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Temporal variations in the flowering of 'Honeycrisp' apple grafted on eight different rootstocks

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Abstract: *Malus domestica* is one of the world's most important deciduous fruit trees. Over a four-year period (2017–2020), temporal variations in flowering were evaluated in 'Honeycrisp' apple trees on eight rootstocks (G.30, G.969, G.202, G.41, G.11, M.9T337, M.26 EMLA and V.6) planted in 2014 in Chihuahua, México. Among the variables evaluated were the probability of late-spring frost, winter chill units, growing degree days, flowering period, foliar nutrient concentrations, trunk cross-sectional area, number and weight of fruit per tree, and production efficiency. Significantly different chill unit accumulations occurred over the four years, with values falling between 974 and 1 415, where for the latter value, the start of flower opening was earlier, but there was a higher risk of damage by temperatures ≤ –2 °C. There was no effect of rootstock on the time of onset and end of flowering. The most productive combinations were 'Honeycrisp' on G.969, G.11 and V.6 with yield estimates of 35 300, 34 200 and 33 600 kg/ha, respectively. The commercial production of 'Honeycrisp' apple trees requires the evaluation of their agronomic performance with different rootstocks. Flowering is particularly important since this phenological stage is so closely linked to productivity and is strongly affected by variations in winter temperatures.

Keywords: chill units; climate change; foliar nutrients; *Malus domestica*; trunk cross-sectional area; Weibull model

Fossil fuels (coal, oil, and gas) are by far the largest contributors to global climate change, accounting for over 75% of global greenhouse gas emissions and nearly 90% of all carbon dioxide emissions (Li et al. 2020). Climate change is the abiotic factor likely to pose the greatest threat to global food security in the future. One channel for this threat is via limitations in the geographical distribution and productivity of many food plants. It is considered that such limitations will apply more strongly to horticultural crops than to arable crops (Ru et al. 2023). Thus, the yield and the quality of many fruit and vegetable species depend on the stability of the air temperature and solar radiation intensity and the frequency, evenness and amount of rainfall (Ahmadi, Baaghideh 2018). However, temperate fruit trees have been

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the most affected group. In their annual growth cycle, these crops have a specific requirement for chilling during the winter months (Kishore et al. 2015). This requirement has not been fully satisfied lately in most areas that were only marginally suitable for temperate fruit cultivation.

The domesticated apple (*Malus domestica* Borkh.) is a major fruit crop in many countries, including many countries and/or regions in which the climate is unideal or even marginal for this species. Especially in these marginal regions, instabilities in temperature and rainfall already lead to problematic variabilities in cold-unit accumulation and elevated risk of damage to flowers by late-spring or fruit by early-autumn frosts (Ramírez-Legarreta et al. 2011). Furthermore, continual exposure of a tree to environmental stress also has more complex effects on productivity, with such environmental pressures generating conditions in which the occurrence and severity of challenges from various pests and diseases also rise (Singh et al. 2016). Climate change is expected to exacerbate rather than alleviate most of these challenges.

Numerous studies have shown that fruit quality and yield in apples are affected by winter chill and also by the more general weather conditions during the about six-month growing season (Petri, Leite 2003; Singh et al. 2016; Ru et al. 2023). Flowering is the most important phenological phase for most fruit crops, with the sequential stages of flower induction, initiation, opening, pollination and fruit set al. being especially susceptible to the vagaries of the weather (Campoy et al. 2019). In temperate fruit trees, including apple trees, it has been demonstrated that exposure to high winter cold accumulation leads to early flowering but increases the risk of damage from late-spring frosts (El Yaacoubi et al. 2014). Conversely, the flowering process is prolonged, negatively impacting agronomic management and tree architecture, and significantly reducing productivity and fruit quality (Drepper et al. 2022). Apple's sensitivity to temperature differs among the various commercial apple cultivars. Moreover, the tree's temperature sensitivity is also affected by the choice of rootstock. Ideally, apple requires significant periods of low winter temperatures (~1 200 chill units at temperatures between 1.4 °C and 12.4 °C) to overcome winter dormancy, and this should be followed by a longish period (~3 500 h) of warmer, stable temperatures during fruit development (Ramírez-Legarreta et al. 2011). In many regions where apples are grown, these climatological requirements are not sufficiently met, making it necessary to apply chemicals to trigger bud-break, and/or implement early- or late-season frost controls, and/or erect hail nets, and/or apply cocktails of fungicides and pesticides to mitigate crop variability. However, all these crop management interventions raise both the cost of production and also, in many cases, the cost to the environment (Li et al. 2020).

In México, around 58 000 ha of apple trees are established in arid and semi-arid regions and at altitudes between 1 900 m and 2 700 m above sea level (SIAP 2023). Here, the winter cold accumulations are highly variable, resulting in the chill requirements of many cultivars not being fully met. In such cases, sprouting promoters are sprayed (Petri, Leite 2003). However, some of these chemicals are carcinogenic and/or have negative impacts on the environment. In the short term, these spray strategies help growers remain profitable. However, they still affect economic sustainability through the cost of agrichemicals and the labour and diesel needed to apply them (Petri et al. 2014).

Among the numerous apple cultivars grown in North America and Europe, 'Honeycrisp' is widely appreciated for its bicoloured fruit with unique quality and palatability characteristics of juiciness, crisp texture and distinct flavour (Marini et al. 2020). It is also among the most important commercial cultivars, being second only to 'Gala' and 'Red Delicious' (Sherif 2022). However, in México, production and management of this cultivar are complicated by its high susceptibility to physiological disorders, including alternate bearing, sunburn, epidermal scald and bitter pit (Al Shoffe et al. 2020; Serra et al. 2020). One or another of these disorders can affect between 20 and 75% of all fruit produced. Ways to diminish their impacts include pruning, fruit thinning, foliar applications of Ca^{2+} and careful balancing of soil nutrients, including K^{+}, Mg^{2+}, P, Ca^{2+} and N (Valverdi, Kalcsits 2021).

Tree anchorage and mineral nutrient uptake are the basic functions of the root system of any rootstock (Fazio et al. 2016). Rootstocks are used extensively in fruit tree production systems, including in apples. The different commercial rootstocks available differ in their levels of adaptation to soil conditions, including salinity, pH, moisture content, pathogen resistance and nutrient uptake, and they also influence the size, vigour and health of the scion tree (Valverdi et al. 2019). Furthermore, the rootstock-cultivar interaction can significantly influence fruit

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quality, including its flavour, colour, mineral content, bioactive compounds and size, and the mineral content of the leaves (Valverdi, Kalcsits 2021).

The occurrence and severity of physiological disorders in 'Honeycrisp' are due mainly to the physicochemical properties of the soil, the prevailing climatic conditions (high ambient temperatures and solar radiation and low relative humidities) and the rootstock (Al Shoffe et al. 2020; Li et al. 2020; Marini et al. 2020). These sets of factors limit the planted area of 'Honeycrisp' in México and so create the need to generate basic information to help understand the effects of seasonal temperature variation on the accumulation of winter chill units and to minimise irreversible cell damage to the floral structure caused by late-spring frosts. Therefore, the objective of this research was to evaluate seasonal variations in flowering in 'Honeycrisp' apples in response to winter chill accumulations on a selection of rootstocks.

MATERIAL AND METHODS

Study area, plant material and experimental design. This study was conducted over the 2017–2020 fruiting cycles using cv. 'Honeycrisp' apple trees, established in 2014 on eight different rootstocks G.30, G.969, G.202, G.41, G.11, M.9T337, M.26 EMLA and V.6. Trees were trained to a tall spindle training system and spaced at 1.22 m within the row and 3.66 m between rows (2 240 trees/ha). The study orchard is part of the multinational project NC-140 (Canada-México-USA) and is located in Cuauhtemoc, Chihuahua, México (28°28'33.2688"N, 106°58'57.50"W) at an altitude of 2 129 m, with a mean annual temperature of 18 °C and mean annual precipitation of 496 mm. The physicochemical properties of the soil in the arable depth (0–30 cm) were soil texture, sandy clay loam, comprising sand 56%; silt 20%; clay 24%; pH 6.8; organic matter 5.5%; 1.39 dS/m EC (electrical conductivity), 8.8 mg/kg NO_3^-; Ca 2 963 mg/kg (low); Fe 79.8 mg/kg (moderately low); Cu 2.17 mg/kg (moderately low). Nutrient supplements were applied by surface applications of dry fertiliser (140 N : 80 P_2O_5 : 100 K_2O). Standard commercial practices for weed control and irrigation scheduling were followed throughout the trial.

The trial was set up as a completely randomised design. The eight rootstocks, G.30, G.969, G.202, G.41, G.11, M.9T337, M.26 EMLA, and V.6, were planted randomly along a row with five replications per rootstock. Each 'Honeycrisp' scion/rootstock combination was considered an experimental unit.

Late-spring frosts, chill units and growing degree days. The occurrence of late-spring frosts was estimated using the method described by Ortiz-Solorio (1987), involving recordings of the hourly temperatures below –2 °C (between January and April, from 2000 to 2020). This temperature threshold corresponds to the point at which apple blossoms are vulnerable to irreversible damage by late-spring frosts. Data were recorded using a Las Quintas Lupita weather station owned by the Union Agricola Regional de Fruticultores del Estado de Chihuahua (UNIFRUT) to verify and validate the temperature.

To calculate the off-season chill units (CU), the model is based on the accumulation of CU, where 1 CU equals 1 h exposure at 6 °C. The chilling contribution becomes less than 1 as temperatures rise above or drop below the optimum value. A negative contribution to the CU accumulations occurs at temperatures above 15 °C, and a zero contribution occurs below 0 °C. The procedure described by Richardson et al. (1974) was used from the beginning, consisting of the accumulation of CU in autumn until February 28th in 2016–2017, 2017–2018, 2018–2019 and 2019–2020 from hourly temperature data obtained with a RHT10 datalogger sensor (Extench Instruments®, USA).

Growing degree days (GDD) were similarly recorded from March 1st to April 30th (petal fall) based on the procedure proposed by Anderson et al. (1986) for the calculation of growing degree hours, which consists of two cosine equations.

$$GDH = FA/2\ (1 + \cos\ (\pi + \pi\ (TH - TB)/(TU - TB))) \quad (1)$$

If the values of TH are greater than TU, equation 2 is used.

$$GDH = FA\ (1 + \cos\ (\pi/2 + \pi/2\ (TH - TU)/(TC - TU))) \quad (2)$$

where: GDH – the accumulation of growing degree hours during an hour; FA – full activity (the maximum GDH contribution per hour) [F – assumed to be 1.0 unless the tree is under stress; A – the amplitude of the growth curve (A = TU – TB)]; π – the mathematical constant (π = 3.1416); TH – hourly temperature; TB – base temperature (4 °C); TU – optimum temperature (25 °C); TC – critical temperature (36 °C).

Flowering. This parameter was recorded over the period from 2017 to 2020, for which the number of flower buds per experimental unit was counted

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every third day. A relationship between GDD and flowering kinetics for each 'Honeycrisp'/rootstock combination was constructed using a modified Weibull model (Mora-Aguilera et al. 1993). Briefly,

$$Y = 1 - e^{[-(t/z)^{W}]} \quad (3)$$

where: Y – the proportion of flower opening (0–1) per unit of time, e – mathematical notation for the constant (e = 2.71828); t – time in growing degree days (GDD); z – the estimator of flower open rate in its inverted form ($1/z$) by GDD; w – a dimensionless parameter controlling the curve shape.

Sampling of leaves. From the mid-canopy of each 'Honeycrisp'/rootstock combination, 70 leaves were collected during the growing season on July 26th, 2017–2020. Leaves were collected from vegetative shoots, and the leaf samples from the four cardinal directions were pooled for analysis. The collected leaf materials showed no signs of mechanical damage, pests or diseases.

Leaf mineral nutrients. The leaves were transported for analysis to the Plant Physiology Laboratory at the Autonomous University of Chihuahua, Chihuahua, México, where extraction and quantification of mineral nutrients were carried out using the method of Ontiveros-Capurata et al. (2022). Briefly, leaves were triple washed with (*i*) tap water, then (*ii*) 4N HCl and lastly (*iii*) deionised water. Surface water was allowed to evaporate completely at room temperature, and leaves were then dried at 75 °C for 24 h in a Heratherm VCA 230® oven (Thermo Scientific, Waltham, USA). Each sample was homogenised in a Willey R-TE-650/1 mill with a 1 mm mesh (Tecnal, São Paulo, Brazil). The extraction and quantification of total N were determined by the Kjeldhal method (Novatech®, USA and Micro Kjeldahl Labconco®, USA) and total P by the ammonium metavanadate method (NH_4VO_3) (Thermo Scientific™, USA). The extraction of K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+} and Zn^{2+} was carried out by triacid digestion (HNO_3, $HClO_4$ and H_2SO_4) (25 mL of the mixture in a 10 : 10 : 25 ratio) using 25 mL of the acid mixture on a hot plate under a fume hood. Analyte quantifications were carried out using an Analyst 100® atomic absorption spectrophotometer (PerkinElmer®, USA). Results are reported as g/kg for macronutrients and mg/kg for micronutrients.

Trunk cross-sectional area. Trunk cross-sectional area (TCA, cm²) was determined for each tree by recording trunk girth (C, cm) 30 cm above the graft union and calculating TCA as $C^2/4\pi$, where π is the mathematical constant.

Fruit number and fruit weight per tree, and production efficiency. The number of fruit (n) per experimental unit was counted, and fruit weights (kg) were measured using an electronic digital balance Scout® Pro SP602 (OHAUS, USA, capacity 0.6 kg, approximate resolution of 0.01 g). Production efficiency (kg/cm²) per tree and per hectare was estimated by dividing fruit weight (kg) by TCA (cm²).

Statistical analyses. Homogeneity of variances was assessed with the Kolmogorov-Smirnov test. The analysis of variance was carried out, and when a significant treatment effect was detected, using Tukey's multiple comparison of means test ($P \leq 0.05$). If heterogeneous variances were detected in the data, the Kruskal-Wallis non-parametric test was performed, and the separation of medians was carried out with the Mann-Whitney test ($P \leq 0.05$). The statistical analysis package SAS version 9.1 statistical software (SAS Institute Inc., North Carolina, USA) was used for all analyses.

RESULTS

Consistent occurrences of chill (air temperatures between 1.5 °C and 12.5 °C) started on about October 22nd in 2016–2017, 2017–2018 and 2018–2019, and on about November 15th in 2019–2020, with an interannual variation of about 24 days. The CU accumulations for each of the off-seasons were: 1 019 (2016–2017), 974 (2017–2018), 1 415 (2018–2019) and 1 011 (2019–2020). Fitting these chill accumulations with the modified Weibull model revealed significant variations between chill accumulation rates year by year (Table 1).

The end of the off-season is characterised by a trend for gradual increases in air temperature that favour the progression of seasonal phenology in temperate plant species, including bud break, flowering and fruit set. Here, the passage of physiological time was estimated from the accumulation of GDD after March 1st, arbitrarily taken as the starting point of the growing season. The simple linear model significantly adjusted ($P \leq 0.01$ and $R^2 \geq 0.99$) the parameters of heat accumulation and Julian days (JD). It can be seen (Table 2) that the rate of heat accumulation was higher during the spring of 2017 than in the later years of the study.

The analysis of 2000–2020 temperature data indicates an above 30% probability of occurrence

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Table 1. Start date and number of days of chill unit accumulations for the different periods of this study, and accumulation adjustment with the modified Weibull model

Period (September to February)	Start date	Number of days	CU	Weibull model $Y = 1 - e^{[-(t/z)^W]}$	Indicators of model fit			
					slope (1/*z*)	*P* > *F*	R^2 (%)	*CV* (%)
2016–2017	22/10	142	1 019	$1 - e^{[-(t/133.1)^{2.08}]}$	$^{b}7.5\times10^{-3}$	< 0.01	99.6	5.3
2017–2018	22/10	142	974	$1 - e^{[-(t/143.6)^{2.66}]}$	$^{c}6.9\times10^{-3}$	< 0.01	99.5	6.5
2018–2019	22/10	142	1 415	$1 - e^{[-(t/102.3)^{2.42}]}$	$^{a}9.8\times10^{-3}$	< 0.01	99.0	7.8
2019–2020	15/11	105	1 011	$1 - e^{[-(t/138.5)^{2.88}]}$	$^{bc}7.2\times10^{-3}$	< 0.01	99.6	4.9

CU – chill units accumulation by period; *Y* – the proportion of flower opening (0–1) per unit of time; *e* – mathematical notation for the constant (*e* = 2.71828); *t* – time in growing degree days (GDD); *z* – the estimator of flower open rate by GDD; *w* – a dimensionless parameter controlling the curve shape; slope (1/*z*) – rate of increase in chill units by day; R^2 – coefficient of determination; *CV* – coefficient of variation between difference of observed and adjusted values
[a–c]values with the same letter in the slope (1/*z*) column are not significantly different (Student's *t*-test for slope comparison, $P \leq 0.05$)

Table 2. Evaluation of the linear model (GDD = *s* × JD – *k*) during the four years of investigation

Year	GDD = *s* × JD – *k*	*P*-value	Adjusted R^2 (%)
2017	$^{a}12.23 \times JD - 874.94$	< 0.01	0.99
2018	$^{b}10.88 \times JD - 736.02$	< 0.01	0.99
2019	$^{b}10.56 \times JD - 718.11$	< 0.01	0.99
2020	$^{b}10.96 \times JD - 744.54$	< 0.01	0.99

Growing degree day accumulations began on March 1st and ended on April 30th
GDD – growing degree days; *s* – the slope; JD – Julian days; *k* – the mathematical constant; R^2 – coefficient of determination
[a,b]values of *s* preceded by the same letter are not significantly different (Student's *t*-test for slope comparison, $P \leq 0.05$)

of temperatures ≤ –2 °C estimated for March 31st (JD 90). This is interesting in that on more than one occasion, the onset of flowering of 'Honeycrisp' can occur around this time. After this, the probability of frost decreases to 15% (April 10th, JD 100) and 7% (April 18th, JD 108) (Figure 1).

For the flower opening of 'Honeycrisp', there was no significant interaction between years and rootstocks in the GDD requirement. The proportion of open flowers in relationship with GDD among the years evaluated was significantly adjusted ($P \leq 0.05$) using the modified Weibull model. This analysis allows variation to be detected in the onset, the rate of increase and the termination of the flower opening with respect to physiological time so that after an off-season with an accumulation of 1 415 CU, 'Honeycrisp' then required 248 GDD

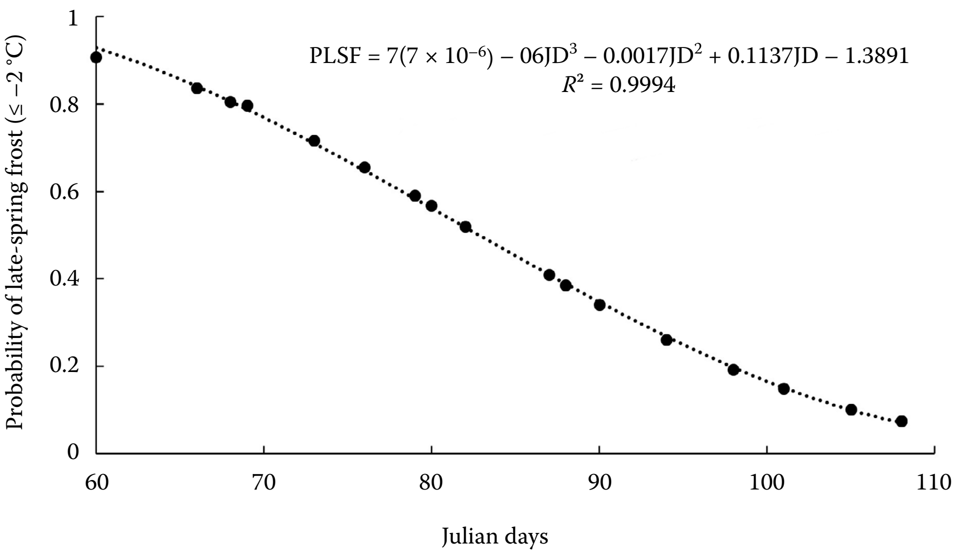

Figure 1. The probability of late spring frost (PLSF, temperatures ≤ –2 °C) and its relationship to Julian days, fitted to a third-degree polynomial model (dashed line = fitting curve) in Cuauhtemoc, Chihuahua, México
JD – Julian days; R^2 – coefficient of determination

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Table 3. Flower opening period in 'Honeycrisp' by evaluation year and the flower opening adjustment with the modified Weibull model

Year	Flower opening		Weibull model	Indicators of model fit			
	start	end	$FO = 1 - e^{[-(t/z)^{w}]}$	slope (1/*z*)	*P*-value	R^2 (%)	*CV* (%)
2017	316[b]	414[b]	$1 - e^{[-(t/392.8)^{11.2845}]}$	[c]25.5 × 10^{-4}	< 0.01	69.0	35.5
2018	364[a]	505[a]	$1 - e^{[-(t/461.0)^{8.8708}]}$	[d]21.7 × 10^{-4}	< 0.01	91.5	16.8
2019	248[c]	313[c]	$1 - e^{[-(t/291.7)^{17.3749}]}$	[a]34.3 × 10^{-4}	< 0.01	99.4	8.0
2020	309[b]	445[b]	$1 - e^{[-(t/370.1)^{7.3019}]}$	[b]27.0 × 10^{-4}	< 0.01	89.4	20.7

The flower opening is obtained by substituting in the equation of the Weibull model the value of *t* in growing degree days (GDD), with the start of calculation on March 1st, until the flower opening ends

FO – flower opening (0–1) (FO × 100 to obtain % flower opening); *e* – the mathematical constant (*e* = 2.71828); *t* – time in GDD; *z* – the estimator of flower open rate; *w* – a dimensionless parameter controlling the curve shape; slope (1/*z*) estimator of the growth rate in its inverted form; R^2 – coefficient of determination; *CV* – coefficient of variation between the difference of observed and estimated values

[a–d]values in the slope column (1/*z*) with the same letter mean not significantly different (Student's *t*-test for slope comparison, $P \leq 0.05$)

to start of flower opening, had a rate of increase of 34.3 × 10^{-4} in the proportion of flowers opening by GDD, a value that differed significantly from the other years evaluated and ended at 313 GDD. Following an off-season with only 974 CU of accumulation, 364 GDD was required to start of flower opening, with a rate of increase of 21.7 × 10^{-4} in the proportion of flowers opening by GDD and ended at 505 GDD. In two of the four years evaluated, with previous off-season CU accumulations of 1 011 CU and 1 019 CU, the GDD requirement for the start of flower opening was intermediate between the years of maximum and minimum CU accumulation (Table 3). In the number of JD, the difference between the low and the high CU accumulation off-seasons differed by up to 12 JD for the start of flower opening and 19 JD for termination. A graphical representation of this is presented in Figure 2.

The different rootstocks don't seem to affect the timing of flowering in the 'Honeycrisp' scions, with no significant differences between rootstocks in the GDD requirement for the onset and the end of flower opening. Therefore, an average of four years was obtained. For flower opening onset (5%), the extremes were detected for rootstocks V.6 with a requirement of 271 GDD and G.11 with a requirement of 303 GDD. To conclude the flower opening period, the requirement was 391 GDD for rootstocks G.41 and 432 GDD for rootstocks V.6 (Table 4). Thus, 'Honeycrisp' on G.969 rootstock has the shortest flower opening period with 99 GDD between onset and the end, and V.6 has the longest flower open-

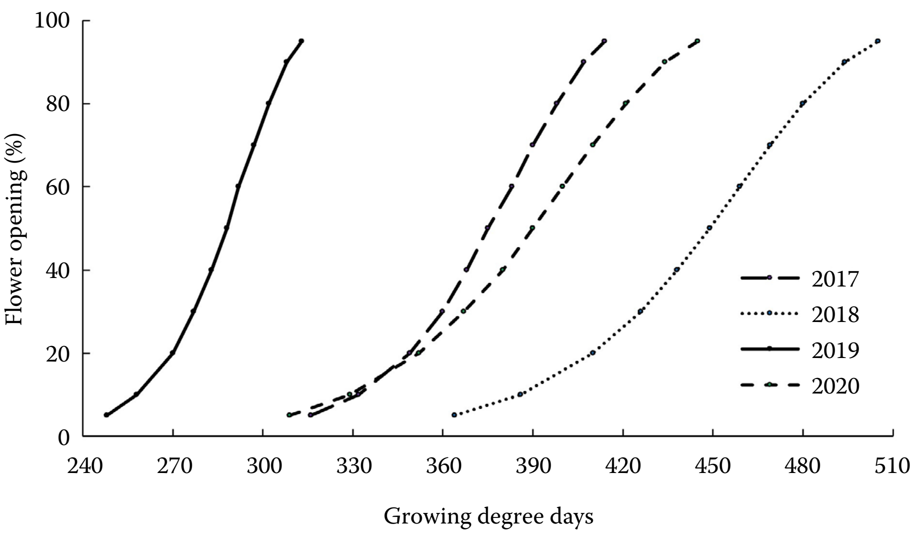

Figure 2. Flowering period in 'Honeycrisp' apple trees evaluated for four years (2017–2020) based on growing degree day accumulation

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Table 4. Indicators of the Weibull model and its goodness of fit for flowering in 'Honeycrisp' apple scions grafted on eight different rootstocks

Rootstock	Flower opening in GDD		Weibull model $FO = 1 - e^{[-(t/z)^{w}]}$	Indicators of model fit			
	start	end		slope (1/z)	P-value	R^2 (%)	CV (%)
G.11	303[a]	411[a]	$1 - e^{[-(t/378.4)^{13.3027}]}$	[f]264×10^{-5}	< 0.01	99.0	37.9
G.202	282[a]	431[a]	$1 - e^{[-(t/384.0)^{9.5268}]}$	[h]260×10^{-5}	< 0.01	99.0	33.9
G.30	276[a]	398[a]	$1 - e^{[-(t/359.9)^{11.1587}]}$	[b]278×10^{-5}	< 0.01	99.0	18.9
G.41	287[a]	391[a]	$1 - e^{[-(t/359.0)^{13.077}]}$	[a]279×10^{-5}	< 0.01	99.0	36.2
G.969	297[a]	396[a]	$1 - e^{[-(t/365.0)^{14.2237}]}$	[d]274×10^{-5}	< 0.01	99.0	48.8
M.26 EMLA	290[a]	411[a]	$1 - e^{[-(t/373.3)^{11.6345}]}$	[e]268×10^{-5}	< 0.01	99.0	38.5
M.9T337	280[a]	401[a]	$1 - e^{[-(t/363.2)^{31.3069}]}$	[c]275×10^{-5}	< 0.01	99.0	34.8
V.6	271[a]	432[a]	$1 - e^{[-(t/379.9)^{8.7343}]}$	[g]263×10^{-5}	< 0.01	99.0	30.8

The flower opening is obtained by substituting in the equation of the Weibull model the value of t in growing degree days (GDD), with the start of calculation on March 1st, until the flower opening ends

FO – flower opening (0–1) (FO × 100 to obtain % flower opening); e – the mathematical constant (e = 2.71828); t – time in growing degree days (GDD); z – the estimator of flower open rate; w – a dimensionless parameter controlling the curve shape; slope (1/z) – estimator of the flower opening rate in its inverted form; R^2 – coefficient of determination; CV – coefficient of variation between the difference of observed and estimated values

[a–g]values in the slope column (1/z) with the same letter mean not significantly different (Student's t-test for slope comparison, $P \leq 0.05$)

ing period with 161 GDD between onset and end. Despite the lack of significance between the onset and end of flowering, the rate of the flower opening by GDD for 'Honeycrisp' was significantly affected by the different rootstocks with rates of the flower opening by GDD fluctuating between 279 × 10^{-5} for rootstocks G.41 and 263 × 10^{-5} for rootstocks V.6. On the other hand, the coefficients of determination (R^2) between observed and adjusted values by the Weibull model were higher than 95.0%, while the coefficients of variation (CV) ranged between 18.9% for rootstocks G.30 and 48.8% for rootstocks G.969. The different values of the slope (1/z) between rootstocks and their significance are shown in Table 4 and Figure 3.

The integration of winter chill accumulation, flowering occurrence, JD and probability of late spring frosts shows that 1 415 CU were accumulated during the winter 2018–2019. Thus, the flowering of 'Honeycrisp' in the spring of 2019 occurred between the 90th and 99th JD, corresponding to the period from March 31st to April 9th, respectively. In this sense, the risk or probability of late spring frost damage on flower structures fluctuated between 0.40 and 0.24 (onset and termination, respectively) with 9 days of duration for this phenological event. While

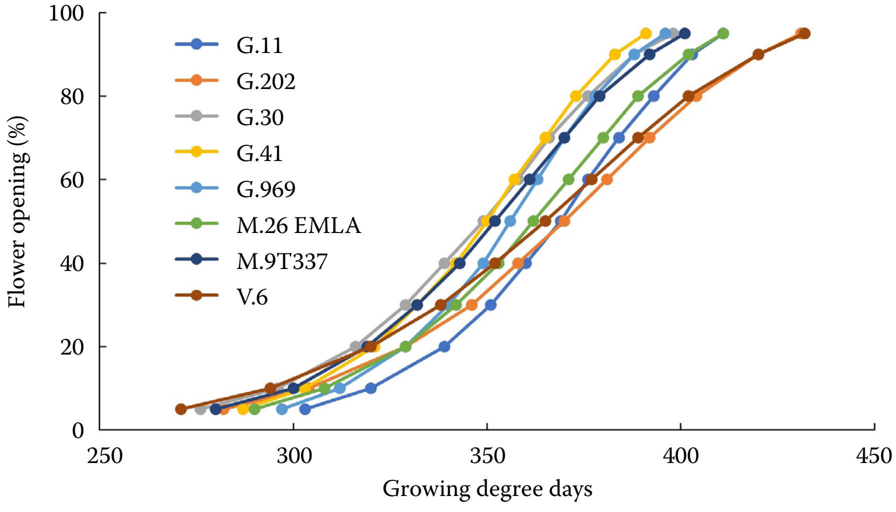

Figure 3. Flowering period in 'Honeycrisp' apple tree rootstocks based on growing degree day accumulation (with onset of the accumulation on March 1st) in Cuauhtemoc, Chihuahua, México

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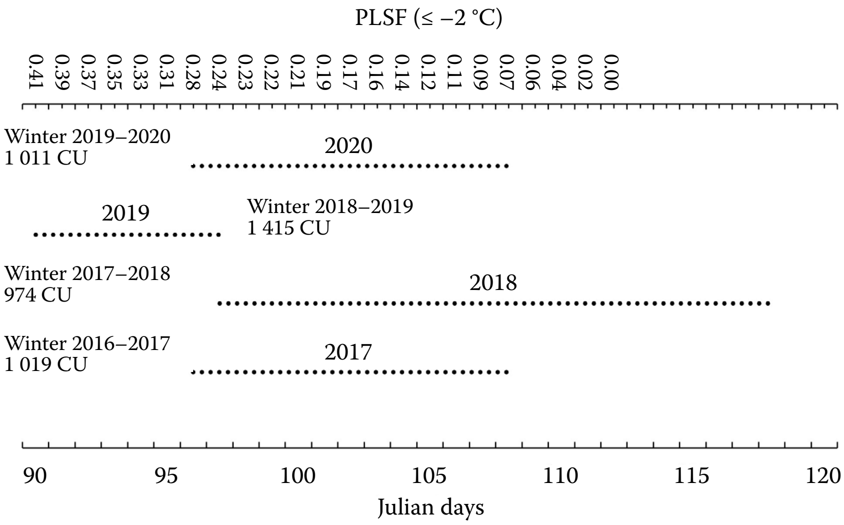

Figure 4. Occurrence of the flowering in 'Honeycrisp' apple trees for different years and its relationship with Julian days and the probability of late spring frost in Cuauhtemoc, Chihuahua, México

PLSF – probability of late spring frost; CU – chill units

974 CU were accumulated in winter 2017–2018, flowering in spring 2018 occurred on JD 99 to 119, corresponding to April 9th and 29th, respectively. This behaviour allowed observation of a probability of frost occurrence between 0.24 and 0 at the beginning and end of flowering. Finally, in the year 2017, 1 019 CU was accumulated, whereas in 2020, 1 011 CU was accumulated; in both conditions, flowering occurred in an intermediate season, ending on JD 109 for both years (Figure 4).

No significant variation ($P \geq 0.05$) was found in leaf concentrations of N-total, P, K, Ca, Mg, Fe, Mn or Zn. Instead, differences were observed with respect to Cu concentration in the leaves of G.202, G.30, G.969, G.41 and G.11, with data ranging from 7.3 to 9.2 mg/kg, where G.41 was the most outstanding (Table 5).

Table 5. Foliar nutrient concentrations in 'Honeycrisp' apple trees grafted on eight selected rootstocks

Rootstocks	Macronutrients (g/kg)				
	N-total	P	K^+	Ca^{2+}	Mg^{2+}
G.202	21.0^a	22.6^a	16.3^a	16.4^a	3.7^a
V.6	19.8^a	23.7^a	16.1^a	13.3^a	4.2^a
G.30	20.9^a	19.5^a	15.0^a	16.4^a	4.2^a
G.969	20.1^a	22.6^a	14.9^a	16.7^a	3.5^a
G.41	20.9^a	21.1^a	14.9^a	19.5^a	4.7^a
M.26 EMLA	21.2^a	22.1^a	14.8^a	13.7^a	4.3^a
M.9T337	20.2^a	22.9^a	14.5^a	18.2^a	4.5^a
G.11	21.1^a	24.1^a	14.7^a	17.8^a	4.8^a
Rootstocks	**Micronutrients (mg/kg)**				
	Fe^{2+}	Cu^{2+}	Mn^{2+}	Zn^{2+}	
G.202	143.0^a	7.5^{ab}	102.5^a	37.7^a	
V.6	214.0^a	7.3^b	100.7^a	39.5^a	
G.30	180.0^a	7.5^{ab}	92.2^a	42.8^a	
G.969	136.7^a	7.7^{ab}	107.8^a	34.7^a	
G.41	172.5^a	9.2^a	103.3^a	43.2^a	
M.26 EMLA	174.8^a	7.0^b	103.5^a	40.5^a	
M.9T337	158.0^a	7.5^{ab}	101.3^a	42.2^a	
G.11	183.0^a	7.7^{ab}	96.2^a	39.2^a	

Data are expressed on a dry weight basis and correspond to the average obtained between 2016 and 2020

$^{a–b}$means with the same letters within each column do not differ significantly (Tukey's test; $P \leq 0.05$)

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The modified Weibull model significantly adjusted the TCA of 'Honeycrisp' on selected rootstocks, highlighting rootstocks G.30 and V.6 with slopes of 495.6 × 10^{-6} and 495.3 × 10^{-6} units between 2017 and 2020, respectively (Table 6). Rootstocks M.9T337, G.41, G.11, G.969 and V.6 showed slopes not significantly different from one another, while rootstock M.26 EMLA had the lowest rate of increase in trunk cross-sectional area with 493.2 × 10^{-6} units. Regarding trunk cross-sectional area, the rootstocks with the highest vigour were G.30 and V.6 with TCAs of 28.13 cm^2 and 23.09 cm^2, respectively. While the rootstock M.26 EMLA was the least vigorous, with a TCA of 10.88 cm^2.

Data on the number of fruits per tree, yield per tree, and production efficiency are shown in Table 7. One of the aspects to consider when selecting a rootstock is associated with its effect on tree vigour, fruit number or fruit size, which are highly correlated with yield. Under the climatic and agronomic management conditions of the experimental area, rootstocks G.969, G.11, V.6, M.9T337 and G.30 showed the highest number of fruits per tree (NFPT) with values ranging from 43 to 46 fruits. With the exception of G.30, these

Table 6. Indicators of the modified Weibull model and goodness of fit for trunk cross-sectional area (2017–2020) in 'Honeycrisp' apple trees grafted on eight rootstocks

Rootstock	Weibull model $Y = 1 - e^{[-(t/z)^{w}]}$	Indicators of model fit				
		slope (1/z)	$P > F$	R^2 (%)	CV (%)	TCA (cm^2/tree)
G.30	$1 - e^{[-(t/2017.61)^{1107.6}]}$	$^{a}495.6\times10^{-6}$	< 0.02	96.6	5.9	$^{a}28.13$
M.9T337	$1 - e^{[-(t/2022.0)^{438.85}]}$	$^{bc}494.6\times10^{-6}$	< 0.04	89.6	8.5	$^{b}13.74$
G.41	$1 - e^{[-(t/2022.70)^{438.85}]}$	$^{b}494.9\times10^{-6}$	= 0.02	95.7	6.1	$^{b}13.67$
G.11	$1 - e^{[-(t/2020.84)^{480.05}]}$	$^{b}494.8\times10^{-6}$	< 0.01	99.8	1.1	$^{ab}15.18$
G.969	$1 - e^{[-(t/2020.40)^{531.46}]}$	$^{b}494.9\times10^{-6}$	< 0.01	98.0	4.1	$^{ab}15.38$
M.26 EMLA	$1 - e^{[-(t/2027.50)^{208.07}]}$	$^{c}493.2\times10^{-6}$	< 0.01	98.4	1.7	$^{b}10.88$
V.6	$1 - e^{[-(t/2019.0)^{854.98}]}$	$^{ab}495.3\times10^{-6}$	< 0.02	79.9	18.4	$^{ab}23.09$
G.202	$1 - e^{[-(t/2027.15)^{215.63}]}$	$^{c}493.3\times10^{-6}$	< 0.01	98.6	1.6	$^{b}9.87$

Y – trunk cross-sectional area; e – the mathematical constant (e = 2.71828); t – time in growing degree days (GDD); z – the estimator of flower open rate; w – a dimensionless parameter controlling the curve shape; slope ($1/z$) – estimator of the growth rate in its inverted form; R^2 – coefficient of determination; CV – coefficient of variation; TCA – trunk cross-sectional area

$^{a-c}$values in column slope (1/z) and TCA with the same letters within each column do not differ significantly (Tukey's test; $P \leq 0.05$)

Table 7. Number of fruits, average production per tree and estimated production efficiency per tree and hectare for 'Honeycrisp' apple grafted on some rootstocks

Rootstock	NFPT	FW	PPT	YE	EPH
G.969	47^{a}	276	12.7^{a}	0.825	35 300^{a}
G.11	43^{a}	286	12.3^{ab}	0.810	34 200^{ab}
V.6	43^{a}	281	12.1^{ab}	0.524	33 600^{ab}
M.9T337	41^{a}	243	11.2^{ab}	0.815	31 100^{b}
G.30	38^{a}	244	10.5^{b}	0.373	23 300^{b}
M.26 EMLA	31^{b}	277	8.6^{b}	0.790	28 400^{b}
G.202	31^{b}	245	7.6^{b}	0.770	25 300^{b}
G.41	–	–	–	–	–

Production values for G.41 rootstock were highly variable and not considered in this analysis

NFPT – number of fruits per tree; FW – fruit weight (g); PPT – production per tree (kg); YE – yield efficiency (kg/cm^2 based on the trunk cross-sectional area); EPH – estimated production per ha (kg)

$^{a-b}$means with the same letters within each column do not differ significantly (Tukey's test; $P \leq 0.05$)

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rootstocks also showed the highest production per tree (PPT) with values of 12.7, 12.3, 12.1 and 11.2 kg, respectively. However, when estimating the production efficiency per ha with the densities proposed for each rootstock, only G.969, G.11 and V.6 showed significant values.

DISCUSSION

The effects of climate change are global, but the effects will also be felt quite differently in different regions, as climate is the result not only of synoptic factors (weather systems) but also of local ones (geographical features). Thus, a mountain range has strong local effects, including rainfall patterns, cloud cover, insolation, and wind strength. Overall, climate change will directly affect the geographical distributions of most food plant species and will also affect plant productivity as the climates of traditional growing regions for particular species will become gradually more (or less) marginal. As already noted above, climate change will likely most affect the horticultural crop plants (Valverdi et al. 2019), including vegetable crops and tree fruit crops, such as apples.

In México, apple orchards have been established in areas at altitudes ranging between 1 900 m and 2 600 m above sea level. In 1994, the first reports appeared on the high interannual variations in winter temperature in these areas and their negative effects on CU accumulations (Ramírez Legarreta et al. 2011). The interannual variations are in the range of around 30%. This wide variation in CU accumulations is also accompanied by wide variations in daily temperature and precipitation during the growing season. Fernandez et al. (2022) suggest that climate change may have further impacts on socioeconomics and food security in many nations, introducing yet another dimension of uncertainty into agricultural production.

The weather affects winter dormancy and a whole range of other physiological and phenological processes in tree crops (El Yaacoubi et al. 2014). In this study, the seasonal variation in CU accumulation onset shows a difference of 24 days. This picture is compounded by significantly different rates of CU accumulation between years. Combining these two sources of variation (CU onset timing and CU accumulation rates) results in interannual CU accumulations that can differ by as much as 440 CU, a 31% difference between the minimum and maximum annual CU accumulations, and which can even occur between consecutive winters. In this study, the average CU for the four seasons (2017–2020) was 1 104. On average, winter 2016–2017 had the lowest number of CU (974 CU), while 2018–2019 had the highest (1 415 CU). It is clear that the apple-growing region in México does not receive adequate winter cold. On the other hand, the cold requirements for ‘Golden Delicious’ (1 093 ± 180 CU), the main apple cultivar in México, are partially covered (Hauagge, Cummins 1991). However, for ‘Honeycrisp’, the reported cold requirements are expressed in chilling hours, and there is no published data for CU, which makes it difficult to compare them due to the different methods and data used for their calculation. Our CU data compared to other major apple producing regions such as Shandong, China (± 1 500 CU), Washington State, USA (± 2 500 CU), and Rancagua, Chile (± 1 600 CU), suggest that our study area is mainly a ‘warm winter’ apple-growing region (Cook et al. 2017). The complexity of CU accumulation makes it difficult to explore how projected climate change scenarios will interact (Ru et al. 2023). The resulting climate uncertainty clouds the thinking of government decision makers, industry planners and producers alike. This situation of uncertainty contrasts with a simple trend for warmer winters, i.e. for reduced CU accumulations (Fernandez et al. 2022), which are estimated to reduce CU accumulations by 26% to 30% (Benmoussa et al. 2020).

During our short study, the weather data show marked interannual differences during the four seasons (2016/2017, 2017/2018, 2018/2019 and 2019/2020). During the 2017–2018 winter, only 974 CU were accumulated, while for 2018–2019, the accumulation was 1 415 CU. Similarly wide fluctuations in CU accumulation have been reported in other latitudes where temperate fruit trees are grown (Ghrab et al. 2014). In these circumstances, the use sprouting promoters has been adopted to promote more uniform sprouting, but it does not completely solve the problem of low CU accumulation because it does not eliminate the bud weakness, bud deformation, bud abscission and (later) the reduction in fruit set, which accompany low CU accumulations (Kishore et al. 2015; Ahmadi, Baaghideh 2018). Moreover, the additional spraying and additional product costs further reduce profitability, posing a special threat to growing regions that are already commercially marginal, such as those we are dealing with here (Serra et al. 2020).

Once winter dormancy is complete, trees require heat accumulation to allow bud break. Both the

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chill and heat requirements of apple are cultivar and rootstock dependent and are widely used to model flowering and dormancy dates, and so predict the probability of successful adaptation of a particular new cultivar to a particular growing region (Campoy et al. 2019). A common symptom of sub-optimal chilling is poor and/or protracted bud-break, which leads to an extended flowering period (Pertille et al. 2022). Our results show that in the main apple producing area in México, a high CU accumulation is associated with an early and compact flowering period. This comes with the obvious increased risk of damage by a late spring frost, a situation already described by Pfleiderer et al. (2019) and Drepper et al. (2022). Conversely, a low CU accumulation is associated with a reduced risk of damage from a late spring frost because flowering is both delayed and prolonged. However, higher temperatures around flowering (above 24 °C) increase the risk of fire blight (Ramírez-Legarreta et al. 2011) and pollen grain dehydration (DeLong et al. 2016). Meanwhile, windspeeds above 20 km/h decrease pollinator activity (mostly honey bees) and damage flowers due to petals dehydration and so increases flower drop. The combination of all these random weather effects badly affects apple production, reducing both fruit yield and fruit quality (Ghrab et al. 2014). Climate change is predicted to increase overlap between the apple flowering period and the incidence of late spring frosts (Drepper et al. 2022), but information for apple on the effects of late spring frosts during flowering and fruit set is limited (Ru et al. 2023).

Rootstocks are well known to affect both tree vigour and tree anchorage, and so play a major role in fruit tree production through their influence on uptake of water and minerals (Fazio et al. 2020). Therefore, at the time of planting, it is important to match the rootstock characteristics not only with the soil characteristics of the proposed new orchard but also with its climate and with the proposed scion (Nimbolkar et al. 2016). Unfortunately, there is little information on nutrient and water uptake efficiency and their effects on the growth and production of these rootstocks (Fazio et al. 2016).

Fruit mineral analyses help producers manage nutrient imbalances while also providing a possible predictive tool. Our leaf nutrient analyses for 'Honeycrisp' apples on different rootstocks show no significant differences in nutrient concentrations of N-total, P, K, Ca, Mg, Fe, Mn and Zn, although those of N-total on V.6 and B.969, and of P on G.30 were slightly lower. Crassweller et al. (2019) report leaf macronutrient concentration in 'Honeycrisp' on M.26 (the usual rootstock in the USA to obtain low-vigour trees). However, this information is only generally useful, as soil minerals are normally amended based on the leaf mineral levels of the scion for each scion/rootstock combination (Fazio et al. 2016; Al Shoffe et al. 2020).

Tree vigour is usually indicated by the increment in TCA. Here, 'Honeycrisp' on G.30 and V.6 were the most vigorous trees, while the G.969 and G.11 produced trees of medium vigour and M.9T337, G41, M.26 EMLA, and G202 produced trees of lowest vigour. Similar results for the vigour of 'Honeycrisp' on these rootstocks have been reported by Cline et al. (2021) and Sherif (2022).

The productivity of 'Honeycrisp' on each rootstock is also affected by both climate and soil (while the latter is usually managed by the grower). At the test site, 'Honeycrisp' was most productive on G.969, G11 and V.6 rootstocks with estimated yields of around 35 000 kg/ha based on the recommended planting density per hectare for each rootstock. The outstanding performances of rootstocks G.969 and V.6 confirm the results obtained in different regions in the USA (Cline et al. 2021). Our estimated yields may be relatively low by world standards, but they are commercially acceptable here and are understandable given the rather marginal environmental conditions under which apple trees are grown in Chihuahua. It is usually assumed that each rootstock-cultivar combination expresses its highest potential under optimal conditions of climate and soil (Fazio et al. 2020), hence the results presented here for the production efficiency of 'Honeycrisp' on different rootstocks, offers a new reference for commercial apple producers under our edaphic and climatological conditions.

In this study, fruit weights varied between 243 g and 286 g, with the fruits on the rootstocks G.11 (286 g), V.6 (281 g) and G.969 (276 g) being the largest. Fruits produced on trees with low fruit loads and large fruits, as in our case, are usually prone to Ca deficiencies and raised incidences of bitter pit (Fazio et al. 2020; Islam et al. 2022). The results shown here could be compared more objectively with those obtained in other regions of the world, with 'Honeycrisp' apple grafted on these same rootstocks, if the management of the trees were carefully standardised and the particular climatic and edaphic conditions to which each experimental site is subjected were described in more detail.

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CONCLUSION

At the study site, trees were subject to a 30% variation between maximum and minimum chill accumulation during late summer and winter. Under conditions of maximum chill accumulation, 'Honeycrisp' had an earlier and shorter flowering period and was therefore subject to a higher risk of late spring frost damage. Conversely, under lower chill accumulations during late summer and winter, the flowering periods were later and longer, with higher risks of flower damage from high temperatures and strong winds. There were no significant rootstock effects on the flowering period of 'Honeycrisp'. Based on the recommended planting density for each rootstock, the highest estimated yields per hectare for 'Honeycrisp' were on the rootstocks G.969, G.11 and V.6, and were around 35 000 kg/ha. By international standards, these yields are relatively low but are understandable and acceptable, given the marginal production conditions under which apples are grown in Chihuahua.

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