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Flower and fruit formation of *Hexachlamys edulis* in Buenos Aires, Argentina

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Abstract: *Hexachlamys edulis* (O. Berg) Kausel & D. Legrand, "ubajay" is a Myrtaceae species autochthonous of South America. It is a prominent species, with potentially nutraceutical fruits, leaves and other organs with important uses with great benefits for human health and new alternatives for production systems. Levels of polyphenols and pigments together with the antioxidant activity allow us to consider *H. edulis* fruit as a functional food. Nevertheless, there are not yet enough scientific studies on its physiology and productive capacity. The objectives of this work were i) describe the development of flower bud to mature fruit of *H. edulis* in the agro ecological conditions of the locality of Moreno (Buenos Aires provinc); ii) study of pollination methods; iii), evaluate the floral phenology evolution and iv) compare the climatic conditions of Moreno (Buenos Aires province) with Federación (Entre Ríos province), which is the original place of the plants. Nine floral phenological stages (B, C, D, E, F1; F2, F3, G, H) have been recognized during the blooming period. Results of pollination treatments were very diverse. SP and CPS did not produce fruits while OP and CPA produced 5.6% and 20.0% respectively. Climatic condition of Moreno was very appropriate during the year 2018, when the levels of flower anthesis and fruit set were the highest. Fruits ripened during the last three weeks of December, without significant differences between 2019 and 2020. Despite the climatic differences between the site of origin and the experimental plot, it can be confirmed that the biological cycle of *Hexachlamys edulis* was perfectly fulfilled without anthropic intervention in the Moreno site, confirming that this species presents a high plasticity and that fruit production could be incremented with appropriate cultural practices.

Keywords: *ubajay, floral phenology, plasticity, nutraceutical species.*

Introduction

Southern Hemisphere of the planet has a great biodiversity of autochthonous or endemic plant species that are not only important as a food source but also constitute an invaluable source of substances with therapeutic and dyeing properties. It is known that man has used plants for medicinal purposes since ancient times and according to the World Health Organization (Petrovska, 2012), about 75% of the world's population depends almost exclusively on the use of plants for health caring.

Hexachlamys edulis (O. Berg) Kausel & D. Legrand, “ubajay” is a Myrtaceae species included in the tribe Myrteae that is the largest tribe in the plant family with fleshy fruits. In fact, among its representatives with edible fruit are feijoa (*Feijoa sellowiana*), guava (*Psidium guajava*), jabuticaba (*Plinia cauliflora*), Surinam cherry (*Eugenia uniflora*), strawberry guava (*Psidium cattleyanum*), camu camu (*Myrciaria dubia*), arazá (*Eugenia stipitata*), and rumberry (*Myrciaria floribunda*). All these species, included *H. edulis,* have a wide distribution in tropical and warm-temperate regions of the South America. *H. edulis* is certainly a prominent species, with potentially nutraceutical fruits, leaves and other organs with important uses that make it a tree that can provide great benefits for human health and new alternatives for production systems (Povilonis et al., 2021). Levels of polyphenols and pigments together with the antioxidant activity allow us to consider *H. edulis* fruit as a functional food (Arena et al., 2021). Nevertheless, there are not yet enough scientific studies on its physiology and productive capacity.

The timing of phenological events such as bud burst, leaf-expansion, abscission, flowering, fertilization, seed set, fruiting, seed dispersal and germination can be quite sensitive to environmental conditions mainly climatic, being temperature, solar radiation, and water availability the key of factors that control plant phenology (Guesmi, 2021). Study of reproductive phenology and its interactions with the ecosystem provides the necessary tools for understanding the biology of a species (Kebede and Isotalo, 2016). For this purpose, floral phenology shows the natural evolution of flower buds in all their stages until the formation of the fruit in relation to the climatic conditions (Barret et al., 2021). In addition, analysis of phenology is a determining factor for the study of climate change, which also allows predicting the distribution of tree species through the biological processes of survival and reproductive success as suggested by Chuine and Beaubien (2001). Also, it is interesting to explore the plasticity of the species that possibility to enlarge the production area. In effect, some previous studies showed that ubajay could be grown in Moreno (Buenos Aires province) (Quintavalle, 2020¸ Arena et al., 2021). In fact, a well-known example was the plasticity of the species *Berberis microphylla* that was well adapted to extremely diverse conditions of its place of origin as well as the climate of Moreno (Radice et al., 2018). It is known that changes in climatic and edaphic factors strongly influence the growth and reproduction of a species. Thus, phenology patterns displayed by plants are adaptations to the surrounding abiotic and biotic environments (Radice and Arena, 2018). In summary, phenotypic plasticity is the capability of a genotype to produce diverse phenotypic expressions under different environments. In fact, plants can react with changes in stomata structure and functionalities, chlorophyll content, type of leaves, shoot elongation (Zunzunegui et al., 2009) or leaf morphology and structure (Radice and Arena, 2014). The objectives of this work were i) describe the development of flower bud to mature fruit of *H. edulis* in the agro ecological conditions of the locality of Moreno (Buenos Aires province); ii) study of pollination methods; iii), evaluate the floral phenology evolution and iv) compare the climatic conditions of Moreno (Buenos Aires province) with Federación (Entre Ríos province), which is the original place of the plants.

Materials and Methods

Plant material and growing conditions

Plants were obtained from seeds of fruits collected in Federación, Entre Ríos (Argentina) (30° 59' SL, 57° 55' WL, 50 m.a.s.l.), and they were grown in the nursery of the experimental field of the University of Morón placed in Moreno (34° 39' SL, 58° 47' WL, 14 m.a.s.l.) for two years. Then, they were planted with 3 m between them in the row in the same experimental field in 2009. Plants grew without cultural management, or any supplementation of fertilizers or irrigation, and they were 9 years old at the beginning of this study; all of them were in the adult phase since they had flowers and fruit production. Experiment was carried out from September to December between the years 2018 and 2020 in a plot of fifteen *H. edulis* plants.

Measurements

Changes observed on the buds were registered and morphological floral changes were considered to recognize every phenological stage according to a modification of the Fleckinger scale (Fleckinger, 1955).

To study the pollination methods of this species, 100 branches were randomly marked and four pollination treatments were assigned: open pollination (OP), flowers in natural state; self-pollination (SP), where flowers were isolated with cloth bags; cross pollination, where emasculated flowers were pollinated with pollen from the same plant (CPS), and cross pollination, were flowers were emasculated and pollinated with pollen from another plant (CPA). Results were analyzed by ANOVA based on a binomial model for the response variable presence/absence of fruit.

Phenology evolution was evaluated through a relative percentage scale according to Radice (2005). At the same time, evolution of these stages was registered weekly during the three consecutive years (2018 to 2020). Fruits of each plant were harvested in three weeks corresponding to the second, third and fourth week of December of the years 2019 and 2020. Fruit yield was analyzed by ANOVA between years.

Climatic conditions between 2018 and 2020 were analyzed for comparison with phenological data. Mean, maximum and minimum air temperatures, number of days with frost and cumulative rainfall were registered daily on the Moreno locality, and in hourly frequency by the meteorological station of INTA (Instituto de Clima y Agua, Castelar). Temperature was analyzed by ANOVA and Tukey Test, and cumulative rainfall and frost day by χ^2 using Rstudio. Moreover, historical climatic condition of Moreno and Federación were described for the period 1991-2021, and all the climate data are based on ECMWF data (https://es.climate-data.org/info/sources/) (Fig. 7).

Soil of the experimental field of Moreno was sampled and analyzed. Soil nitrogen (N) was determined using the Kjeldahl technique using a Bϋchi K350 (Bϋchi, Flawil, Switzerland), while carbon (C) and phosphorus (P) soil concentration were determined with a plasma emission spectrometry (ICPS 1000 III, Shimadzu, Kyoto, Japan). Finally, soil of Moreno and Federación were described through the classification made by USDA Soil Taxonomy.

Results

Flower and fruit development

Flowers are typical of Myrtaceae; appear in the leaf axils in apical and axillary position, are white, solitary or in cluster and hermaphroditic (Fig. 1-2).

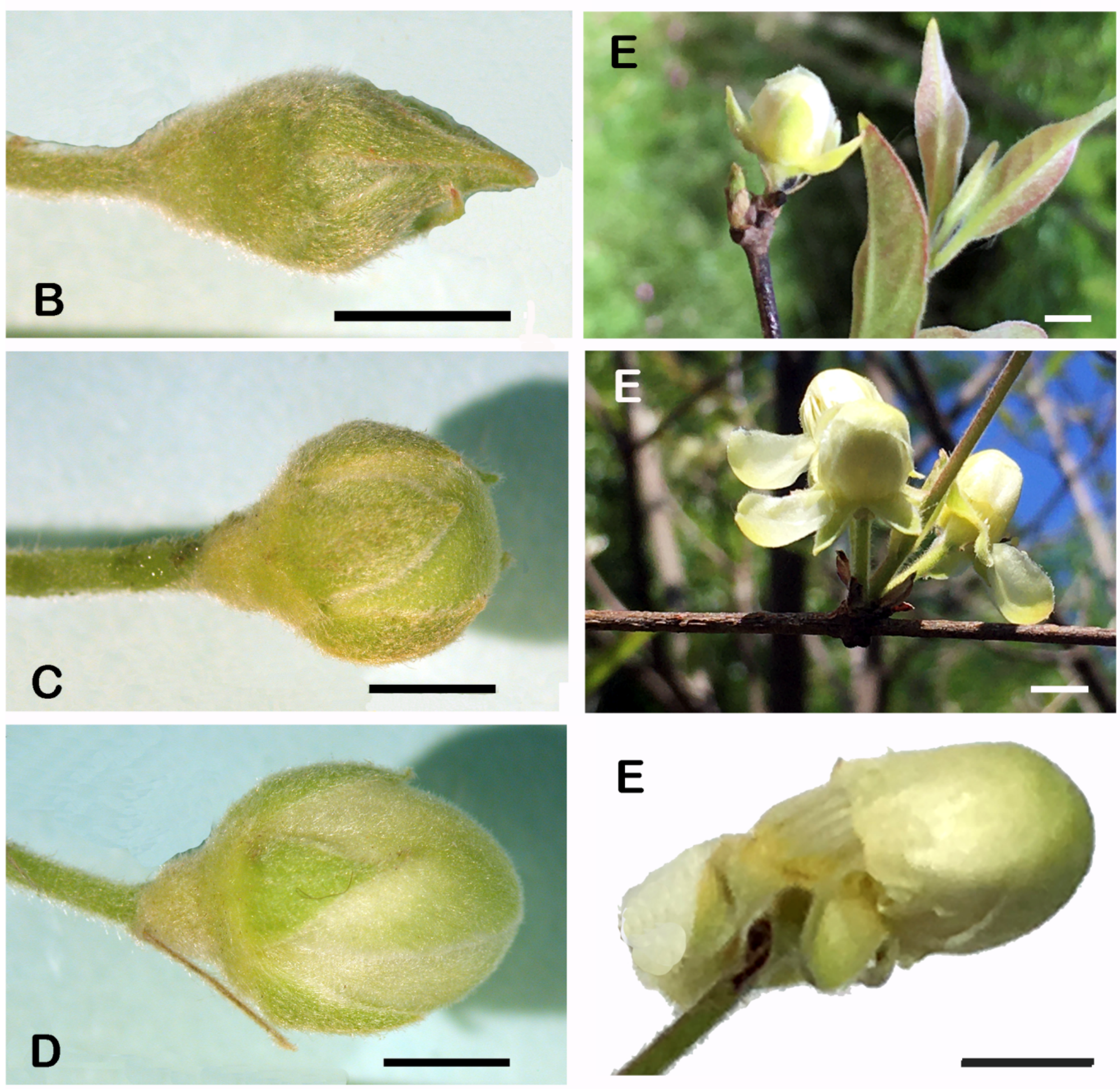

Figure 1: Flowers of Hexachlamys edulis in different pre anthesis stages. Letters indicate the different phases B, C, D and E. Bars = 4mm.

Figure 2: Flowers of Hexachlamys edulis *in anthesis stages (F_1, F_2, F_3)* ***Bars*** *= 5mm*

Nine floral phenological stages (B, C, D, E, F1; F2, F3, G, H) have been recognized during the blooming period. Green flower buttons appear as a first step of growing floral scale (stage B, Fig. 1) and quickly increase their diameter (stage C, D, Fig. 1). Previously to the anthesis stage, petals increase in long 2-3 times respect to the sepals (stage E, Fig. 1). In this stage, stamens show still non dehiscent anthers (Fig. 2) and the pistil shows receptive stigma. End of stage E occurs when the petals move from the center towards the outside leaving exposed the reproductive organs or in some cases come off from the button (Fig. 1). Floral button enlarges very rapidly and some hours or one day after its apparition, anthesis occurs (stage F_1, Fig. 2). Suddenly, stamens appear slightly yellow (stage F_1, Fig. 2) to turn gold after sun exposition while becoming dehiscent (stage F_2, Fig. 2). Anthesis end is manifested with anthers dehydrates (stage F_3, Fig. 2). The next stage (G) is shown as a greased receptacle with a remainder of the pistil in which the stigma and upper part of the style are necrotic (Fig. 3, 10-19, 10-28, see arrows). Finally, fruit formation is recognized when receptacle turn like sphere (stage H, Fig. 4-A).

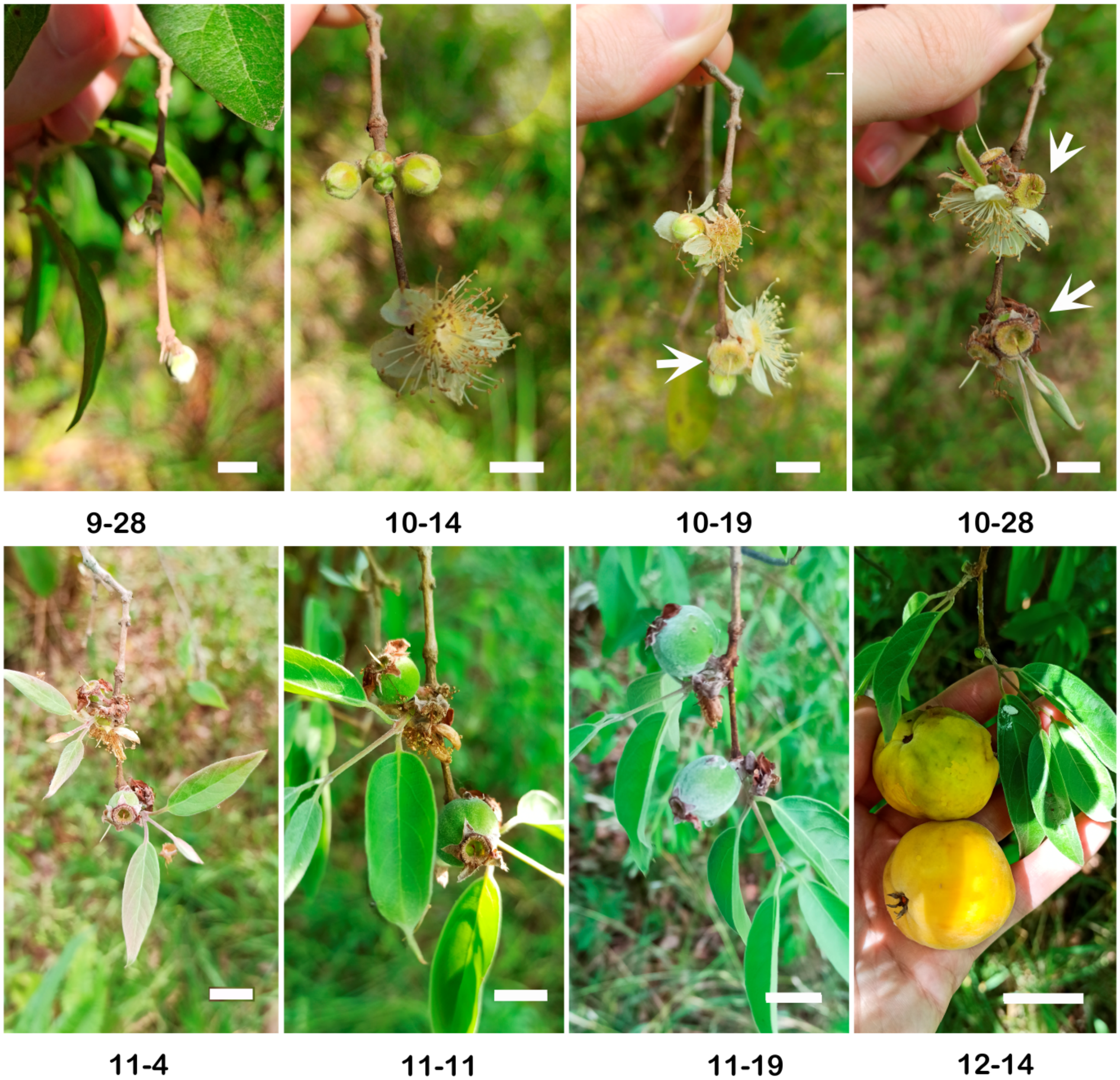

Figure 3: Branch of Hexachlamys edulis *with flowers and fruit in its development period according to the dates indicated from September 28 (9-28) to December 14 (12-14). Arrows indicate G phase.* ***Bars*** *= 1 cm.*

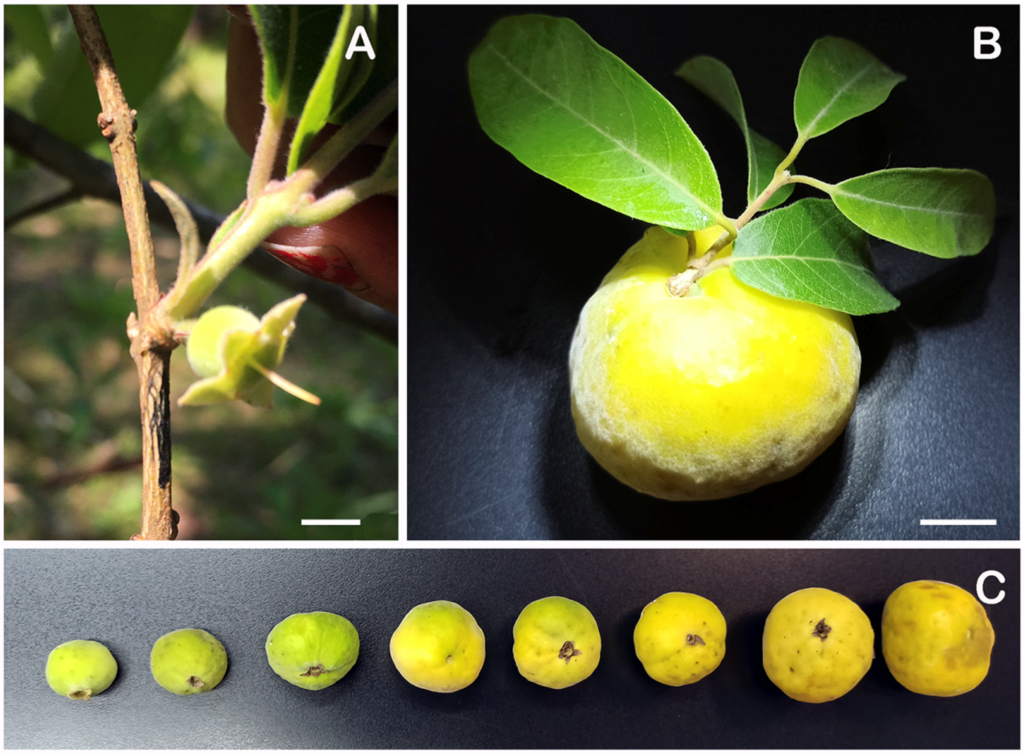

Figure 4: Fruits of Hexachlamys edulis*. **A**, first stage of development; **B**, mature fruit; **C**, fruits in different stages of growth on harvest day. **Bars** = 1 cm*

Results of pollination treatments were very diverse. SP and CPS did not produce fruits while OP and CPA produced 5.6% and 20.0% respectively. Treatments with fruits were compared by ANOVA and not significant differences were observed (p= 0.0736).

Flower phenology

Evaluation of flower phenology in plants grown in Moreno plot during the three years of study is represented in the Figure 5.

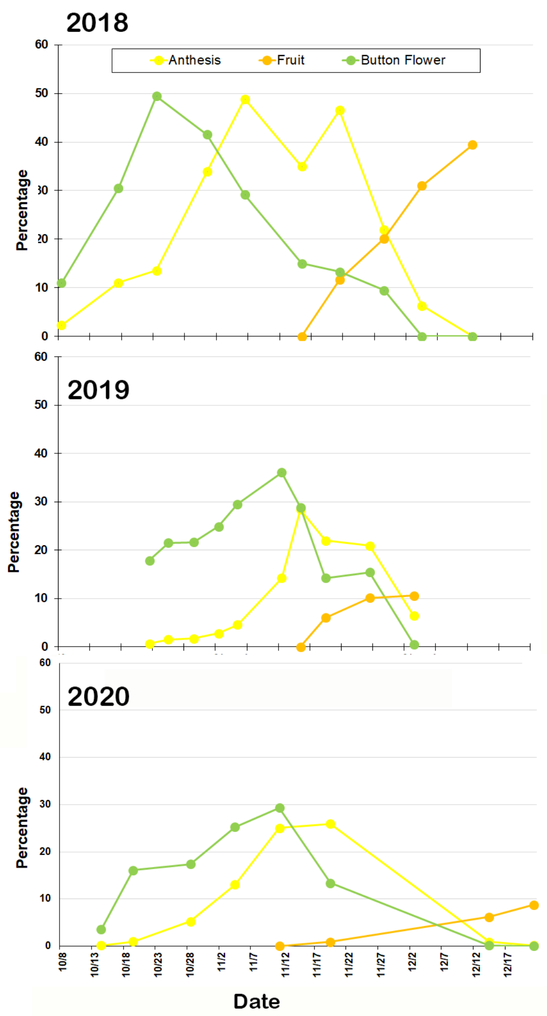

Figure 5: Phenology of Hexachlamys edulis *registered on Moreno, province of Buenos Aires, from October 8 (10/8) to December 17 (12/17) during 2018, 2019 and 2020 years.*

During 2018, 10% of button flowers were recorded on October 8, and a maximum value of 50% was observed on October 23. On this year, anthesis stage started on October 17 with 10%; then the flower opened and increased until November 6 with a maximum value of 49%. A fall of anthesis flowers was observed on November 15 with 35% followed by a new increase to 47% on November 21, showing a double maximum value of flowers in anthesis stage, and then disappeared the first week of December. Fruits began their growth at the end of November and 40% of fruit set was registered on December 11.

On October 15 of 2019, the first button flowers were recorded (18%). A scarce appearance of flowers in anthesis were observed between October 15 and November 8 with less than 10% of flower in this stage, and its maximum expression was on

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November 15 with a poor value of 28%. First grown fruits were registered on November 26 and only 11% of fruit set was registered.

In 2020, the first button flowers were counted on October 14 (3.5%). Anthesis stage, also started on October 14 and the maximum values being recorded on November 11 and 19 (25 and 26%, respectively). On November 19, growing fruits were observed, and fruit set was 39% on December 2.

During December 8-14 of 2019, only 1 plant (149 g) presented ripened fruits (Fig. 6). Then, during the two following weeks, 7/15 and 14/15 plants had ripened fruits, totaling 2512 and 5614 g, respectively. In 2020, during December 8-14, 3/15 plants had ripened fruits to be harvested (458 g), while during the following weeks, 13/15 and 15/15 plants produced ripe fruits (3817 and 6823 g, respectively). There is no statistical evidence to affirm that there are differences between years in fruit yield (p = 0.057).

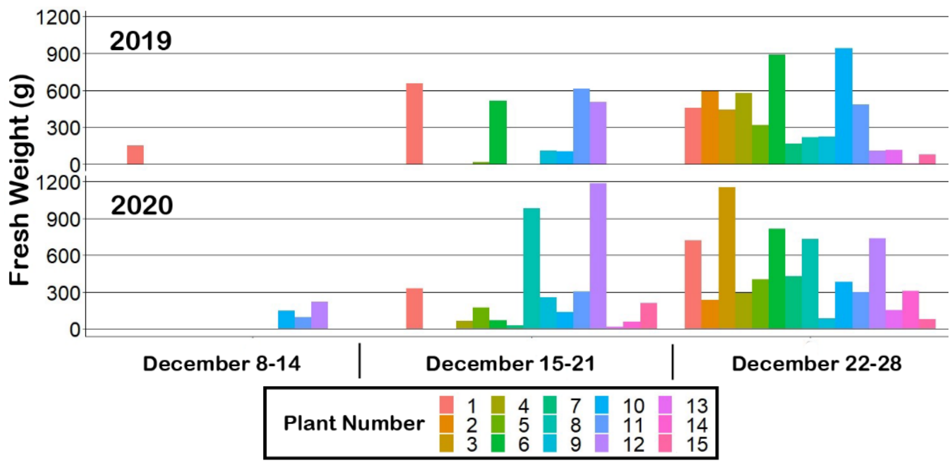

Figure 6: Fresh weight of ripe fruits per plant of Hexachlamys edulis *during three weeks of December (8-14, 15-21 and 22-28) in 2019 and 2020.*

Growing conditions

Moreno's climate is classified as warm and temperate. According to Köppen (1936), this climate is classified as Cfa i.e., temperate rainy climate. Moreno is a locality with significant rainfall without driest month. Historical average of mean annual temperature is 17.3 °C, with average maximum of 28.1 °C in summer and average minimum of 7.9 °C in winter; in addition, annual rainfall is 1,100 mm approximately (Fig. 7). Federación (Entre Ríos) is considered a subtropical climate without a dry season, with average mean temperatures ranging between 24 and 25 ºC in summer and between 12.5 and 14.2 ºC in winter, and rainfall close to 1,400 mm per year (Fig. 7). Although the summer in Federación is rainier than Moreno, its relative humidity is lower than Moreno (Fig. 7).

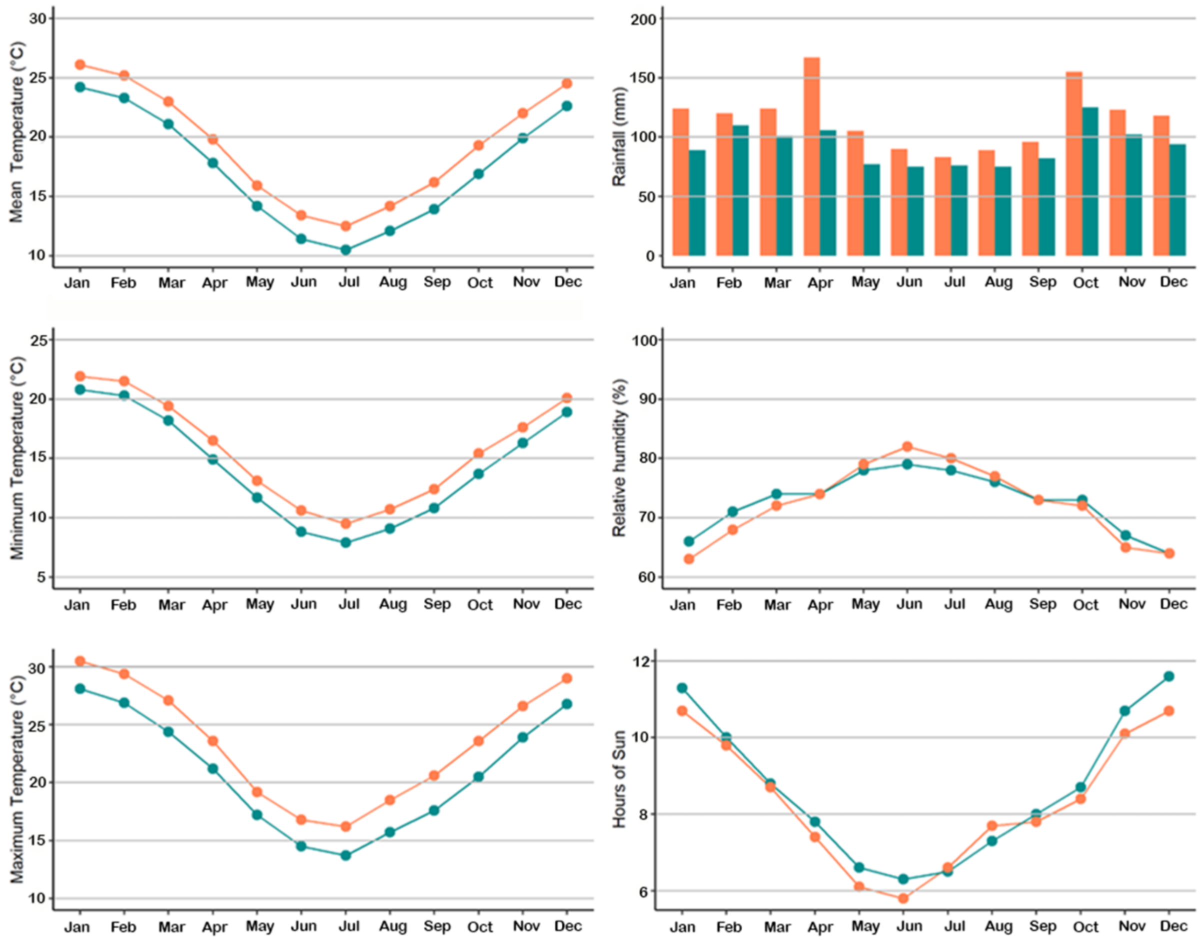

Figure 7: Historical average for Mean, Maximum and Minimum air monthly temperatures, cumulative rainfall, relative humidity and hours of sun for Moreno, province of Buenos Aires (green) and Federación, province of Entre Ríos (orange).

Between the three years, significant differences are observed in climatic parameters. In effect, monthly mean temperatures in 2018 were significantly higher for April and September with 23 and 16.5 ^{0}C, although significant lower in June (9.2 ^{0}C) compared to the same month of the other two years studied (13.1 and 11.5 ^{0}C for 2019 and 2020 respectively) (Fig. 8). On the other hand, minimum temperatures were significant different for March, April, May, June and in particularly for September of 2018, when 11.97 ^{0}C was observed compared to 8 and 7 ^{0}C for 2019 and 2020, respectively (Fig. 9). In addition, number of days with frost between the three years was also significant different for June, July and September (Fig. 9). Total frost days for 2018 were nine but no frosts were registered during the month of September. On the contrary, seven total frost days were registered on 2019 with the last on September 3 and fifteen total frost days on 2020 with the last one on September 20 (Fig. 9).

During April, May and December of 2018, rainfall exceeded 200 mm per month (Fig. 10), while on September, 110 mm were recorded and 154 mm in November, although 115 mm were accumulated only in two days. The remaining months accumulated around 50 mm or less. Unlike the other years studied in 2018, June and October were months with scarce rainfall, with 11 and 33 mm respectively. During 2019, rains were registered with a greater frequency in June and October with 150 mm and 142 mm on December. The records for 2020 were very different. During the months of March and April, the rains were abundant, 150 and 104 mm respectively, while in the following months the rains were lower than the records of previous years. Monthly accumulated rainfall was significantly different between the years studied (Fig. 10).

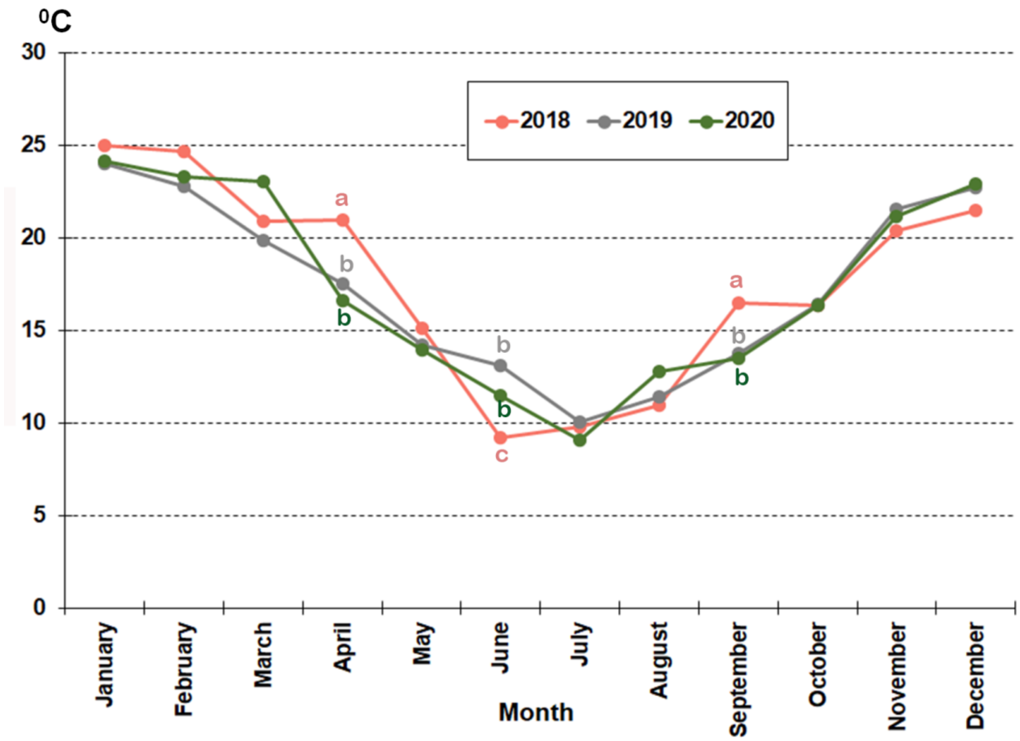

Figure 8: Mean monthly temperature of Moreno (province of Buenos Aires) for the three years of study. Different letters indicate significant differences for the values of the same month.

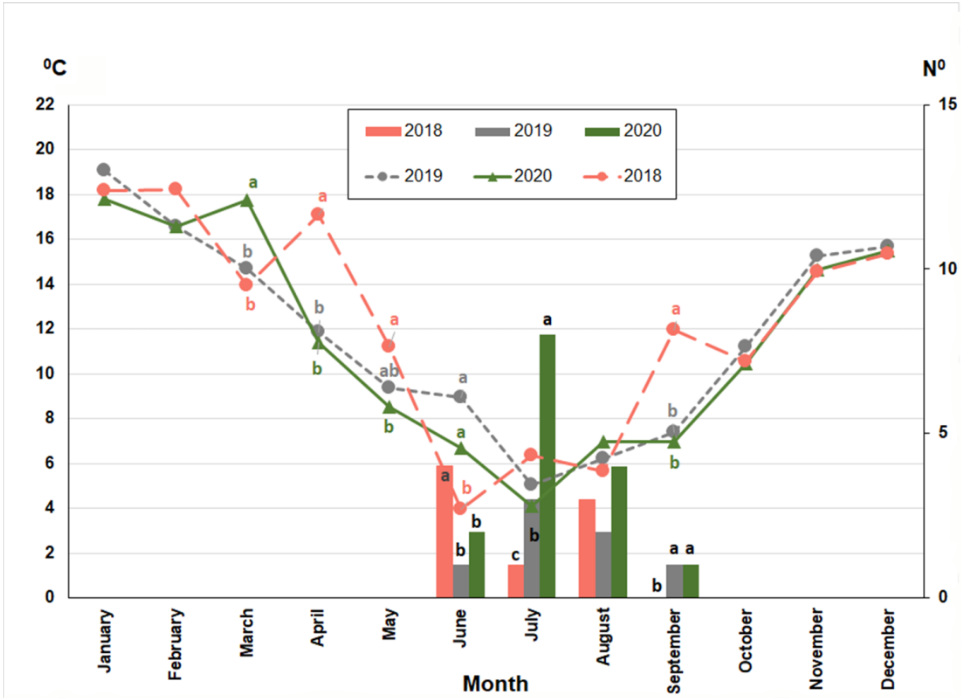

Figure 9: Minimum monthly temperature (lines) and total frost days (bars) of Moreno (province of Buenos Aires) for the three years of study. Different letters indicate significant differences for the values of the same month. Color letters for minimum temperatures and black letters for total frost days.

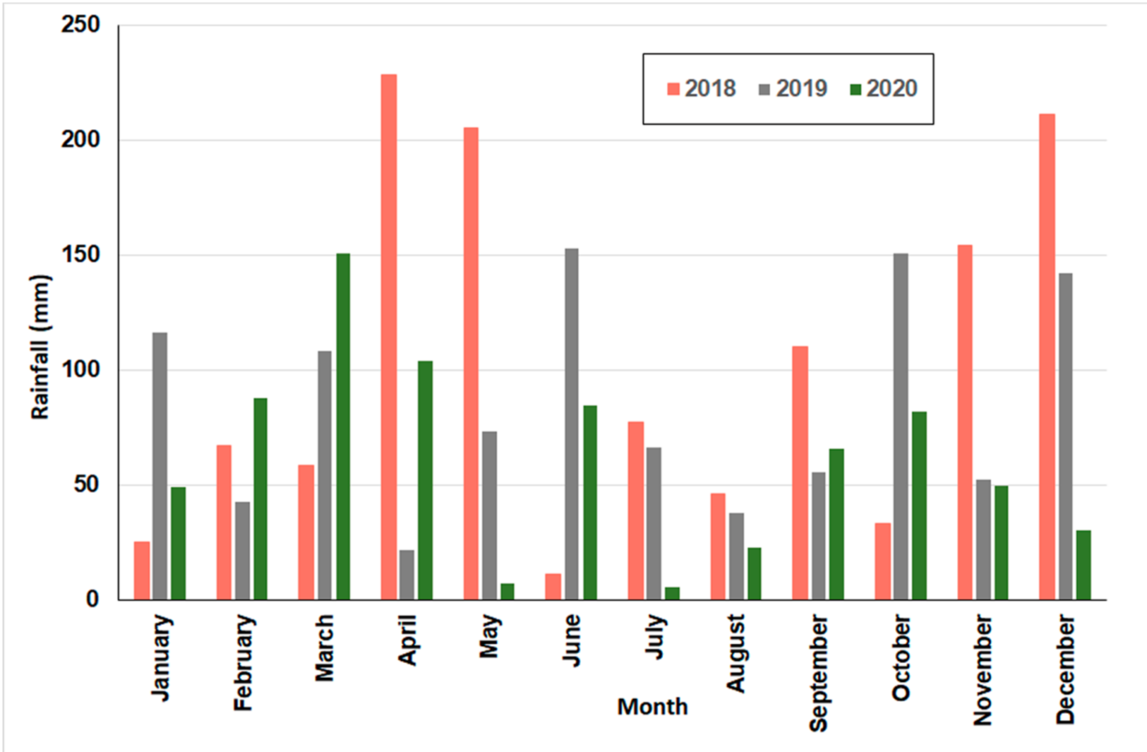

Figure 10: Cumulative monthly rainfall of Moreno (province of Buenos Aires) for the three years of study. Different letters indicate significant differences for the values of the same month.

Moreno soils are typically Mollisols, ergo dark fertile soil with very thick A horizons and without B horizons, unlike to Federación soils that belong to the Entisol order characterized by deep, reddish or sandy brown and with a high content of fine-textured pebbles and lack well-developed horizons (Baillie, 2001). In particular, the soil analyzed in the experimental field of Moreno showed 3.72% of organic material, 2.15% of carbon, 0.21% of nitrogen and 34.97% of assimilable phosphorus. Finally, the C: N ratio calculated was 10.23.

Discussion

Flower morphology was in correspondence with typical as described for the family (Rotman, 1982). However, in this experiment placed in Moreno (Buenos Aires, province), solitary flower development was more frequently observed than the described in the literature. In coincidence with other species of this family, *H. edulis* proved to be self-incompatible due to the fact that no fruits were obtained by self-pollination (Pound et al., 2002; Finatto et al., 2011).

Bloom occurs at different times, and its beginning and duration seem to depend on ecological factors. In fact, the length of the anthesis stage is closely related to the climatic conditions (Nyéki and Soltész, 1996) and alteration of plant phenology is one of the most readily observable ecosystem reactions to climate change (McEwan et al., 2011). In particular, two picks of the anthesis stage showed on 2018 year was a consequence of an abundant rainfall fallen previous days that caused the soil to remain with excess water for several days. Fruit trees suffer from lack of oxygen due to flooding for some days. In consequence, plants increasing the ethylene level and suffers changes in their physiological mechanisms. In this particular case, it is well known that plants react detaching flowers that are the most demanding organs of nutrients (Taiz et al., 2015). After the soil drainage that occurs after day November 14, the anthesis stage restarts from the reserve button flowers.

On the other hand, the important differences observed in the number of flowers that appeared in 2018 with respect to the other two studied, are largely due to the low temperatures recorded during the months of September and October for 2019 and 2020. In effect, during these last two years, mean temperatures of September were lower than 10 ^{0}C, and significantly lower to the historical average. In addition, on 2020 cumulative rainfall were significant poorer respect to the other two years, which, added to the low temperatures, caused all the floral expression to be delayed and some button flowers were lost due to death. In coincidence with Guesmi (2021), it can be said phenology is directly influenced and controlled by climate especially by duration of sunlight, precipitation, temperature and other life-controlling factors. In fact, between results observed on 2018 and the others two years studied, one month advance was occurred of the anthesis stage. These results are in contrast with Bradley et al. (1999) who argue that flowering in many species is conditioned by photoperiod and therefore will not respond to climate warming. Conversely, changing in temperatures and rainfall affect resource availability for woody plant species (Chuine and Beaubien, 2001; Barret et al., 2021) and it is evident that the reproductive response of the species is linked in some way to the temperature (Scranton & Amarasekare, 2017).

Similarly to other species of Myrtaceae family, *H. edulis* showed a self-incompatibility (Beardsell et al., 1993). Fresh weight of fruit harvested on 2019 and 2020 were not significant different but in 2019, only one plant produced fruit in the first week of December, while in 2020 fruit were harvested from three plants. This difference could be explained to the greater supply of flowers in anthesis throughout 2020, while in 2019, there was an abrupt drop in button flower and flowers in anthesis stages.

Growing conditions of *H. edulis* in Moreno location are different compared to the site where mother plants grew. In effect, climatic conditions showed that the historical temperature of Federación was always a few degrees superior to that of Moreno, with more rain and drier summers. On the other hand, it can be confirmed that plants can grow over the soil textures described. Despite not having a detailed analysis of the soil fertility of the Federación, the higher proportion of sand would indicate a lower fertility. So, soils of Federación are lighter and well drained two conditions that could be favorable for root growth. These important differences on the growth condition surely could alter the physiological cycle of the species studied (Taiz et al., 2015). In fact, level of nitrogen to plants is clearly exemplified by its effects on growth of leaves, senescence, root system architecture and flowering time, among other aspects (Luo et al., 2020). In general, poor nutrition promotes flowering (Cho et al., 2017) event that is manifested in Federación with a copious flowering. In addition to its effect on plant growth and plant development processes, the nutritional status of the plant is an important factor in the resistance or susceptibility of various crops to certain pathogens (Spann and Shumann, 2010). Despite all these differences, the plants that currently have fourteen years of cultivation do not present serious physiological or pathogenic alterations that impede the continuity of life.

Conclusions

Climatic condition in Moreno (Buenos Aires province) was very appropriate during 2018, when the levels of flower anthesis and fruit set were the highest. However, in the following years atypical situations were fulfilled since the temperatures of the spring period were lower than those calculated in the historical records and particularly in the year 2020 with later frosts and water shortage. Considering the historical climatic values of the town of Moreno (Buenos Aires province), the possibility of cultivation in this geographical area can be guaranteed. According to the results observed in the study area, the biological cycle of *Hexachlamys edulis* was perfectly fulfilled without anthropic intervention, confirming that this species presents a high plasticity. Production of fruits could be increased with the appropriate cultural practices of the crop either with the introduction of irrigation, drainage channels, fertilization and adequate pruning. Finally, this study shows that the selection of individuals with better adaptation to lower temperatures and later spring frosts is recommended for the introduction of the crop in this area.

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