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Short note

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Use of the biostimulant Retard Cherry® as a strategy to delay blooming period in sweet cherry trees

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Spring frosts are a limiting factor in sweet cherry production in central-southern Chile. Sweet cherry trees cv. 'Regina' and 'Sweetheart' were studied to evaluate the effect of foliar application of a biostimulant (Retard Cherry®) prior to leaf fall on the bloom delay, fruit set, fruit drop, yield and quality. Data were compared to a non-product control. The study was conducted in the Maule Region, Chile. Results showed that the use of Retard Cherry® delayed full bloom by 6-8 days between cultivars compared to the control; however, there was no delay in the harvest date. The climatic conditions favored high fruit set (37%-49%) and low fruit drop (63%-70%) between cultivars in both treatments. Regarding fruit quality, no differences in size, soluble solids concentration and color were observed with the product, but a decrease in firmness were observed for 'Regina'. These results show that Retard Cherry® is an effective tool in delaying bloom, providing trees with more favorable climatic conditions for pollination and fruit set.

1. Introduction

Sweet cherry (*Prunus avium* L.) is a fruit tree of temperate climate, native to Asia Minor (Iezzoni *et al.,* 2017). Its cultivation has been widely distributed in Mediterranean climate countries such as Turkey, Italy and Spain, in the United States and in Middle Eastern countries such as Iran (Bujdosó and Hrotkó, 2017). In the southern hemisphere, Chile is the main exporter, with a volume of 350 thousand tons in the 2020/21 season in a cultivated area of 48,960 ha (iQonsulting, 2021; ODEPA, 2021).

Emergence from recess and regulation of blooming time in deciduous fruit trees involves a combined process of winter cold and spring warmth accumulation (Fadón *et al.,* 2020). In sweet cherry trees, temperature conditions during the cold accumulation phase are estimated to be the main driver of bloom (Fadón *et al.,* 2021). In mild winter areas, insufficient cold accumulation can cause delayed bloom, floral malformations, and low fruit production in the trees. In contrast, when high cold accumulation accompanied by spring warmth occurs in this period, bloom may be advanced and flower opening concentrated, increasing the risk of spring frost damage (Herrero *et al.,* 2017), which can damage flowers and buds (Miranda *et al.,* 2005) and generate a large decrease in crop production and profitability (Kaya *et al.,* 2021). Moreover, low temperatures can affect blooming synchrony between variety and pollinizer, limiting pollinator activity, delaying pollen tube development and fruit set (Guo *et al.,* 2015).

Climate change in the near future is expected to generate warmer springs with greater thermal fluctuations, which may alter plant phenology and increase the risk of spring frost damage in species of temperate climate (Augspurger, 2013). Some tools used to reduce spring frost damage are overhead sprinkler irrigation, wind towers with heaters and heated macro-tunnels. However, these technologies have a high implementation cost and are not always effective enough in control (Yuri *et al.,* 2017).

A complementary strategy to avoid frost damage in fruit trees is to delay tree bloom, shifting it to a period with greater climatic stability, more favorable for pollination and fruit set (Liu and Sherif, 2019). Plant growth regulators evaluated in stone fruits can extend bud dormancy (Durner and Gianfagna, 1991), delay bloom (Ebel *et al.,* 1999) or synchronize bloom with another variety, as well as delay harvest (Basak *et al.,* 1998). In some cases, however, the application of these products can cause flower abscission, low fruit set and yield (Crisosto *et al.,* 1990; Liu and Sherif, 2019). On the other hand, the use of foliar biostimulant, as sustainable alternatives to plant growth regulators, could increase flower bud resistance to winter cold and delay bloom, without detrimental effects on fruit production.

This study aimed to evaluate the effect of foliar application of the biostimulant Retard Cherry® on bloom delay before leaf fall as well as on fruit set, fruit drop and fruit quality in 'Regina' and 'Sweetheart' sweet cherry trees in central Chile.

2. Materials and Methods

Plant material and experimental site

The study was conducted during the 2018/19 growing season in two commercial orchards of sweet cherry (*Prunus avium* L.) located in San Clemente, Maule Region, Chile (35°32' S, 71°27' W, 230 m a.s.l.), at less than 5 km between them. In one orchard, 'Regina' sweet cherry trees on 'Gisela-6' rootstock were evaluated; they were planted at 4.0 × 1.8 m in 2015. In the other, 'Sweetheart' sweet cherry trees on 'Colt' rootstock were evaluated; they were planted at 5.0 × 2.5 m in 2010. Both cultivars were trained in Central Leader. Orchards management were carried out according to commercial standards in the region. Seasonal environmental conditions are summarized in Table 1.

Climatic data was recorded by an automatic weather station Vantange 2 (Davis Instruments, Hayward, CA, USA) near the orchards. During the

Table 1 - Environmental conditions 2018, San Clemente, Chile

Variable	Annual	May 1-Jul 31	Aug 1-Dec 25	October
Air temperature (°C)				
Mean	13.6	7.4	13.7	13.3
mean maximum	21.4	13.3	21.3	20.2
mean minimum	7.3	3.1	7.2	7.6
Maximum	35.6	25.2	35.4	26.4
Minimum	-5.1	-5.1	-5.1	1.5
Relative humidity (%)				
mean minimum	46.1	66.8	42.1	44.5
Precipitation (mm)	493	234	205	37.8
Solar radiation (MJ m^{-2})	5.718	573	2.677	829
Chill Hours	1.433	857		
Chill Units (Utah)	478	1.308		
GDH	72.507		30.59	6.65
GDD (base 10°C)	1.807		697	123

Note: Chill Hours: Weinberger, 1950. Chill Units (Utah): Richardson *et al.*, 1974. GDH: Anderson and Seeley, 1992. GDD: Stanley *et al.*, 2000).

blooming period, environmental conditions favourable to bee activity were calculated, defined as the number of hours per day with air temperature higher than 15°C and solar radiation higher than 300 W m^{-2}.

Experimental design

The experimental design was a randomized by complete block divided into two treatments (5,000 m^2 per treatment). The treatments were: (1) control without product; (2) foliar application of Retard Cherry® (AM Ecological S.A., Chile). The product was applied twice at doses of 1.0 and 0.5 L/ha, prior to 50% leaf drop: for 'Regina' on March 26 and April 9; for 'Sweetheart' on March 15 and 30. Applications were made with a conventional hydro-pneumatic sprayer (Parada SpA, Santiago, Chile) with a spray volume of 1,200 L/ha. All measurements were made in 10 replicates, each consisting of two branches per replicate, trees per replicate, considering three edge rows per side.

Bloom delay

Blooming evolution (%) was determined weekly from stage 'first white' to 'full bloom', counting the number of open flowers per date. The full bloom date was defined when 80% had flowered. Bloom delay was determined by subtracting the days between the full bloom date of the control and that of the treatment.

Fruit set, fruit drop and yield

Fruit set and fruit drop were monitored on the same branches studied at bloom. Fruit set (%) was evaluated 20 days after the full bloom evaluation by counting the number of fruits formed in relation to the total number of flowers per branch. Fruit drop (%) was determined by the number of fruits that did not reach harvest in relation to the number of fruits formed. Yield (kg/tree) was also determined from individual trees.

Fruit quality

Harvest date was determined on the basis of fruit color. Evaluation of weight (g), diameter (mm), color, firmness, and soluble solids concentration (SSC) considered a sample of 50 fruits per treatment. Color was determined visually by scale (light red = 1, red = 2, mahogany red = 3, dark mahogany = 4 and black = 5). Firmness (g mm^{-1}) was measured with a FirmTech II texturometer (BioWorks Inc, Wamego, USA). SSC (°Brix) was measured on the same fruits with a digital refractometer (Atago, PLAS-BX/ACID5, Japan).

Statistical analysis

The data obtained underwent an analysis of variance (ANOVA) and the means were compared with the Tukey test (P≤0.05). When necessary, a transformation of the data was carried out. Analysis was performed with the Statgraphics Centurion XVI program (Warrenton, Virginia, USA) and the figures were generated using SigmaPlot 10 software (WPcubed GmbH, Germany).

3. Results

Sweet cherry trees treated with Retard Cherry® showed a 6-8 day delay in the full bloom date with respect to the control, with a greater delay in the case of 'Regina' (Table 2, Fig. 1). Although the application of Retard Cherry® delayed bloom, it had no effect on harvest date (Table 2).

A similar level of fruit set was maintained

Table 2 - Effect of foliar application of Retard Cherry® on the date of phenological stage in sweet cherry trees 'Regina' and 'Sweetheart'

Cultivar/treatments	80% full bloom	Harvest
'Regina'		
Control	09-Oct	25-Dec
Retard Cherry	17-Oct	25-Dec
'Sweetheart'		
Control	27-Sep	21-Dec
Retard Cherry	02-Oct	21-Dec

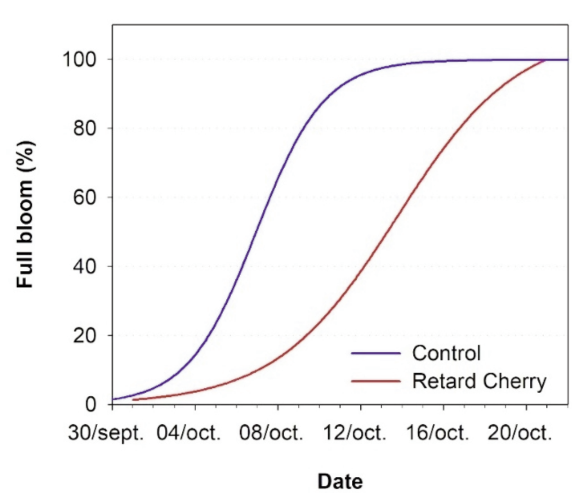

Fig. 1 - Effect of foliar application of Retard Cherry® on blooming dynamic in sweet cherry trees cv. Regina.

between treatments with an average of 49% for 'Regina' and 37% for 'Sweetheart' (Table 3). Even though fruit drop was numerically higher in the treatment with Retard Cherry®, it was not statistically significant, which is reflected in tree yields, with a mean of 8.6 kg/tree for 'Regina' and 14 kg/tree for 'Sweetheart' (Table 3).

The 'Regina' sweet cherry trees treated with Retard Cherry® showed 18% lower firmness than the control, with no change in fruit color, while 'Sweetheart' showed a higher incidence of fruit with lower color, although this was not noticeable to consumers. Fruit weight, diameter, and SSC were not affected by treatment (Table 4).

Table 3 - Effect of foliar application of Retard Cherry® on fruit set, fruit drop and fruit yield in 'Regina' and 'Sweetheart' sweet cherries

Cultivar/treatments	Fruit set (%)	Fruit drop (%)	Yield (kg/tree)
'Regina'			
Control	50 a	65 a	8.6 a
Retard Cherry	47 a	75 a	8.6 a
P-value	0.45	0.16	-
'Sweetheart'			
Control	33 a	ND	14.0 a
Retard Cherry	41 a	63	14.0 a
P-value	0.41	-	-

Means in a column followed by the same letter do not differ statistically, according to Tukey test (P≤0.05). n= 10/treatment. ND= no detected.

Table 4 - Effect of foliar application of Retard Cherry® on fruit quality in 'Regina' and 'Sweetheart' sweet cherries at harvest

Cultivar/ treatments	Weight (g)	Diameter (mm)	Color (1-5)	Flesh firmness (g mm^{-1})	SSC (°Brix)
'Regina'					
Control	12 a	28 a	4.0 a	231 a	20 b
Retard Cherry	12 a	28 a	4.4 a	189 b	21 a
P-value	0.41	0.31	0.09	0.00	0.01
'Sweetheart'					
Control	13 a	29 a	4.2 a	274 a	21 a
Retard Cherry	12 b	29 a	3.6 b	271 a	20 b
P-value	0.04	0.46	0.01	0.80	0.00

Means in a column followed by the same letter do not differ statistically, according to Tukey test (P≤0.05). n = 50/treatment.

4. Discussion and Conclusions

The results of fruit set are in concordance with those of Raffo and Curetti (2021) who reported a delay of up to 10 days in leaf emergence and full bloom of several sweet cherry cultivars in Rio Negro, Argentina. A greater assimilation and subsequent transport of reserves to the plant, prior to leaf fall, would allow an adequate dormancy and a more homogeneous bloom, effect that could be favored by the foliar application of biostimulants in autumn.

Fruit set in sweet cherry trees is normally low, and highly dependent on pollen availability and climatic conditions during and after pollination (Hedhly *et al.*, 2007). In Chile, sweet cherry growers use the following scale of fruit set intensity: high 34-40%; medium 15-20%; low 8-10% (C. Tapia, pers. comm, October 11, 2021).

Sagredo *et al*. (2017) found that the effective pollination period in 'Regina' sweet cherry cultivars was about 5 days post anthesis, with the highest fruit set levels occurring 2-3 days post anthesis. Similarly, Zhang *et al.* (2018) showed that the peak pollen germination and stigma receptivity of certain sweet cherry cultivars occurred 2-3 days post anthesis under three ambient temperature scenarios. In addition, the pollen tube required at least 48 h to reach the ovule.

Regarding to bee activity, more than 100 bee visits per minute were observed by Koumanov and Long (2017) with proper hive management, temperatures above 18°C and wind speed less than 16 km h^{-1}. On the other hand, Vicens and Bosch (2000) indicated that bee activity (*A. mellifera*) was fully active with air temperature above 14°C and solar radiation greater than 300 W m^{-2}.

The high fruit set obtained in this study could have been favored, among others, by the prevalence of suitable climatic conditions for bee activity during blossom and for fruit set of that season (Table 5; Fig. 2). No frost was observed during blossom in both orchards. Post-bloom environment of the trees treated with Retard Cherry® was more stable, with an average daily mean air temperature about 1-2 °C higher than that measured in the control trees one week earlier.

Table 5 shows the great difference in the conditions for bee flight at blooming between the two cultivars. The application of Retard Cherry, by delaying blooming, had a much greater effect on 'Sweetheart', as it distanced it from the riskiest date of low tem-

Table 5 - Environmental conditions in the seven days since full bloom for both cultivars and treatments

Cultivar/Treatment	Period	Air temperature (°C)			Relative humidity (%)	Days with rainfall	Rainfall (mm)	Bee activity (h) [(x)]
		mean	max	min				
'Regina'								
Control	9 Oct - 15 Oct	11.8	18.8	5.4	69.4	0	0	30
Retard Cherry	17 Oct -23 Oct	13.8	20.8	7.7	64.4	2	3	37
'Sweetheart'								
Control	2 Sept - 3 Oct	11.0	16.7	6.2	71.8	4	18	17
Retard Cherry	2 Oct - 8 Oct	11.9	19.5	6.1	72.8	1	11	26

[(x)] Bee activity (hours with air temperature > 15°C and solar radiation > 300 W m^{-2}).

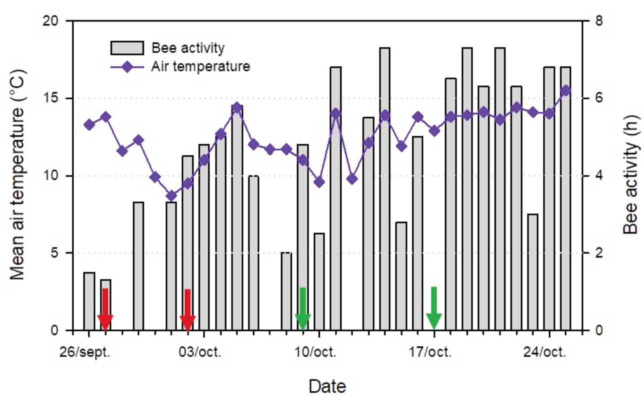

Fig. 2 - Mean daily air temperature and hours favorable for bee activity (>15°C and > 300 W m^{-2}) per day from 26 September to 25 October 2018.
Arrows indicate full bloom date: green for 'Regina' and red for 'Sweet-heart'. The first arrow corresponds to the control for both cultivars.

peratures and frost in early spring; something similar occurred in the case of 'Regina', although with less intensity, as it is a later blooming cultivar.

Harvest date was not affected using Retard Cherry® on sweet cherry trees, although there was evidence of lower fruit firmness on Regina and slightly less coloration on 'Sweetheart' (Tables 2 and 4). The fruit ripening stage is characterized by a rapid increase in weight and size, due to an increase in cell size, leading to a reduction in firmness. Sugar content increases, keeping acids relatively constant; however, color is the one that shows the greatest changes, being a relevant factor in determining the harvest date in sweet cherries (Tudela *et al.,* 2005; Muskovics *et al.,* 2006). In previous studies, Raffo and Curetti (2021) reported that the use of Retard Cherry® showed a marked delay in color development of 'Sweetheart' sweet cherries. The use of GA_3 allows delay harvest in sweet cherry trees and has also proven to increase fruit size, firmness and SSC (Basak *et al.,* 1998; Horvitz *et al.,* 2003; Raffo and Curetti, 2021). Therefore, GA_3 would be a complementary tool to the use of foliar biostimulant Retard Cherry®, to extend the harvest window, with good quality fruit and better prices.

Therefore, it can be concluded that the use of Retard Cherry® on 'Regina' and 'Sweetheart' sweet cherry trees is an effective tool for delaying bloom to avoid frost event and favor conditions for bee flight. No delay in the harvest date was observed. Fruit set and fruit drop percentages were not affected by the treatment. At harvest, fruit from trees treated with Retard Cherry® showed no differences in size and SSC but showed lower firmness for 'Regina' and less color for 'Sweetheart' compared to those harvested from control trees.

Since this study was carried out under specific climatic conditions, further investigations will be necessary to consolidate the results obtained.

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