

## Temperature and floral development in the formation of parthenocarpic fruits in mango ‘Ataulfo’

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### Abstract

One of the main problems in mango ‘Ataulfo’ is the so-called ‘mango child’ to refer to the high production of parthenocarpic fruits. The objective was to determine the factors of the climate that affect the presence of parthenocarpic fruits and in which part of the development of the inflorescence and fruit are affecting. The study was carried out from 2017 to 2018 in a commercial orchard of mango ‘Ataulfo’, with an incidence of 80% of parthenocarpic fruits, 10 trees were selected and in each tree 50 inflorescences were marked, in each flow of flowering presented, which they were distributed around the tree and in the middle part of the treetop. The variables evaluated were the development of the inflorescence, percentage and type of fruits (parthenocarpic and with seed), embryo development and temperature. Two flowering flows were presented. The second flowering period lasted for a period slightly longer than 70 days and the stage of full flowering until fruit set coincided with extreme temperatures, minimum of 15 °C on average and maximum above 35. The highest production of parthenocarpic fruit was presented in the second flowering flow with 75%, while in the first the production of fruits with seed exceeded the parthenocarpic. In Nayarit, the presence of parthenocarpic fruits is related to extreme temperatures ( $\leq 15$  and  $\geq 35$  °C) during the period of full flowering until fruit mooring, causing embryo abortion.

**Keywords:** *Mangifera indica* L., development of embryo, fruits without seed, inflorescence, mooring of fruits.

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## Introduction

One of the main problems in mango 'Ataulfo' is the so-called 'mango child' to refer to the high production of parthenocarpic fruits. In Nayarit, the presence of these has been increasing in recent years reaching an incidence of up to 90% of production in the main producing municipalities of 'Ataulfo' (Pérez *et al.*, 2007). The absence of seed in the fruit, greatly affects the size of them, reaching up to three times less than the fruits with seed. The above is reflected in low yields and poor fruit quality. The absence of seeds can be attractive in fruits that have many seeds such as citrus, prickly pear, papaya or in those that have a large as mango (Baker *et al.*, 1973; Varoquaux *et al.*, 2000); However, its development promotes cell expansion via the synthesis of hormones or other compounds not fully determined, giving a larger size (Gillaspy *et al.*, 1993).

Seedless fruits are called parthenocarpic; in this respect Vardi *et al.* (2008) comment that there are several types of parthenocarpy currently recognized, including stenospermocarpy in which pollination and fertilization occurs, but the newly fertilized zygote aborts, leading to the formation of seedless fruits. Based on this, the lack of seed in mango fruits is known as stenospermocarpy (Sedgley and Griffin, 1989; Davenport, 2009).

On the other hand, the temperature in the reproductive phase of the plants, is one of the most important factors for the development of the seed and the fruit. The response of the plants to an extreme environment includes alternations in the quality of the flower according to Scaven and Ranfferity (2013). In mango parthenocarpic fruits, contrasting results are known due to the effect of temperature. Studies by several authors (Sukhvibul *et al.*, 2005; Shaban and Ibrahim, 2009; Patel-Gaurang *et al.*, 2015) indicate that low temperatures (15/10 °C, day/night) during the flowering and fertilization period, are causing the production of parthenocarpic fruits, by reducing pollen viability and pollen tube growth or by causing embryo abortion in various mango cultivars.

These temperatures also reduce the number of hermaphroditic flowers, which is closely related to the mooring of fruits and the abortion of the embryo. Other studies indicate that abortion of the embryo in mango has an important role in inducing the process of abscission in the early stages of fruit development, although also problems with pollination and fertilization lead to abortion of embryos (Beasley *et al.*, 1999; Polito, 1999; Rajan, 2012). Gehrke-Velez *et al.* (2012) found fruits with mango 'Ataulfo' seed with cross-pollination, but the self-fertilized fruits contained malformed or necrotic embryos, suggesting a delayed self-incompatibility, giving rise to a parthenocarpic fruit production.

On the other hand, Salazar-García *et al.* (2016) observed that cold temperatures,  $\leq 13$  °C,  $\leq 14$  °C and  $\leq 15$  °C, in prefloration seem to be the cause of parthenocarpic fruits in 'Ataulfo' and found no evidence of stenospermocarpy.

Other authors mention that abortion of the embryo influences, in addition to high and low temperatures, some chemical treatments with bioregulators, chromosomal aberrations and genetic factors as genes that control meiosis (Lin *et al.*, 1984; Vardy *et al.*, 1989). More recent studies in the molecular context indicate that the expression level of the MaMADS gene is very low in aborted embryos compared to normal embryos, so this gene must have an important role

in the development of the embryo to give rise to a fruit normal (He *et al.*, 2012). The objective of the present work was to determine the critical factors of the climate that affect the production of parthenocarpic fruits in the Ataulfo cultivar and in which part of the development of the inflorescence and fruit is affecting.

## Materials and methods

The study was carried out from 2017 to 2018 in a commercial orchard of mango ‘Ataulfo’, with an incidence of 80% of parthenocarpic fruits. The orchard is located in the town of Las Palmas municipality of San Blas, Nayarit, with a warm subhumid climate (Aw1), at the coordinate’s latitude 21° 35’ 58.12’’ and longitude 105° 08’ 59.63’’, with an altitude above sea level of 135 m. The trees with 20 years of age, are grafted on creole rootstocks of the region and established at distances of 10 x 10 m between rows and trees with a density of 100 trees per ha.

The orchard was managed with drip irrigation (three irrigations per week for three h), cube-type mechanical pruning (approximately 50 cm pruning) and fertilized using 2 kg of calcium nitrate per tree at the beginning of the rainy season (June ) and 2 kg of organic fertilizer (chicken manure) per tree applied at the end of the rainy season. The health of the garden was based on preventive applications for anthracnose (*Colletotrichum gloesporioides*) with fungicides such as copper octanoate (1 to 1.5 L ha<sup>-1</sup>) and *Basillus subtilis* (200 g/100 liters of water), making three applications of each of alternate form.

In this orchard 10 trees of uniform vigor were selected. In each tree 50 inflorescences were marked in each presented flowering flow. The flows were marked with strips of different color, which were distributed around the tree and in the middle part of the treetop. The variables evaluated were the development of the inflorescence, percentage and type of fruits (parthenocarpic and with seed), embryo development and temperature.

### Development of inflorescences

In each marked inflorescence, the date and stage of mango flower development was recorded considering the external scale generated by Pérez-Barraza *et al.* (2009), which considers 12 stages (E) from E 1, vegetative bud completely closed until E 12 before; adding two stages, E 13= fruit setting (fruits between 4 and 5 mm in length) and E 14= mooring (fruits between 1 and 2 cm in length), for this study the inflorescences were marked from E 3.

### Percentage of parthenocarpic fruits and with seed

For the evaluation of this variable, the inflorescences marked from E 3 (swollen bud) in the different flowering flows were considered. When the inflorescences reached the mooring of fruits (E 14), the number of fruits with seed (fcs) and parthenocarpic fruits (fp) was counted for each inflorescence. Subsequently, the percentage of each of them was calculated by marked inflorescences.

The procedure was as follows: total fruits (TF)=  $\Sigma$ fcs, fp; percentage of fruits with seed (FCS%)=  $(fcs \times 100)/TF$ ; percentage of parthenocarpic fruits (FP%)=  $100-FCS\%$ .

## **Embryo abortion**

In the 10 selected trees, fruits with a parthenocarpic appearance were sampled every 15 days from curdling, mooring and fruit development when it reached 5 cm in length. In each sample, 20 fruits were collected, the seeds were extracted and placed in FAA fixative (50% 100% ethanol + 5% glacial acetic acid + 10% formaldehyde + 35% distilled water) for 8 days, then rinsed with ethanol 50 and 70% (4 h in each) and were placed in GAA fixative (25% glycerol + 50% ethanol + 25% distilled water). Once in the laboratory, they were dehydrated with ethanol, transparent with xylene and embedded in paraffin and anatomical sections were made in a rotary microtome (8 micrometers). They were observed in a microscope to follow the development of the embryo in three stages of the development of the fruit 1) fruit set, between 3 and 5 mm in length; 2) mooring of fruits, between 1 and 2 cm in length; and 3) fruits in development with a length between 4 and 5 cm.

## **Maximum and minimum temperature**

Data from the network of agrometeorological stations in Nayarit were used, which consists of automated equipment that collects fifteen minute data on temperature, precipitation, solar radiation and wind (Adcon Telemetry, model A753, Klosterneuburg, Austria). The station selected for characterizing the conditions of the study area was El Verdineño, located at 21.702 north latitude, 105.132 west longitude and 43 masl. The station is located 10 km from the experimental plot.

## **Quantification of thermal thresholds**

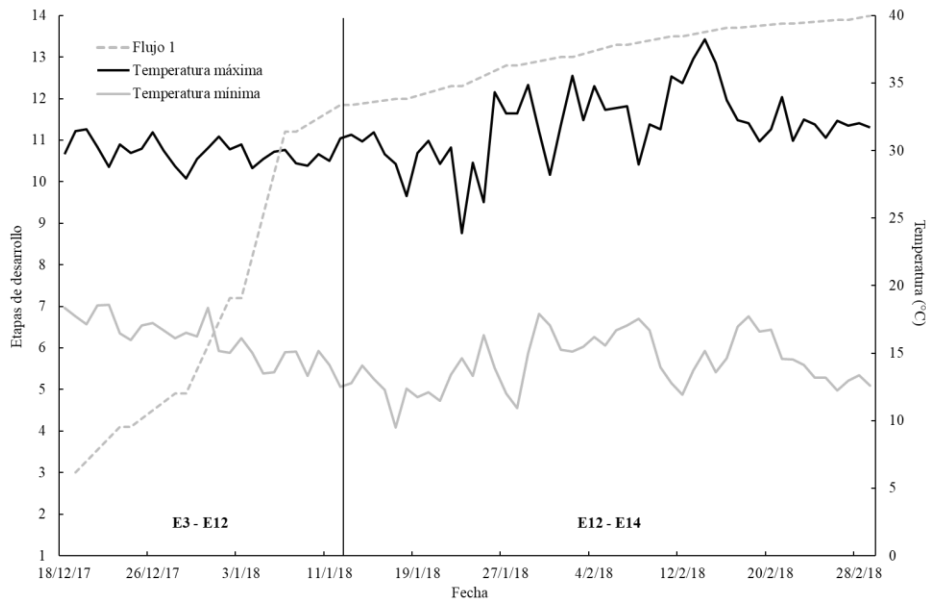
Using the 2013 access database engine (Microsoft Office 2010, Redmond, Washington, United States), the maximum (T max) and minimum temperature (T min) data were integrated, calculating the daily average. These temperatures were considered during the period of development of inflorescences E3-E14. In each vegetative flow presented a database was integrated with temperatures, phenological stage or stage and date in which this stage was presented, later the number of days with temperatures that passed the thermal thresholds was quantified.

# **Results**

## **Development of inflorescences and temperature**

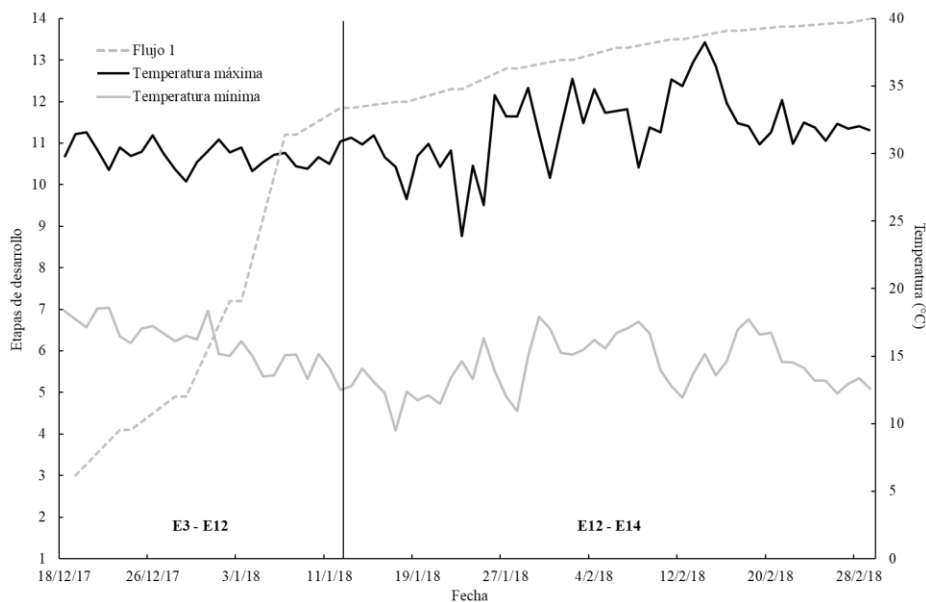
In Nayarit, the stages of development of the inflorescence of the first flow, were presented in December 2017. E3, started on December 15, 2017 and reached full flowering on January 14, 2018 with a duration of 29 days. The time elapsed from E12 (full flowering) to the mooring of fruits was 42 days, the whole process lasted 71 days (E3 to E14) (Figure 1).

In the Figure 1, the minimum temperatures presented in the phenological stages of E3 to E12 were perceived, these fluctuated between 13.3 and 18.6 °C. In the development stage E3, swollen bud, the minimum temperature was about 18 °C, then it gradually descended during the 29 days of duration until E12, reaching a minimum of about 13 °C. During the development stages of E12 to E14, the minimum temperature ranged between 12 and 18 °C except for January 17, which went down to 9.5 and January 28 to 10.8 °C.



**Figure 1. Stages of floral development, first flow, and maximum and minimum temperature presented during the development of the mango ‘Ataulfo’ inflorescence, Nayarit, 2018.**

With regard to maximum temperatures, they fluctuated between 28 and 31.5 °C during E3 to E12, after full flowering (E12) to fruit harvests (E14) the temperatures fluctuated between 24 and 38 °C. The stages of development of the inflorescence of the second flow, were presented until January 2018 (Figure 2), E3 started on January 23, 2018 and reached full bloom on February 26, 2018 with a duration of 34 days. The time elapsed from E12 (full flowering) to the mooring of fruits was 41 days, the entire process lasted 75 days (E3 to E14).



**Figure 2. Stages of floral development, second flow and maximum and minimum temperature presented during the development of the mango ‘Ataulfo’ inflorescence, Nayarit, 2018.**

In the Figure 2, we observe the minimum temperatures presented in the phenological stages of E3 to E12, these fluctuated between 11 and 18°. In the development stage E3, the minimum temperature was 16.3 °C and they were mostly above 15 °C, presenting a drop-in temperature at the end of January and 12 February (approximately 11 and 12 °C, respectively). During the stages of development from E12 to E14, the minimum temperature ranged between 8 and 15 °C.

With regard to maximum temperatures, these fluctuated between 24 and 38 °C during E3 to E12, after full flowering (E12) to fruit harvests (E14) the temperatures fluctuated between 31 and 40 °C.

### Quantification of thresholds

The minimum temperature thresholds presented during the development stage of the inflorescence are shown in Table 1. In the first flowering flow, 30 days were presented with minimum temperatures below 19 °C during E3 (swollen bud) up to E12 (full flowering), in that same period there were from 16 to 26 days with temperature thresholds of less than 16 and 18 °C, while for minimum temperature thresholds less than 15, 14 and 13 °C, they were 9, 7 and 3 days respectively.

**Table 1. Thresholds of minimum temperature and phenological stage, in two flows of flowering in mango ‘Ataulfo’, Nayarit 2018.**

Flow of Flows	Phenological stages	Days with minimum temperature below the thermal threshold (°C)												
		20	19	18	17	16	15	14	13	12	11	10	9	8
Flow 1	E3 to E12		30	26	22	16	9	7	3	0	0	0	0	0
	E12 to E14			44	39	32	27	21	12	5	2	1	0	0
Flow 2	E3 to E12			35	30	23	18	12	5	2	1	0	0	
	E12 to E14						40	38	31	18	12	2	1	0

During the period of full flowering (E12) to fruit harvests (E14) events greater than 44 days with minimum temperatures of less than 18 °C occurred, in thresholds with minimum temperatures below 15 °C the number of events reached 27. In this same period, 1 event of up to 10 °C was recorded, minimum temperature.

In the second flow of flowering, during stage E3 to E12 the number of events with minimum temperatures below 18 °C was 35, with an event recorded at 11 °C. In contrast, during the period from E12 to E14 the number of events with minimum temperatures below 15 °C was 40 and even an event with a minimum temperature of less than 9 °C was registered.

The maximum temperature thresholds presented during the development stage of the inflorescence can be observed in Table 2. The maximum temperature oscillated during the period of development of the inflorescences of 27-40 °C. In the first flowering flow, from E3 (swollen bud) to E12 (full flowering) there were 30 days with a temperature higher than 27 °C reaching up to 31 °C (six days with maximum temperature at this threshold). During the period of full flowering (E12) to fruit harvests (E14) there were 41 days of events with a maximum temperature greater than 27 °C, highlighting 13 days with maximum temperature greater than 33 °C, being 37 °C the highest temperature record, but only one day.

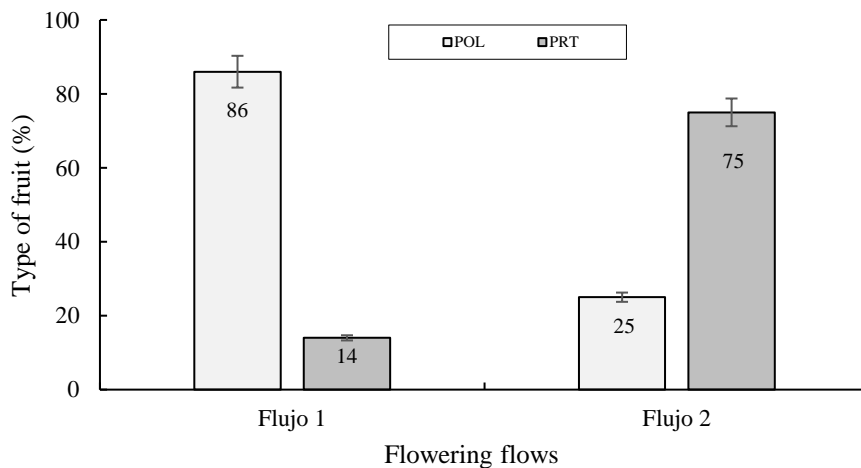
For the second flow of flowering, during the period of E3 to E12, the number of events with maximum temperatures greater than 28 °C was 33 days, being 38 °C the maximum temperature value and only occurred for one day. Contrary to this, during the period of E12 to E14 the number of events with maximum temperatures greater than 40 °C was 3, and even more than 30 events (days) with maximum temperatures greater than 35 °C were quantified.

**Table 2. Thresholds of maximum temperature and phenological stage, in two flows flowering mango ‘Aaulfo’, Nayarit 2018.**

Flow of Flows	Phenological stages	Days with maximum temperature greater than the thermal threshold (°C)													
		27	28	29	30	31	32	33	34	35	36	37	38	39	40
Flow 1	E3 to E12	30	29	25	15	6									
	E12 to E14		41	38	35	30	22	13	9	5	3	1			
Flow 2	E3 to E12		33	31	30	27	21	13	9	5	3	1			
	E12 to E14					41	38	36	34	32	24	15	9	4	3

**Percentage of parthenocarpic fruits and seeds**

Regarding the type of fruit produced by inflorescence (first flow, started in December 2017), in Figure 3 it was observed that the percentage of parthenocarpic fruits was 14%, while the production of normal fruits; that is, with seed, it was about 86%, in inflorescences that emerged in that same time. The opposite occurred in the second flowering flow, started on January 23, 2018, where 75% of the fruits produced were parthenocarpic (second flowering flow) and only 25% corresponded to seed-bearing fruits.

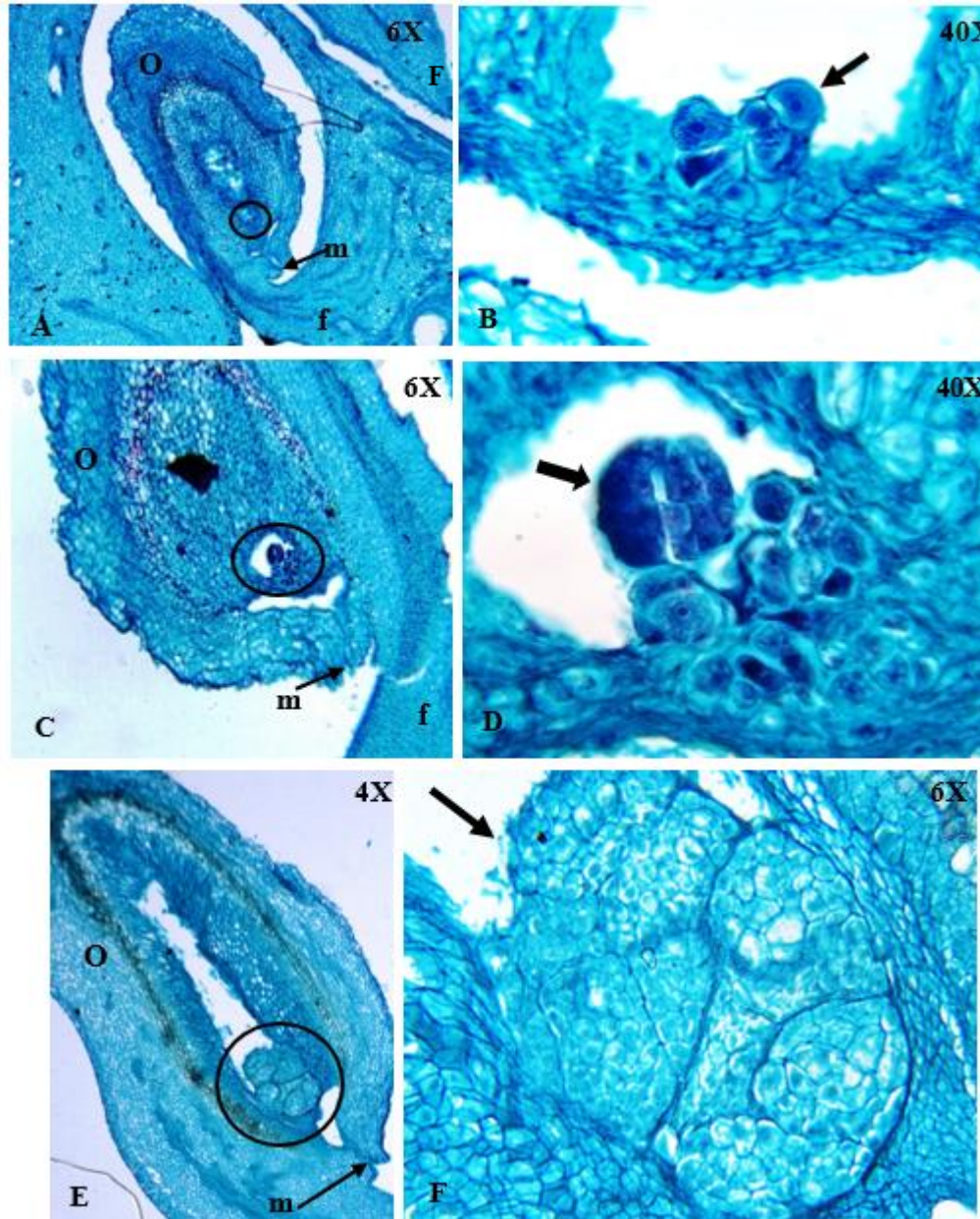


**Figure 3. Percentage of fruits with seed (POL) and parthenocarpic (PRT) by inflorescence, Nayarit.**

**Embryo development**

The results obtained in fruits collected in the different stages of development, are shown in Figure 4. The fruits presented an anatopic ovule with the degenerated embryo sac, however, the aborted embryo was observed from the early stage of fruit development, in curd (E 13) with fruits between 4 and 5 mm in length (Figure 4A and 4B), in tie or E 14 (Figure 4C and 4D) even the embryo

aborted in the globular stage was observed (fruits with a length between 1 and 2 cm). Finally, in developing fruits with a length between 4 and 5 cm, the presence of numerous embryos was analyzed (Figure 4E and F).



**Figure 4.** Embryo abortion observed, in three stages of development of parthenocarpic fruits. A= stage of fruit setting (fruits between 3 and 5 mm in length), the circle indicates the presence of the aborted embryo (6X); B= aborted embryo observed on a larger scale (40X). C= mooring (fruits between 1 and 2 cm), the circle indicates the presence of the embryo in an aborted globular state (6X); D= aborted embryo globular (40X). E= fruits in development (between 4 and 5 cm), the circle indicates the presence of numerous aborted embryos (4X) and F= same embryos on a larger scale (6X), corroborating that ‘Ataulfo’ is a type of polyembryonic fruit. O= anatopic ovule; m= micropyle; f= funiculus. Nayarit 2018.



## Discussion

In Nayarit the first flow of flowering, from start to fruit harvest, occurred in the presence of minimum temperatures of 14 °C on average and maximum around 33.5 on average. It is important to point out that, of the two flows presented, the second of them lasted for a period slightly longer than 70 days, especially the stage of full flowering (E12) until the fruit mooring (E14) and coincided with minimum temperatures in average of 12 °C and maximums above 35.5, what are considered extreme temperatures during this phenological stage.

According to Ruiz *et al.* (1999), the mango demands average annual temperatures between 24 and 27 °C, so extreme temperatures (15, 35 °C), in addition to low relative humidity during the flowering period can cause damage to floral structures and fruits in development (Singh, 2005; Ravishankar *et al.*, 2011). These last authors mention that, in the stage of full flowering to the mooring of fruits, the fertilization of the ovule occurs, and the embryo develops and subsequently the growth in size of the fruit.

Regarding the temperature thresholds presented during the entire development of flowering, in the first flow presented in Nayarit, most of the events were with minimum and maximum temperatures considered appropriate according to Singh, (2005); Ravishankar *et al.* (2011), while in the second flow, had a greater number of events with temperatures considered extreme and that cause damage to both flowering and fertilization and fruit development, these are minimum temperatures below 15 °C and greater than 35 °C.

Regarding the type of fruit produced, in Nayarit, the highest production of parthenocarpic fruits was presented in the second flowering flow, where the quantity of them increased to more 70% with respect to the first flow presented. The above is related to the period in which the fruit set (E 13) and mooring (E 14) of fruit in the second flowering and the presence of minimum temperatures lower than 15 °C and greater than 35 °C during that stage, since several studies coincide with these results.

According to Sukhvibul *et al.* (2005) exposure to low temperatures (20/10 °C, day/night) three days after manual pollination, caused an abortion of the embryo and increased the percentage of seedless fruits (nubbin= child) in mango cultivars 'Irwin', 'Kensington' and 'Nam Dok Mai', mentioning that polyembryonic cultivars were more susceptible to low temperature than monoembryonic cultivars. What coincides with the cultivar 'Ataulfo' which is a polyembryonic cultivar and is likely to be susceptible to both low and high temperatures during the flowering period.

Based on the above, surely the temperatures presented in the period of full flowering and fruit binding damaged the development of the embryo causing its abortion, since it is at this stage where the fertilization, development of the embryo and the subsequent growth takes place of the fruit as mentioned by Singh (2005); Ravishankar *et al.* (2011). On the other hand, Issarakraisila *et al.* (1992) found that 60% of the mango flowers of the Kensington cultivar, which developed at night temperatures between 7 and 15 °C, had short styles and poor development of pollen tubes.

In the same cultivar, temperatures lower than 15 and above 33 °C during the flowering period reduced the viability of pollen grains and night temperatures below 10 °C decreased its germination by more than 50% (Issarakraisila and Considine, 1994). However, in this study the greatest number of events with minimum temperature thresholds below 15 °C and maximum above 35 °C, occurred in full flowering until fruit tie in the second flowering flow.

The aborted embryos found in the different stages of development of mango ‘Ataulfo’ fruits, indicates that in these there was pollination and fertilization and that possibly the embryo was damaged by temperatures less than 15 °C and greater than 35 °C presented from E 13 to E 14, which coincides with that reported by Wilely *et al.* (1988); Sukhvibul *et al.* (2000a) defining these fruits as stenospermocarpiacs, a type of parthenocarpy in which there is pollination and fertilization but the newly fertilized embryo is aborted (Vardi, *et al.*, 2008). Contrary to this, Salazar-García *et al.* (2016) found no evidence of stenospermocarpy in fruits without mango ‘Ataulfo’ seed; however, in their work they did not perform histological studies and only observed macroscopic characteristics of longitudinally sectioned seedless fruits.

On the other hand, Huang *et al.* (2010) mention that there was no sexual reproduction in mango fruits ‘Tainong 1’ when they develop at maximum daytime temperatures <20 °C, due to slow growth of the pollen tube and low fertilization rate. In fruits with seeds, through histological studies of high quality, they observed the development of the embryo in its different stages of development, from globular to torpedo shape. In this study, in seedless fruits of ‘Ataulfo’ embryos were observed in globular state in the first stages of development, but aborted, and even in degenerated ova, multiple aborted embryos were observed, which is related to the type of polyembryonic fruit to which the cultivar ‘Ataulfo’ belongs.

## Conclusions

In Nayarit, the flowering period, from the beginning to the mooring of the fruit, occurs from mid-December to February or March and it happened in two floral flows.

The second flowering flow lasted for a period slightly longer than 70 days and the stage of full flowering until the fruit felling coincided with extreme temperatures, minimum of 15 °C on average and maximum above 35 degrees.

The highest production of parthenocarpic fruits occurred in the second flowering flow in Nayarit, while in the first the production of fruits with seed exceeded the parthenocarpic ones.

In Nayarit, the presence of parthenocarpic fruits is related to extreme temperatures ( $\leq 15$  and  $\geq 35$  °C) during the period of full flowering until fruit mooring, causing embryo abortion.

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