamber, and for the temperature of 40°, they were stored in a forced air circulation oven for 24 hours.
The evaluation of the number of germinated pollen grains and pollen tube length were performed using a microscope model Olympus® BH2, with 20 x 0.40 magnification. Fifty pollen grains per repetition were counted, totaling 150 pollen grains per treatment, and they were considered germinated when presented a pollen tube length equals to or greater than their diameter. To assess the pollen tube length, DinoCapture® software was used, in which three pollen grains were selected, by repetition, and their lengths were verified.

Experimental design

The experimental design was completely randomized, containing two treatment factors and three replications. The treatment factors consisted of five olive cultivars (Koroneiki, Arbosana, Arbequina, Manzanilla and Picual) and six temperatures (10, 15, 20, 25, 30 and 40°C).

Statistical analysis

The statistical analysis was performed using the R software (2019). The data expressed in percentage were transformed by arcsin [square root (n + 1)] and, then, it was submitted to analysis of variance (ANOVA) by the F test ($p \leq 0.05$). In case that interaction between factors (temperature and cultivar) was not significant, the main factors' effect was considered. The temperature effect was analyzed by polynomial regression, and the cultivar means were compared by the Tukey test ($p < 0.05$).

Climate and phenological stages

The phenology of cultivars Koroneiki and Arbosana was evaluated from a database containing nine-year records. The phenological scale was proposed based on the BBCH 61, BBCH 65 and BBCH 68 stages. The beginning of flowering (10% of open flowers), full flowering (at least 50% of open flowers) and the end of flowering (most petals fallen) were characterized according to the methodology used by Conde-Innamorato et al. (2019). For the phenology of Arbequina, Manzanilla and Picual cultivars, the research results were used, which carried out the evaluation over a period of ten years.
To calculate the probability of temperatures occurrence between 0 and 40°C, a dataset of hourly average temperature data, from September 26 1998 to November 15, 2019 was used. From every hour recorded temperature, it was observed the occurrence of the following values: 0-5ºC, 5-10ºC, 10-15ºC, 15-20ºC, 20-25ºC, 25-30ºC, 30-35ºC and 35-40°C. The number of times the temperature remained within the observed range, for each hour and day, was related to the number of years (22 years). Thus, the hourly probability of occurrence of the observed range temperature was calculated. The data were plotted on a box-plot to represent the probability for each temperature range.
Regarding the climatic data analysis from Uruguay's historical series on maximum and minimum temperatures, daily temperature during the flowering period does not exceed 25°C, as shown in Figure 1. Daily minimum temperatures were between 10 and 15°C. A probability of approximately 0.3 and 0.35 was observed for the temperature ranges of 10 – 15°C and 15 – 20°C, respectively. This probability indicates that temperatures during the flowering period are concentrated in these two ranges. A lower probability, about 0.10, occurs for temperatures of 20 – 25°C. Temperatures above 25ºC do not occur within the aforementioned olive tree flowering window in Uruguay.

Conclusions

Between the first and last flowering date in the study period, a window of 52 days was observed, the first day being day 268 (25th of September). The optimum temperature for pollen tube germination and growth in the evaluated olive cultivars ranges between 25 and 30°C. Based on the 22-year historical data series, during the flowering period 75% of the days had temperatures below 20 ° C. Low temperatures may limit olive fruit set in Uruguay, so further investigations will be needed to study these factors on "*in vivo*" pollination.

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