

## Prediction models of the floral development of the 'Mendez' avocado

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### Abstract

The availability of prediction models provides an opportunity to forecast important events of avocado flower development. The objective of this research was to develop models of prediction, generation in temperature, floral development in 'Mendez' avocado. The investigation was carried out in two commercial orchards of the company Agro Gonzalez in the south of the state of Jalisco, Mexico. In each orchard 10 trees were selected and in each of them 30 shoots were labeled at the beginning of each vegetative flow (FV) of winter (February 2014 and 2015) and summer (August 2014 and 2015). From each marked tree an apical bud was collected monthly or biweekly as the anthesis date approached. With the record of the minimum temperatures (from 8 to 20 °C) the cold days (DF) were calculated and accumulated for each sampling period of years, denominating them accumulated cold days (DFA). Through the regressions, the temperatures associated with the floral development were identified and mathematical prediction models were obtained. Subsequently, the ability to predict the floral development of the best prediction models for all FV in 2014 against the same FV in 2015 and vice versa was evaluated. After verification of the difference between years, a regression model is obtained for each FV by integrating the information of the years into a single data set. The floral development of apical buds of the winter and summer FV was associated with temperatures  $\leq 17$  (126 DFA) and  $\leq 15$  °C (138 DFA), respectively, and could be modeled mathematically.

**Keywords:** *Persea americana* Miller, cold days, ecophysiology, flowering.

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## Introduction

In the avocado (*Persea americana* Mill.), the time elapsed since the vegetative shoots finish their growth and begin their floral development until anthesis is variable and depends mainly on the cultivar and the climate in which it develops (Salazar-García, 2002). In South Florida the floral development process of the 'Choquette' avocado, from the time the buds were in 'rest' (January) until the maximum floral opening (April) covered three months. In the case of cv. Booth-8 this same process requires four months (December-April) (Davenport, 1982).

In the semi-arid Mediterranean climate of southern California the outbreaks of summer vegetative flow of cv. Hass needed 7.5 months from the flower initiation (August) until before (April). The development of the flowers was preliminarily related to minimum temperatures  $\leq 15$  °C (Salazar-García *et al.*, 1998). In the subhumid semi-warm climate of the state of Nayarit, Mexico, this same avocado cultivar requires almost 12 months for winter outbreaks (before and after the previous year). The summer months, five months younger than those of winter before, 7.5 months before arriving, so that the flowering occurred on the same date for the floral buds of both vegetative flows.

The annual averages of maximum temperatures fluctuated from 26.8 to 33.4 °C and the minimum from 9.3 to 20.4 °C (Salazar-García *et al.*, 2007). In a study conducted in six types of climate in the state of Michoacán, Mexico, the floral development process in outbreaks of winter, spring and summer flow, which produced normal (main) flowering, elapsed time was: 13 to 13.5, 9.5 to 10.5 and 7 to 7.5 months, respectively. In all climates, mean air temperatures vary from 14 °C to 24 °C (Rocha-Arroyo *et al.*, 2011).

The water stress has not been effective to promote the flowering of the 'Hass' avocado. Studies conducted under controlled conditions that had the only factor associated with avocado flower initiation was the decrease in temperature (Chaikiattiyos *et al.*, 1994). In 'Fuerte' avocado, it was found that the absence of high temperatures ( $\geq 20$  °C), more than the presence of low temperatures, was responsible for the floral initiation (Buttrose and Alexander, 1978). In another study in growth chambers, Nevin and Lovatt (1989) promoted the flowering of young 'Hass' trees with low temperature stress (8 h day from 15 °C to 18 °C and 16 h night from 10 °C to 13 °C).

At the end of four or eight weeks the trees were transferred to the greenhouse with an approximate temperature of 24 °C, 12-h day and 19 °C, 12-h night. The intensity of flowering was the same for the plants treated with four or eight weeks of low temperature and the development of floral buds was similar in time, except for the anthesis date, which occurred a week later in the trees that received eight weeks of low temperature. Control trees (24 °C, 12-h day and 19 °C, 12-h night, throughout the experiment) did not flower. In another study in growth chambers, Salazar-García *et al.* (1999) obtained similar results because after four weeks of stress due to low temperature ( $\leq 15$  °C) the apical buds of young 'Hass' trees reached the irreversible determination towards flowering (DIF). Subsequent work carried out in 'Hass' adult orchards in Nayarit, Mexico, resulted in an association between temperatures  $\leq 19$  °C and DIF (Salazar-García *et al.*, 2006).

Prediction models that simulate the growth and development of crops are important tools for modern agricultural research because they allow the simple representation of physiological processes through mathematical equations (López Cruz *et al.*, 2005). Since the floral development of the avocado has been truthfully associated with temperature, prediction models have been developed for several regions of Mexico. In the semi-warm climate of Nayarit, the process of floral development of the cv. Hass with the accumulation of cold days (DFA) with minimum temperatures  $\leq 21$  and  $\leq 19-20$  °C; this for outbreaks of winter and summer vegetative flows, respectively (Salazar-García *et al.*, 2007).

Subsequently, these prediction models were tested in four types of climate of the Michoacán avocado belt (semi-warm dry sub-humid, semi-warm sub-humid, sub-humid temperate and humid temperate). Of all the prediction models developed in Nayarit, only the model for summer shoots DFA  $\leq 19$  °C presented a high predictive capacity ( $R^2 = 0.94$ ) of floral development in summer shoots in the four climates of Michoacán where it was presented this vegetative flow (Salazar-García *et al.*, 2009).

The availability of prediction models provides the opportunity to predict important events or alterations of avocado floral development such as DIF, cauliflower status (elongation of secondary axes of the inflorescence, meiosis has occurred in the anther locules and microspores are evident) or before (the stigma is receptive and pollen can be released) (Salazar-García *et al.*, 1998). With this you can have sustenance for planning and making decisions about some practices of garden management, such as the introduction of pollinating bees, pruning, control of pests and diseases that occur during flowering, fertilizers and authorized bioregulators to increase its effect and its benefit-cost relationship.

The avocado 'Mendez No. 1' (Plant Patent 11,173 USA 2002), known in Mexico as 'Mendez', has economic importance in the south of the state of Jalisco, where there are more than 6 000 hectares planted. In this region, its main annual harvest occurs in the summer, reaching the highest prices of the year, different from the 'Hass' that takes place in autumn-winter. No information is available on the floral development process of 'Mendez', so the objective of this research was to develop prediction models, based on temperature, of floral development in 'Mendez' avocado.

## Materials and methods

### Characteristics of the gardens

The research was carried out during two production cycles (2014-2015 and 2015-2016) in two commercial avocado orchards 'Mendez' grafted on Creole rootstocks of the company Agro González, SPR of RL, with fertigation, Feozem haplico soil and semi-warm climate subhumid [AC(w)] (García-Amaro, 1998) from the south of the state of Jalisco. The garden "Colorin 1" is located in Atequizayan, Municipality of Zapotlan el Grande and the garden "Ocote cuate 2" is in Zapotiltic, Municipality of Zapotiltic. For these orchards, the altitude is 1 556 m and 1 428 m, the distance between trees 7x 3.5 m and 5x5 m, the age at the beginning of the study of 4 and 6 years, respectively. The orchards received the standard handling of the producer.

## Sampling and processing of yolks

In each orchard, 10 trees were selected without cross-linking and with a production history of at least 50 kg tree<sup>-1</sup>. Each tree was marked 30 shoots at the beginning of each vegetative flow of winter (February 2014 and 2015) and summer (August 2014 and 2015). From each marked tree an apical bud was collected at monthly or biweekly intervals as the anthesis approached. For the vegetative flow of winter (both years) the samplings were carried out from February to October and for summer from August to March of the following year.

The buds were fixed in FAA (formaldehyde:acetic, acid: ethanol, 5:5:90, v:v:v) and then placed in a vacuum hood (Nalgene 8040317, Nalgen Company) at 30 KPa for 5 h. Subsequently, they were classified under a stereoscopic microscope (Zeiss Stereomikroskop Mod. Stemi 2000-C, Carl Zeiss, Göttingen, Germany), with the visual scale of Salazar-García *et al.* (1998) which comprises from E-1 (vegetative bud) to E-11 (anthesis).

## Calculation of cold days (DF)

In each orchard, the air temperature was recorded every 15 min with automated recorders HOBO H8 (Onset Computer, Witzprod, Englewood Cliffs, NJ, USA) operated with batteries. Independently for outbreaks of winter and summer vegetative flows, the occurrence of minimum temperatures from 8 to 20 °C was measured in increments of 1 °C. The formula was used:  $DF = (T_{min} \leq T, 1.0)$ ; where:  $T_{min}$  = minimum registered temperature;  $T$  = critical temperature, from 8 to 20 °C. If the temperature condition is met, then the value of DF is 1, otherwise it is 0. Using the Excel program (2010). The values of  $DF_8$  to  $DF_{20}$  were accumulated for each period of sampling of apical buds. These values were called accumulated cold days (DFA). As zero day was considered when the apical buds were in E-1 (vegetative stage), which occurred in February (winter shoots) and August (summer shoots).

## Identification of the temperature associated with floral development

The DFA were used as independent variables and the state of floral development of the apical buds in shoots of each flow as a dependent variable. The Stepwise SAS/STAT procedure (SAS, 2011) was used to select the best model in order of response (second to fifth order) for each selected temperature (from  $\leq 8$  °C to  $\leq 20$  °C, in 1 °C intervals). The criteria for choosing the best prediction models were: 1) higher value of  $R^2$ ; 2) lower mean square error (CME); 3) the value of  $C_p$  (Draper and Smith, 1981).

Once the best prediction models were identified, their mathematical coefficients ( $B_0, \dots, B_n$ ) were calculated by the REG procedure using the DFA, from  $\leq 8$  °C to  $\leq 20$  °C, at intervals of 1 °C.

## Test of the best prediction models

The ability to predict the floral development of the best prediction models obtained for each vegetative flow in year 1 against the same vegetative flow in year 2 and vice versa was evaluated. The predicted floral development values were analyzed by means of a regression against the observed values of the floral development of the year and corresponding vegetative flow, using the

Excel program (2010). The criteria to determine if the values of the two years belonged to a single population were: 1) that the order of the origin of the regression was closest to one ( $B_0= 1$ ); 2), that the slope was closest to one ( $B_1= 1$ ), and 3) the highest value of the coefficient of the adjusted model ( $R^2$ ). This procedure served to debug models and find the best one.

After verification of the no difference between years, a regression model was obtained for each vegetative flow integrating the information of the two years into a single data set.

## Results and discussion

### Floral development

The state of floral development of apical shoot buds of vegetative flows of winter and summer, were used to generate the models of floral development prediction (Tables 1 and 2). On average, floral development (vegetative yolk to anthesis) in outbreaks of winter and summer vegetative flow took 216 and 187 days, respectively. The shorter time required for the 'Mendez' summer buds to complete their floral development could be due to the fact that when they emerged (August-September) the environmental temperature had already started its descent (average of 18 °C), suppressing the vegetative growth and stimulating floral development.

**Table 1. State of floral development of buds of the vegetative flow of winter 2014 and 2015 in 'Mendez' avocado orchards used in the research.**

Orchard "Colorín 1"		Orchard "Ocote Cuate 2"	
Date	Floral development <sup>z</sup>	Date	Floral development <sup>z</sup>
Vegetative flow of winter 2014			
February 27, 2014	1.1	February 27, 2014	1.1
march 29	2	March 29	2
April 28	3.4	April 28	3.2
May 28	4.6	May 28	4.5
Jun 27	4.8	Jun 27	4.9
July 27	6.1	July 27	5.5
August 11	7.8	August 26	7.2
August 25	8.7	September 10	8.7
September 10	10.6	September 25	10.1
September 25	11	October 10	11
Vegetative flow of winter 2015			
February 25, 2015	1.3	February 26, 2015	1.3
March 27	2.3	March 27	2.5
April 26	3.6	April 26	3.4
May 26	4.4	May 26	4.3
Jun 25	4.7	Jun 25	4.5
July 25	5.3	July 25	5.7
August 24	6.5	August 24	6.3
September 08	10.2	September 08	7.8
September 23	11	September 23	10.7
		October 08	11

<sup>z</sup>= according to the visual scale of Salazar-García *et al.* (1998).

**Table 2. State of floral development of buds of vegetative flow of summer 2014 and 2015 in 'Mendez' avocado orchards used in research.**

Orchard "Colorín 1"		Orchard "Ocote Cuate 2"	
Date	Floral development <sup>z</sup>	Date	Floral development <sup>z</sup>
Vegetative flow of summer 2014			
August 26, 2014	1.1	August 26, 2014	1.1
September 25	2.6	September 25	2.4
October 26	3.9	October 26	3.2
November 25	6.1	November 25	4.5
December 28	7.9	December 28	5.6
January 24	9.7	January 24	8.6
February 23	11	February 23	11
Vegetative flow of summer 2015			
August 24, 2015	1.1	August 24, 2015	1.1
September 23	2	September 23	2.1
October 26	2.5	October 26	2.5
November 26	3.2	November 26	3
December 25	5.8	December 25	4.8
January 27, 2016	6.5	January 27, 2016	6.5
February 11	7.3	February 11	8.2
February 18	8.5	February 18	9.7
February 26	10.1	February 26	10.5
March 04	11	March 04	11

<sup>z</sup>= according to the visual scale of Salazar-García *et al.* (1998).

### Effect of environmental temperature on floral development

Of all the minimum temperatures used ( $\leq 8$  °C to  $\leq 20$  °C),  $\leq 17$  and  $\leq 15$  °C were the best associated with floral development in outbreaks of winter and summer vegetative flows (two years in each case), respectively (Tables 3 and 4). Buttrose and Alexander (1978) and Salazar-García *et al.* (1999) found in young trees of 'Fuerte' and 'Hass', respectively, that under controlled conditions constant temperatures  $> 20$  °C did not promote flowering.

Later, in a field study with 'Hass' avocado in Nayarit, Mexico, Salazar-García *et al.* (2007) associated the temperatures  $\leq 21$  °C with the floral development of outbreaks of winter flow and  $\leq 19$  and  $\leq 20$  °C with that of summer shoots. In an investigation carried out in Michoacán (subhumid semi-warm climate) it was found that temperatures of  $\leq 19$  to  $\leq 21$  °C were related to the floral development process in outbreaks of winter and summer flows of cv. Hass (Salazar-García *et al.*, 2009).

In the present study, the floral development process from buds in state E-1 (term of vegetative shoot lengthening) to anthesis (E-11) in winter buds for 2014 and 2015 accumulated 111 and 140 DFA ( $\leq 17$  °C), respectively. In summer outbreaks 133 DFA (2014) and 143 DFA (2015) were

accumulated at temperatures  $\leq 15$  °C. In the two years of the study, an average of 126 and 138 days were accumulated with temperatures  $\leq 17$  and  $\leq 15$  °C for winter and summer outbreaks, respectively.

**Table 3. Selected models of floral development prediction in outbreaks of the vegetative flows of winter 2014 and 2015, which bloomed in summer 2014 and 2015, respectively, in ‘Mendez’ avocado trees.**

Temperature	Bo	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>	B <sub>5</sub>	B <sub>6</sub>	R <sup>2</sup>	Cp	CME
Parameters (Winter buds 2014)										
$\leq 15$ °C	1.51			-6.62E-05	2.65E-06	-2.92E-08	9.92E-11	0.76	4.66	3.07
$\leq 16$ °C	1.55			7.13E-06	-3.42E-08		1.15E-13	0.9	2.83	1.24
$\leq 17$ °C <sup>z</sup>	1.13	0.006		2.77E-05	-4.75E-07	2.83E-09	-5.59E-12	0.99	5.13	0.13
$\leq 18$ °C	1.04		0.001	-1.29E-05		4.243E-10	-1.19E-12	0.95	4.07	0.65
$\leq 19$ °C	1.05		0.001	-1.26E-05		4.10E-10	-1.15E-12	0.95	4.08	0.69
Parameters (Winter buds 2015)										
$\leq 15$ °C	-2.4	0.227		-1.58E-04	3.08E-06	-2.15E-08	5.10E-11	0.8	4.79	2.59
$\leq 16$ °C	0.71	0.00184	0.00158	-2.57E-05	1.65E-07	-3.51E-10		0.89	3.97	1.51
$\leq 17$ °C <sup>z</sup>	1.67	-0.0245		2.89E-05	-4.12E-07	2.08E-09	-3.53E-12	0.98	4.21	0.33
$\leq 18$ °C	1.51	-0.015		2.25E-05	-3.05E-07	1.45E-09	-2.31E-12	0.98	4.1	0.36
$\leq 19$ °C	1.51	-0.0146		2.23E-05	-3.02E-07	1.43E-09	-2.28E-12	0.97	3.03	0.39

<sup>z</sup>= best models.

**Table 4. Selected models of floral development prediction in outbreaks of vegetative flows of summer 2014 and 2015, which bloomed in winter 2015 and 2016, respectively, in ‘Mendez’ avocado trees.**

Temperature	Bo	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>	B <sub>5</sub>	B <sub>6</sub>	R <sup>2</sup>	Cp	CME
Parameters (Summer buds 2014)										
$\leq 13$ °C	2.06	0.3	-0.01	1.44E-04		-1.19E-08	5.49E-11	0.96	6	0.78
$\leq 14$ °C	1.84	0.18	-0.004	3.93E-05	-1.43E-07			0.98	4.6	0.35
$\leq 15$ °C <sup>z</sup>	1.6	0.11	-0.001	1.05E-05	-1.82E-10			0.98	3.02	0.31
$\leq 16$ °C	1.71			4.63E-05	-8.52E-07	5.76E-09	-1.34E-11	0.98	5.28	0.44
$\leq 17$ °C	0.08	0.05			-1.56E-08	2E-10	-5.86E-13	0.97	4	0.47
Parameters (Summer buds 2015)										
$\leq 13$ °C	1.44	0.0559		-2E-04	9.67E-06	-1.41E-07	6.49E-10	0.835	4.27	2.62
$\leq 14$ °C	1.33	0.0113		3.3E-05	-9.33E-07	9.58E-09	-3.20E-11	0.882	3.99	1.88
$\leq 15$ °C <sup>z</sup>	1.51	0.005	0.0011		-5.95E-07	1.07E-08	-5.09E-11	0.943	4	0.91
$\leq 16$ °C	-1.65	0.1973	-0.0038	3.21E-05	-8.23E-08			0.915	4.24	1.34
$\leq 17$ °C	-0.82	0.1123	-0.0016	9.13E-06		-5.98E-11		0.968	4	0.51

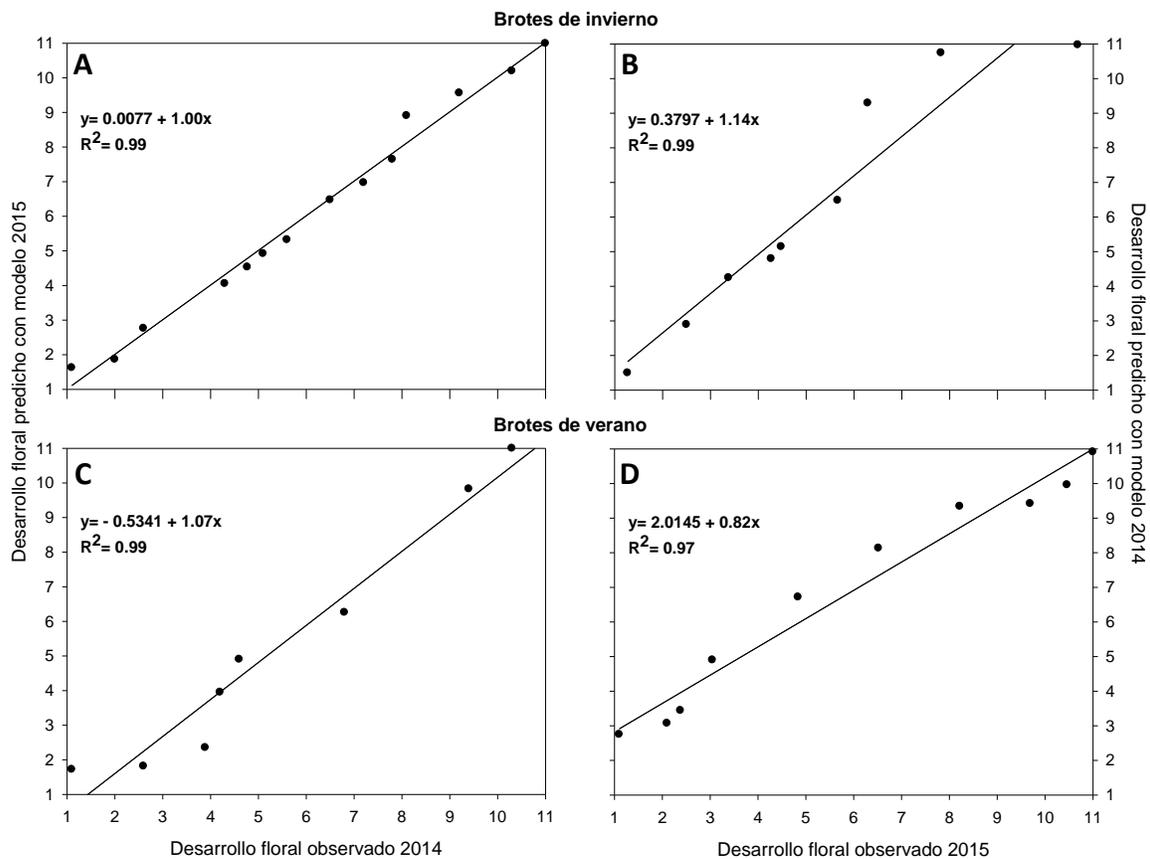
<sup>z</sup>= best models.

The amount of cold accumulated by the apical buds of the shoots of both vegetative flows was lower than that found for ‘Hass’ in the semi-warm subhumid climate of Nayarit for winter shoots (179 DFA with temperatures  $\leq 21$  °C) and summer (183 and 201 DFA with temperatures  $\leq 19$  and

$\leq 20$  °C, respectively) (Salazar-García *et al.*, 2007). In Michoacán, for this same cultivar and type of climate, 375 (winter) and 198 (summer) DFA were required with temperatures  $\leq 19$  and  $\leq 21$  °C (Salazar-García *et al.*, 2009). The differences described show the genetic plasticity of the avocado to different environments in which it is grown and the apparent irrelevance of establishing a unique or critical value of temperature associated with floral development since the “cold” necessary to initiate and complete a development cycle floral depends on the temperatures that occur in each producing region.

### Verification of the no difference between year 1 (2014) vs year 2 (2015)

The verification of the floral development prediction models (adjustment of observed vs. predicted values) was performed reciprocally (year 1 vs year 2 and vice versa), to determine if the data for the two years were statistically equal (Figures 1A-D). For winter outbreaks the DFA model  $\leq 17$  °C of year 2 showed a high predictive capacity of floral development for year 1 ( $R^2= 0.99$ ) (Figure 1A). Something similar was obtained when using the model of year 1 to predict floral development in year 2 ( $R^2= 0.99$ ) (Figure 1B). For summer outbreaks the DFA model  $\leq 15$  °C of year 2 was tested, which showed a good predictive capacity of floral development for year 1 ( $R^2= 0.99$ ) (Figure 1C). Something similar was obtained when using the model of year 1 to predict the floral development in year 2 ( $R^2= 0.97$ ) (Figure 1D).



**Figure 1.** Adjustment of values between the state of floral development observed in year 1 (2014) vs. the floral development predicted obtained for the year 2 (2015) and vice versa in outbreaks of the winter flow with the models DFA  $\leq 17$  (A and B) and summer DFA  $\leq 15$  °C (C and D).

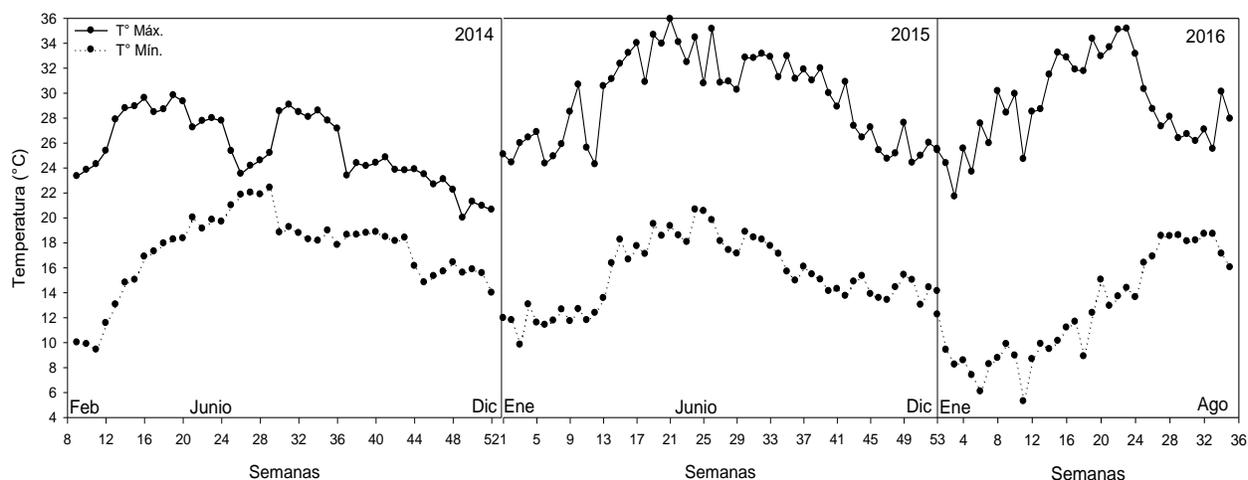
### New models generated with two-year data

The prediction models for outbreaks of the winter and summer flow of year 1 were able to predict the floral development of year 2 and vice versa. Before this, a single data set was integrated with information of two years for each type of outbreak, obtaining two new prediction models: winter  $\leq 17\text{ }^{\circ}\text{C}$  and summer  $\leq 15\text{ }^{\circ}\text{C}$  (Table 5). These models were incorporated into an application ([www.cesix.inifap.gob.mx/frutalestropicales/desarrollofloralmendezjalisco.php](http://www.cesix.inifap.gob.mx/frutalestropicales/desarrollofloralmendezjalisco.php)) which shows the floral development of each vegetative flow of the 'Mendez' avocado. In this way, it is possible to use this information for the best programming of the garden management activities that could inhibit or diminish the intensity of flowering, or plan the introduction to the orchard of pollinating insects. Another use of floral development prediction models is to support students, technicians and researchers involved in the cultivation of 'Mendez' avocado.

**Table 5. Prediction models of the 'Mendez' avocado floral development obtained from data from two years (2014 and 2015) and based on DFA with temperatures  $\leq 17\text{ }^{\circ}\text{C}$  (winter outbreaks) and  $\leq 15\text{ }^{\circ}\text{C}$  (outbreaks of summer).**

Temperature	B <sub>0</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>4</sub>	B <sub>5</sub>	B <sub>6</sub>	R <sup>2</sup>	C <sub>p</sub>	CME
Parameters (Winter buds)										
$\leq 17\text{ }^{\circ}\text{C}$	1.2436	-0.0129		0.000038	-5.24E-07	2.6E-09	-4.3E-12	0.69	3.98	3.43
Parameters (Summer buds)										
$\leq 15\text{ }^{\circ}\text{C}$	1.79412	-0.0399	0.0055	-0.00014	0.0000015	-7.77E-09	1.43E-11	0.85	5.04	2.14

Temperature record. During the study period, the lowest minimum temperatures occurred in the first quarter of 2016 (Figure 2). In week six and 11, 6 and 5  $^{\circ}\text{C}$  were registered, respectively. On the other hand, in 2014 between week 25 and 29 (last of June and July, respectively) the minimum temperatures reached on average 21.8  $^{\circ}\text{C}$ .



**Figure 2. Maximum and minimum air temperatures recorded February 2014 to August 2016. Weekly average of data of the “Colorín 1” and “Ocote cuate 2” orchards.**

## Conclusions

In this research for the first time, the floral development of apical buds of the vegetative flows of winter and summer of 'Mendez' avocado was modeled mathematically at temperatures  $\leq 17$  (126 DFA) and  $15\text{ }^{\circ}\text{C}$  (138 DFA) respectively. The differences between these temperatures and those found for other avocado cultivars, including Hass, show the genetic plasticity of the avocado to adapt to the different environments in which it is grown and the irrelevance of establishing a unique or critical temperature value associated with the avocado floral development since the "cold" necessary to initiate and complete a cycle of floral development depends on the temperatures that occur in each producing region.

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