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Preharvest Gibberellic Acid Treatment Increases Both Modulus of Elasticity and Resistance in Sweet Cherry Fruit (cv. 'Bing' and 'Lapins') at Harvest and Postharvest During Storage at 0 °C

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Abstract: Fruit firmness in sweet cherries (*Prunus avium* L.) is a critical quality parameter highly valued by consumers as it is associated with fruit freshness. In general, firm fruit also cope better with storage and handling. Gibberellic acid (GA) is commonly used by sweet cherry producers to increase firmness, soluble solids content and fruit size. This study evaluated the effects of GA on the rheological properties of sweet cherry fruit at harvest and postharvest storage. Specifically, GA's influence on susceptibility to mechanical damage during handling was evaluated. The following GA treatments were applied to two sweet cherry cultivars 'Bing' and 'Lapins': T0, control, T30—GA at 15 ppm applied at pit-hardening and straw-colour stages; T45—GA at 25 ppm at pit-hardening and GA at 20 ppm at straw-colour; and T60—GA at 30 ppm applied at pit-hardening and straw-colour. The results indicate that GA delayed harvest by two to four days in both cultivars, with 'Lapins' also showing a significant increase in fruit size. Regardless of spray concentration, GA increased the modulus of elasticity and fruit resistance evaluated as stress at the maximum point at harvest. These effects persisted after 35 days of storage at 0 °C and an additional three days of shelf-life at 15 °C. While the strain or deformation capacity of the fruit at bioyield at harvest was constant across treatments, it was, however, lower in the GA-treated fruit than in the controls during storage at 0 °C under the high-humidity conditions of modified atmosphere packaging. The less mature fruit harvested at colour 3.0 (red/mahogany) were stiffer (reduced deformation) and more sensitive to induced mechanical injury than the fruit harvested later at colour 3.5 (mahogany). The GA treatments increased fruit resistance to damage without increasing tissue deformability. Other questions associated with stiffer tissues and lower deformability during storage at 0 °C under high humidity should be further studied, specifically cultivars that are naturally high in box-cracking sensitivity during storage.

Keywords: *Prunus avium*; rheological properties; strain at bioyield; stress; firmness; bruising; postharvest; mechanical damage

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1. Introduction

Sweet cherries (*Prunus avium* L.) are highly valued by consumers for their internal attributes of a distinctive flavour, a pleasing acid/sugar balance and a firm texture. They are also valued for their visual characteristics, such as a heart-like shape, a one-bite size and a deep red colour. Cherries are especially valued if they are without visual defects and with shiny skins and turgid green pedicels (stalks) [1–3]. Fruit firmness is also important, being associated with longer shelf-life (key for distributors) and with greater acceptance by consumers (who associate firmness with freshness and softness with old fruit) [4]. Hence,

it is important to be able to measure fruit firmness. This study deals with the rheological relationships between an external applied force acting on the fruit and the resistance and deformation that results. A viscoelastic behaviour is observed that can be quantified via measurements of the stress–strain relationship with subsequent time-dependent relaxations [5]. For small forces and deformations, the fruit exhibits elastic (reversible) behaviour, and then, when some critical deformation is exceeded, the deformation is plastic (irreversible). Beyond the plastic limit, greater forces and deformations cause tissue damage (cell rupture) [6]. A fruit may exhibit different behaviours depending on how the test is performed. Most methods measure uniaxial force deformations, but biaxial tests have also been performed that are closer to reality since they simulate the actual stresses and strains associated with the fruit's three-dimensional growth [7,8].

Rheological variables vary between cultivars, as has been demonstrated in sweet cherry, so genetic factors are clearly important [9], but the rheological properties also vary in the same fruit depending on temperature, ripeness, transpiration and water uptake [7]. For commercial producers and distributors, sweet cherry firmness is expressed in Durofel, a measurement made using a durometer. A number of other devices have been used to measure fruit firmness. Some of these employ their own measurement units or use special probes, making it difficult to compare results from different workers [10]. The study of the rheological variables of stress, strain, energy and modulus of elasticity in samples at the inflection point, bioyield point and maximum point provides more robust information when characterising the different plant tissues and their sensitivities to mechanical damage [11]; hence, high values of deformation (strain) characterise fruit that are more resistant to mechanical damage [9].

In sweet cherry, fruit firmness changes during development. It increases during growth Stage I, reaches a maximum in Stage II at pit-hardening, and then decreases during Stage III as ripening occurs, reaching a minimum at harvest [12,13]. During the postharvest period, two firmness behaviours have been reported, depending on storage conditions. If fruit are stored in a modified atmosphere packaging (MAP) bag at 100% relative humidity, firmness is maintained or increases slightly [14], but if not, then firmness decreases [15,16], especially when the fruit is removed at high temperature and low moisture conditions. Susceptibility to mechanical damage is related to the rheological properties of the fruit at the time of harvest [17]. Thus, there is a positive relationship between firmness and pitting resistance [18], with higher values of stress and strain at the bioyield point being associated with higher resistance to pitting [9].

The rheological properties of sweet cherry fruit can be modified through the use of various agronomic managements. Thus, firmness is increased by fruit thinning [19,20], by foliar applications of calcium [21,22], and by the application of elicitor compounds such as methyl jasmonate, salicylic acid or melatonin [23–25]. The phytohormone gibberellic acid (GA) is widely used by cherry producers, which increases fruit firmness [26–28]. In a recent study with numerous cultivars and GA application rates, it was found that GA increased firmness, soluble solids and titratable acidity and reduced stem browning and surface pitting. However, the genotype did not have a strong influence on the response [29]. GA inhibits floral bud induction in sweet cherry, leading to a reduction in flower number, with a consequent decrease in yield in the season following application. It has been observed that a double application of 50 ppm or a single application of 100 ppm significantly reduces yield; however, commercial applications do not exceed these thresholds, with rates around 30 ppm [30].

Sweet cherries are classified as non-climacteric fruit, meaning they must complete ripening on the tree and do not experience a significant peak of ethylene during the process. Additionally, other hormones, such as abscisic acid, are involved, increasing prior to maturation and decreasing as harvest approaches [31,32]. The rise in abscisic acid in sweet cherries has been linked to the activation of metabolic pathways associated with ripening, including anthocyanin biosynthesis, decreased firmness and increased sugar content [32–34]. Moreover, treatments with GA have been shown to delay the accumulation

of abscisic acid at the onset of ripening, effectively delaying the natural ripening process of the fruit [35]. The objective of this study is to understand how GA modifies the rheological properties of a sweet cherry at harvest and during storage and how these modifications affect the sensitivity of the fruit tissues to mechanical damage.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiment was conducted in the 2021–2022 season with the sweet cherry cultivars 'Bing' and 'Lapins' in commercial sweet cherry orchards (*Prunus avium* L.) located in Graneros (lat. 34°03′36.7″ S, long. 70°44′01.3″ W) and Mostazal (lat. 34°00′32.0″ S, long. 70°42′25.7″ W) in the central valley of Chile, Sixth Region, respectively. The cultivar (rootstock) combinations 'Bing' (Gisela 12) and 'Lapins' (Colt) were planted in 2013 and 2016, respectively. In both orchards, trees were trained to a Y-shape trellis, and spacing was at 4 × 2 m, with 1250 trees ha^{-1}. Similar soil, climate conditions and agronomics cultural practices were employed with each cultivar in each orchard.

Gibberellic acid (ProGibb ® 40% soluble granule, Valent BioSciences, Libertyville, IL, USA) treatments were named T30, T45 and T60. For T30, GA at 15 ppm was applied at pit-hardening (start of Stage II) and straw-colour (end of Stage II) stages; for T45, GA was applied at 25 ppm at pit-hardening and at 20 ppm at straw-colour; and for T60, GA at 30 ppm was applied at both fruit growth stages. Control trees (T0 treatment) were sprayed with water (Table 1). These rates were chosen based on commercial use by farmers and previous research conducted by Zoffoli et al. [36]. The decision was made to broaden the range to evaluate the effect of higher doses on the behaviour of the rheological variables. The beginning of pit-hardening occurred 28 days after full bloom (DAFB) in 'Lapins' and 'Bing', and straw-colour was reached at 44 DAFB in 'Bing' and 45 DAFB in 'Lapins'. Whole canopies were sprayed using hydropneumatics spraying equipment (NT-2000, Lerpain, Isla de Maipo, Chile) at a rate of 1500 L ha^{-1}, so a notional rate of 1.2 L $tree^{-1}$. The sprays were applied between 7:00 and 9:00 a.m. to avoid dew and at temperatures below 25 °C. Coverage was uniform and complete—i.e., to run-off.

Table 1. Description of gibberellic acid (GA) treatments and harvest dates (days after full bloom, DAFB) at colour 3.5 on 'Bing' and 'Lapins' sweet cherry cultivars.

Cultivar	Treatment	GA (ppm)	Time of Application (DAFB)	Harvest (DAFB)
Bing	T0	0	-	82
	T30	15 + 15	Pit-hardening (28) + Straw-colour (44)	84
	T45	25 + 20	Pit-hardening (28) + Straw-colour (44)	86
	T60	30 + 30	Pit-hardening (28) + Straw-colour (44)	86
Lapins	T0	0	-	87
	T30	15 + 15	Pit-hardening (28) + Straw-colour (45)	87
	T45	25 + 20	Pit-hardening (28) + Straw-colour (45)	91
	T60	30 + 30	Pit-hardening (28) + Straw-colour (45)	91

The experimental units were arranged in a randomised complete block design for each cultivar. Subsequently, three rows were selected, and the treatments were applied in the central row to avoid spray drift from adjacent rows. Four replicates of three trees of similar vigour, size and fruit load were selected and randomly assigned to each treatment. A minimum of six trees were left with respect to the roads to avoid edge effects. Fruit was harvested at two maturity stages when more than 80% of the cherry population achieved the skin colour 3 or colour 3.5 (cherry colour chart scale 2022, Pontificia Universidad Católica de Chile), resembling colour numbers 4 and 5 according to the CTIFL colour chart (Centre Technique Interprofessionnel des Fruits et Légumes, Paris, France), respectively. Two groups of 10 fruit per replicate for each colour stage were collected, one for the evaluation of maturity and the other for the determination of rheological properties.

One hundred fruit per replicate were randomly selected from the exterior of the canopy at the time of harvest with colour 3.5, and the size and colour distribution were determined using an optical vision machine (Cherry roller, PT&I Chile, Santiago, Chile). The size distribution was described in terms of percentage of fruit in each commercial category (Undersize < 22 mm; L 22.0–23.9 mm; XL 24.0–25.9 mm; J 26.0–27.9 mm; 2J 28.0–29.9 mm; 3J 30.0–31.9 mm; 4J 32.0–33.9 mm; and 5J > 34 mm) and the colour distribution by the proportion of fruit in each category (i.e., 3 and 3.5, red/mahogany and mahogany).

2.2. Storage and Fruit Quality Postharvest

One 3 kg group of fruit of colour 3.5 was harvested separately per replicate, placed in a plastic box and hydrocooled with 0 °C sanitised water. The water sanitisation was performed using a 70% calcium hypochlorite solution (Unión Química Spa, Lampa, Chile) and had 80–100 ppm of free chlorine. Then, fruit were immersed in a fungicide solution with a 0.1% fludioxonil (SCHOLAR ® 230 Suspension Concentrated (SC) formulation containing 23% p/v of fludioxonil; Syngenta Crop Protection Inc., Omaha, NE, USA), and 2.5 kg of fruit that were free of visible damage and of uniform diameter (26–28 mm) were selected and packaged in a modified atmosphere bag (Crystal Cherry 826, San Jorge Packaging, Santiago, Chile) after one day of storage at 0 °C. The fruit was then stored for 35 days at 0 °C to simulate the time it takes for Chilean sweet cherries to reach consumers in the Asian market, the most significant market for the Chilean cherry industry. Following this, a shelf-life period of 3 days at 15 °C was included to simulate the time until the fruit is consumed.

Fruit quality after 35 days at 0 °C was characterised in terms of decay, orange-skin disorder, bruising and pitting, and incidences were calculated and expressed as percentages from samples of 70 fruit. There was only one category of damage assessed following the method described by Zoffoli and Rodriguez [37]. The severity of damage was assessed using an arbitrary scale: mild = 1, moderate = 2 and severe = 3. The severity was calculated as the sum of the number of fruit in each category (n_1, n_2 and n_3) multiplied by each factor 1, 2 and 3, respectively, and the total was divided by the number of damaged fruit.

2.3. Fruit Growth and Evolution of Maturity Parameters

Samples of eight fruit of 'Bing' or 'Lapins' from the exterior of the canopy were identified on each of the four trees (replicate) 23 and 25 days after full bloom, and the increases in fruit diameter (mm) were determined weekly. At the same times, groups of 10 fruit per replicate were transported to the laboratory, where composite juice samples were created, and soluble solids (%) and titratable acidity (%) were determined using a digital thermo-compensated refractometer (PAL-1, Atago Co. Ltd., Tokyo, Japan) and by titration with NaOH 0.1 N until pH 8.1 (Edge HI2002, Hanna Instruments, Woonsocket, RI, USA), respectively.

The crude cell wall content was assessed in colours 3 and 3.5 by the alcohol insoluble residue (AIR) method as described by Choi et al. [38] and modified by Param and Zoffoli [9] from a sample of 25 g of ground fruit flesh.

2.4. Rheological Properties and Increased Sensitivity to Damage

The rheological properties of the fruit tissue were determined by a compression test using a Texturometer TA.XT plus analyser (Stable Micro Systems Ltd., Godalming, England) fitted with a 5 mm diameter cylindrical steel probe with a hemispherical end with a contact surface area of 19.6 mm^2. The fruit's mechanical parameters of modulus of elasticity, stress and strain were obtained using the protocol of Param and Zoffoli [9] at two harvest stages—colour 3 and 3.5, in storage after 35 days at 0 °C and after 35 days at 0 °C plus 3 days of shelf-life at 15 °C. The measurements were carried out on the cheek of each fruit (10 fruit per replicate) after previously homogenising the fruit at 15 °C. The compression force was applied to the major axis of the fruit's equatorial diameter. The loading rate was 0.3 mm s^{-1} for a maximum penetration depth set at 5 mm (maximum point) to avoid tissue

disruption. The modulus of elasticity (E) (MPa) is the ratio between stress and strain at the inflection point (just before the start of plastic deformation), and this value indicates how resistant the fruit is to elastic deformation. It was calculated as:

$$E = \frac{FL}{A\Delta L} \quad (1)$$

where F is the force (N), A is the probe area (mm^2), L is the initial length of the fruit (mm) and ΔL is the change length of the fruit (mm) after the test. The stress (σ) (kPa) was calculated as the ratio between the applied force and the area of the probe (σ = F/A), and the strain (ε) (%) was calculated as the ratio ΔL between L (ε = ΔL/L). These variables were calculated from the force/distance curve at the inflection point, at the maximum point (5 mm) and at the bioyield point, which occurs where there is an increase in deformation with a decrease or no change in the force, or the point at which flesh cells begin to rupture but without visible external damage [39].

The compression damage sensitivity was assessed in the same fruit as that in which the compression test was performed because the conditions of this test are those that induce compression damage. On the other hand, the impact damage was evaluated on the day of the harvest on 10 fruit per replicate at 15 °C by dropping a 10 g stainless steel rod of 5.4 mm hemispherical head diameter from a height of 10 cm onto one side of each fruit; the device used was described by Zoffoli et al. [40]. After performing the compression test and impact test, the fruit was placed on a tray inside a polyethylene bag and stored for 10 days at 0 °C and 100% relative humidity. To calculate the fruit damage index, a 5-point scale was used where 0 = no pitting and 4 = very severe pitting; the method and visual scale used were those proposed by Param and Zoffoli [9].

2.5. Statistical Analysis

Statistical analyses were performed using the InfoStat v 2020 software (InfoStat Group, National University of Córdoba, Córdoba, Argentine), the data were analysed using analysis of variance (ANOVA) and mean separations were performed using Fisher's least significant difference (LSD) test when the applicable p-value ≤ 0.05. The data in the figure were graphed with SigmaPlot v 11.0 (Systat Software Inc., San Jose, CA, USA) and are presented as the means and standard error.

3. Results

3.1. Crop Yield, Fruit Growth and Quality Parameters at Harvest

Gibberellic acid treatments delayed the harvest date for 'Bing' at colour 3.5 by two days for T30 and by four days for T45 and T60. Similar four-day harvest delays occurred for 'Lapins' with the higher GA rates. Furthermore, this delay in fruit pigmentation was observed during the fruit ripening period, 77 and 79 days after full bloom for 'Bing' and 'Lapins', respectively (Figure 1). The untreated fruit of 'Bing' was harvested at 82 DAFB and of 'Lapins' at 87 DAFB (Table 1). Data obtained from the optical vision machine for random sampling at harvest confirmed that more than 80% of the fruit on the tree attained the 3.5 colour at harvest. The average production of 'Bing' was between 22.5 and 23.9 kg $tree^{-1}$, and the average fruit weight was similar between GA and control fruit (in the range of 8.3 and 8.5 g $fruit^{-1}$). On the contrary, for 'Lapins', the average weight (8.6 g $fruit^{-1}$) of the control fruit was significantly lower (p-value = 0.0001) than the weight (10.3 g $fruit^{-1}$) of GA. In addition, fruit production per tree was also increased in 'Lapins', with values of 10 kg $tree^{-1}$ in controls and 14.5 kg $tree^{-1}$ in GA-treated trees (p-value = 0.0406).

Figure 1. Colour expression of sweet cherry, cv. 'Bing' and 'Lapins', at 77 and 79 days after full bloom, respectively, for fruit treated with GA. T0: control and T60: GA at 30 ppm applied at pit-hardening and straw-colour stages.

The GA treatments did not affect soluble solids accumulation at harvest (colour 3.5) in 'Bing', with average soluble solids of 24.4% or in 'Lapins', with an average of 22.1%. The average titratable acidity in 'Bing' across all treatments was 1.23%, and in 'Lapins', between 0.85% and 1.02%.

Fruit diameter was similar among GA treatments and control in 'Bing', but in 'Lapins', control fruit were smaller than GA fruit. The main differences showed up in the last stages of development, where fruit diameter reached ca. 26 mm in controls compared with 28 mm in GA-treated ones (Figure 2).

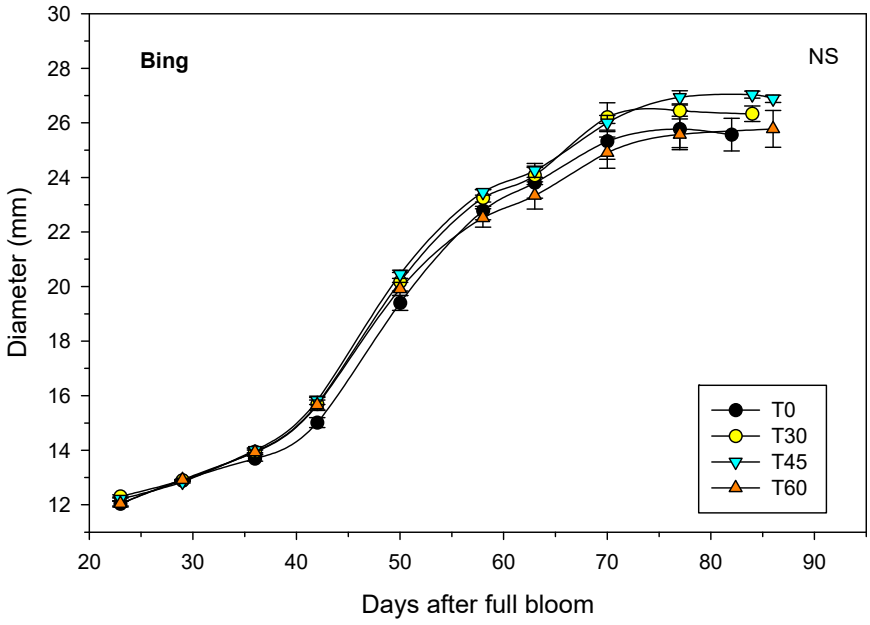

Figure 2. *Cont.*

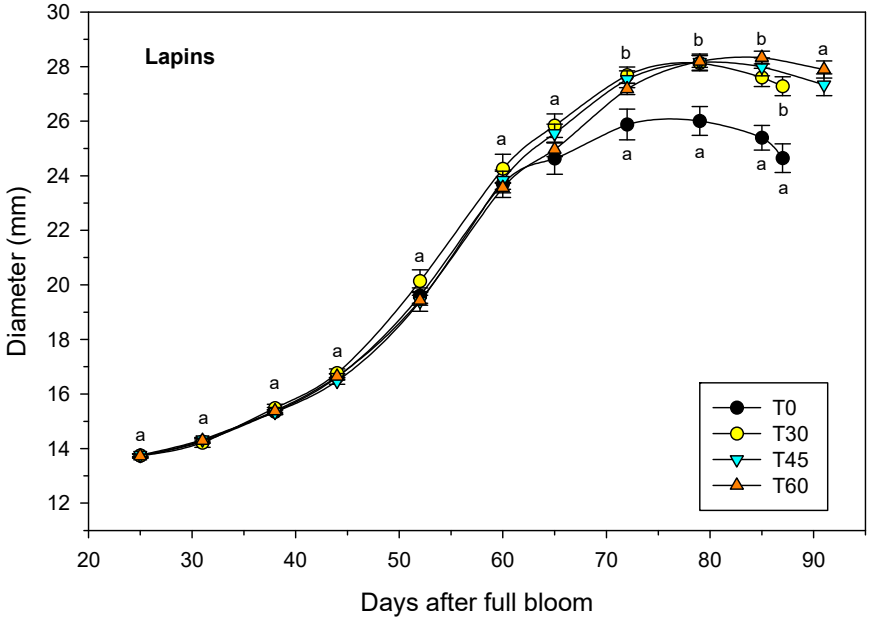

Figure 2. Growth in fruit diameter during development for control and gibberellic acid (GA)-treated fruit of cv. 'Bing' and 'Lapins' sweet cherries. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour). Different letters for each day show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

As we expected from above, the size distribution of 'Bing' fruit was not affected by GA treatment, but 97% of the population of GA-treated 'Lapins' fruit had diameters greater than 26 mm, while only 76% of the population of control fruit had diameters greater than 26 mm (Figure 3).

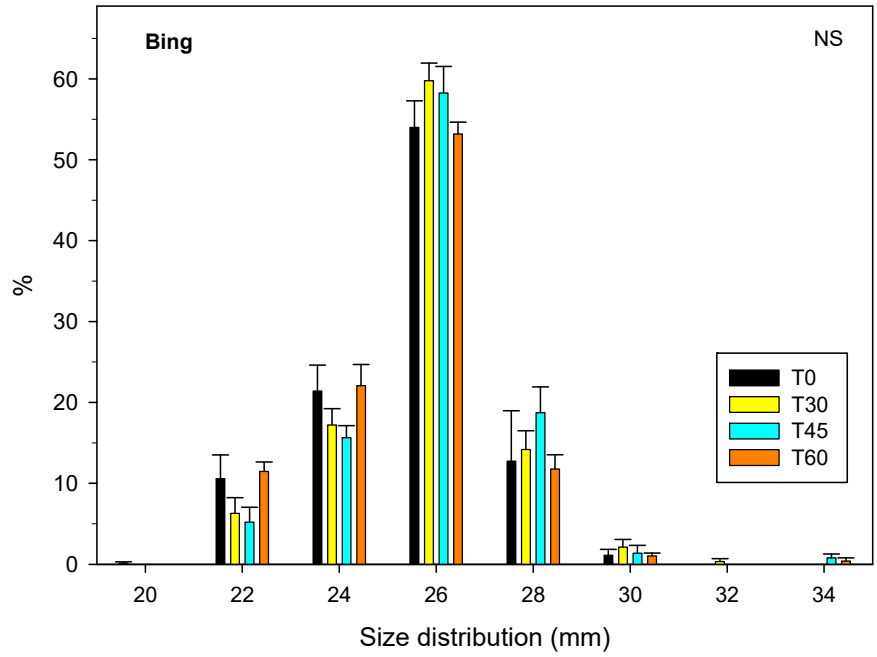

Figure 3. *Cont.*

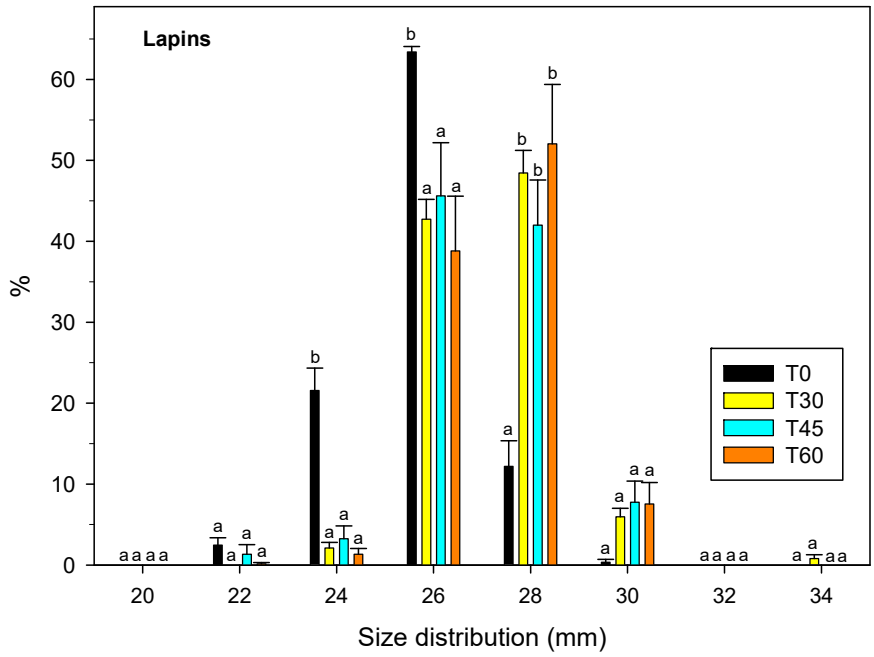

Figure 3. Fruit size distribution at harvest for cv. 'Bing' and 'Lapins' sweet cherries depends on the rate of gibberellic acid (GA) application. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour). Different letters for each size show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

3.2. Rheological Properties at Harvest and Postharvest

In 'Lapins', the application of GA increased the modulus of elasticity at harvest. Treatment T60 induced the highest modulus of 1.92 MPa compared to 1.23 MPa for the control. There were no significant differences among the different GA treatments. 'Bing' behaved similarly, with the modulus of elasticity of the control being 1.73 MPa and the GA treatments all increasing it, with T30 giving the highest modulus of elasticity at 2.19 MPa. The strain at bioyield had a mean value of 11.0% for 'Bing' and 10.4% for 'Lapins', without significant differences among treatments. In 'Bing', the maximum stress value was increased by GA treatment, with 262.4 kPa being reached with T30 compared to the 223 kPa in the control. In 'Lapins', GA increased the maximum stress by an average of 21% compared with 185.8 kPa in the control (Table 2).

Evaluations after 35 days of storage at 0 °C showed similar patterns in terms of the rheological properties of the fruits. The modulus of elasticity and maximum stress were significantly higher in the GA treatments than in the controls. In the 'Bing', the modulus of elasticity and stress were 1.93 MPa and 242.9 kPa, respectively; in the control, they increased by about 32% and 16% in the GA-treated fruit. No significant differences were found among the different GA treatments. In 'Lapins', a dose-dependent effect was observed in which the highest rates induced the highest modulus of elasticity and stress. The modulus of elasticity of the control was 1.38 MPa, and the maximum stress was 194 kPa. The T60 treatment increased these values by about 97% and 38%, respectively. In both cultivars, the strain at bioyield was significantly higher in the controls than in the GA treatments. In 'Bing', the control was 10.37%, and T30 was 8.33%, distinct from the other GA treatments. In 'Lapins', the control was 10.09%, and the GA treatments averaged 8.74% (Table 3).

Table 2. Effect of gibberellic acid (GA) treatments on the rheological properties of 'Bing' and 'Lapins' sweet cherries at harvest in colour 3.5. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.73 a	10.65	223.0 a
	T30	2.19 b	11.01	262.4 b
	T45	1.92 ab	11.12	245.3 ab
	T60	1.92 ab	11.30	244.3 ab
p-value		0.0257	NS	0.0366
Lapins	T0	1.23 a	10.95	185.8 a
	T30	1.71 b	10.11	224 b
	T45	1.74 b	10.21	220.8 b
	T60	1.92 b	10.14	231.5 b
p-value		0.0016	NS	0.0021

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

Table 3. Effect of gibberellic acid (GA) on the rheological properties of 'Bing' and 'Lapins' sweet cherries of colour 3.5 after 35 days of storage at 0 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.93 a	10.37 c	242.9 a
	T30	2.51 b	8.33 a	275.1 b
	T45	2.51 b	8.80 b	287.6 b
	T60	2.64 b	8.86 b	281.8 b
p-value		0.0011	<0.0001	0.0403
Lapins	T0	1.38 a	10.09 b	194.0 a
	T30	2.29 b	8.83 a	241.7 b
	T45	2.45 bc	8.64 a	255.4 bc
	T60	2.72 c	8.75 a	267.7 c
p-value		<0.0001	0.0380	0.0001

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05.

Gibberellic acid treatments did not affect the incidence of postharvest decay; incidences were 0.28% in 'Bing' and 0.7% in 'Lapins', and average pitting values were 32% in 'Bing' and 23% in 'Lapins'. The incidence of bruising in 'Bing' was lower for the higher rates of GA (T45 and T60), and damage severity was significantly lower in the GA treatments than in the control. In 'Lapins', bruising incidence was lower in T30 and T60 than in the control, but the lowest damage severity was in T45 (Table 4). The incidence of orange-skin disorder was high in both the controls and the GA treatments, with an average value of 97% in 'Bing' and 99% in 'Lapins'.

Table 4. Effect of gibberellic acid (GA) treatment on bruising in 'Bing' and 'Lapins' sweet cherries in colour 3.5 after 35 days of storage at 0 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Bruising	
		Inc. [1] (%)	Sev. [2] (1–3)
Bing	T0	32 b	2.27 b
	T30	30 b	1.78 a
	T45	16 a	1.69 a
	T60	19 a	1.71 a
p-value		0.0001	0.0193
Lapins	T0	27 b	2.54 c
	T30	8 a	2.43 bc
	T45	18 ab	1.76 a
	T60	13 a	2.01 ab
p-value		0.0185	0.0118

[1] Inc. refers to the incidence, that is, the proportion of fruit affected. [2] Sev. refers to the severity of the damage, which was evaluated with an arbitrary scale where 1 = mild, 2 = moderate and 3 = severe. Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05.

After postharvest storage at 0 °C and three days of shelf-life at 15 °C, the modulus of elasticity and the maximum stress were significantly different, with the GA treatments being higher than the controls. In 'Bing', there were no differences in the modulus of elasticity and the maximum stress between the GA treatments, but in 'Lapins', the modulus of elasticity was highest for T60 (76%), while the maximum stress was higher (31%) than the control. The strain at bioyield in 'Bing' was significantly higher in T45 and T60, with an average value of 9.09% compared with the control of 8.12%. In 'Lapins', the control value was 10.75%, and the GA treatments averaged 9.25% (Table 5). The GA treatments did not affect soluble solids at postharvest in 'Bing', with average soluble solids of 23.7%, and the 'Lapins' average was 21.9%. The average titratable acidity in 'Bing' across all treatments was 1.08%, and in 'Lapins', it was 0.9%.

Table 5. Effect of gibberellic acid (GA) treatments on the rheological properties for 'Bing' and 'Lapins' sweet cherries at harvest in colour 3.5 after 35 days of postharvest storage at 0 °C and three days of shelf-life at 15 °C. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

Cultivar	Treatment	Modulus of Elasticity (MPa)	Strain at Bioyield (%)	Maximum Stress (kPa)
Bing	T0	1.84 a	8.12 a	236.7 a
	T30	2.35 b	8.67 ab	288.0 b
	T45	2.49 b	8.91 b	312.7 b
	T60	2.54 b	9.27 b	312.7 b
p-value		0.0276	0.0168	0.0031
Lapins	T0	1.40 a	10.75 b	211.3 a
	T30	1.97 b	9.06 a	241.4 b
	T45	2.26 bc	9.38 a	260.2 bc
	T60	2.46 c	9.31 a	276.4 c
p-value		0.001	0.0317	0.0011

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05.

3.3. Effect of Maturity on Rheological Properties and Induced Mechanical Damage

The incidences of impact and compression damage were higher in colour 3 than in colour 3.5 in both 'Bing' and 'Lapins', with significant differences except for with the impact on 'Lapins'. If 'Bing' is allowed to ripen to colour 3.5, the impact damage was 33% less. For compression damage, reductions of around 40% and 27% were achieved with GA for 'Bing' and 'Lapins', respectively. During fruit ripening from colour 3 to 3.5, the modulus of elasticity decreased by 17% and 18% for 'Bing' and 'Lapins', respectively, while the strain increased by 8% and 13%, respectively. Maximum stress decreased by 11% in 'Bing', but decreases were not significant in 'Lapins' (Table 6). Gibberellic acid treatments did not affect the fruit damage index (see Supplementary Material Table S1).

Table 6. Effect of maturity (colour indices 3 and 3.5) on rheological properties and induced mechanical damage (fruit damage index) by compression and impact tests on 'Bing' and 'Lapins' sweet cherry cultivars.

		Fruit Damage Index [1]		Rheological Properties		
Cultivar	**Colour**	**Compression Test**	**Impact Test**	**Modulus of Elasticity (MPa)**	**Strain at Bioyield (%)**	**Maximum Stress (kPa)**
Bing	Colour 3	3.54 b	1.44 b	2.34 b	10.24 a	275.08 b
	Colour 3.5	2.13 a	0.96 a	1.94 a	11.02 b	243.76 a
	p-value	<0.0001	0.0052	<0.0001	0.0108	0.002
Lapins	Colour 3	3.74 b	1.38	2.01 b	9.19 a	233.08
	Colour 3.5	2.73 a	1.24	1.65 a	10.35 b	215.55
	p-value	<0.0001	NS	0.0054	0.001	NS

[1] Each fruit was evaluated on an arbitrary 5-point scale where 0 = no pitting, 1 = mild pitting, 2 = moderate pitting, 3 = severe pitting and 4 = very severe pitting. Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with p-value < 0.05. NS: non-significant at p-value < 0.05.

3.4. Alcohol Insoluble Residues (AIR)

In 'Bing', the concentration of AIR in colour 3 increased as the rate of GA increased: the controls had the lowest AIR value of 1.49 g 100g^{-1} FW, and T60 had the highest value of 2.06 g 100 g^{-1} FW. However, the AIR content per fruit was 178.03 mg/fruit, compared to 139.6 mg/fruit in the control, without significant differences among the GA treatments. In 'Lapins', the behaviour was similar at colour 3: the highest concentration of AIR was in T60 with 1.81 g 100 g^{-1} FW, compared with the control with 1.73 g 100 g^{-1} FW. The lowest fruit content was in the control, with 169.58 mg/fruit, and the highest was in the T60 treatment, with 204.87 mg/fruit. For colour 3.5, there were no significant differences in AIR between controls and treatments, which had average AIR concentrations for 'Bing' and 'Lapins' of 2.07 g 100 g^{-1} FW and 1.95 g 100 g^{-1} FW, respectively, with average AIR contents of 204.51 mg/fruit and 204.43 mg/fruit, respectively (Table 7).

Table 7. Effect of gibberellic acid (GA) treatments on cell wall concentrations of alcohol insoluble residues (AIR) in 'Bing' and 'Lapins' sweet cherries at colours 3 and 3.5. Treatments: T0 (control), 0 ppm GA; T30, 15 + 15 ppm GA (pit-hardening + straw-colour); T45, 25 + 20 ppm GA (pit-hardening + straw-colour); T60, 30 + 30 ppm GA (pit-hardening + straw-colour).

		Colour 3		Colour 3.5	
Cultivar	**Treatment**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**
Bing	T0	1.49 a	139.60 a	1.89	188.07
	T30	1.76 b	172.43 b	2.12	205.62
	T45	1.86 bc	174.63 b	1.96	193.96
	T60	2.06 c	187.03 b	2.32	230.40

Table 7. *Cont.*

Cultivar	Treatment	Colour 3		Colour 3.5	
		AIR (g 100 g^{-1} FW)	**AIR (mg/fruit)**	**AIR (g 100 g^{-1} FW)**	**AIR (mg/fruit)**
p-value		0.0004	0.0149	NS	NS
Lapins	T0	1.73 b	169.58 a	2.09	199.15
	T30	1.53 a	170.85 a	1.81	199.12
	T45	1.69 ab	187.22 ab	1.96	215.78
	T60	1.81 b	204.87 b	1.94	203.68
p-value		0.0385	0.0461	NS	NS

Different letters for each cultivar and in each column show significantly different mean values for Fisher's LSD test, with *p*-value < 0.05. NS: non-significant at *p*-value < 0.05.

4. Discussion

In this trial, the 'Bing' and 'Lapins' trees were 9 and 6 years old, respectively, and both varieties exhibited full production, judging by the yields recorded during the season. The average production from the previous season was 9400 kg/ha for 'Bing' and 12,300 kg/ha for 'Lapins'. Regardless of the trees' age, fruit load plays a much more critical role in influencing the size and quality of the fruit, as shown in other studies [20].

Regardless of the application rate, GA delayed harvest by between 2 and 4 days in 'Lapins' and 'Bing', as has been reported previously [12,41]. This harvest delay has significant implications: early-season producers may experience lower prices, while mid-season and late-season producers may find it advantageous for extending their harvest season. Additionally, for larger farms, this delay serves as a strategy to stagger the harvesting of different cultivars. This delay in anthocyanin synthesis is explained by the lower activity of the phenylalanine ammonia-lyase enzyme, which is hindered by GA [42,43]. Additionally, the delay in sweet cherry ripening and the modification of quality parameters may be attributed to the interaction between exogenous gibberellins (GA) and abscisic acid (ABA). This interaction leads to a reduction in ABA levels at the onset of sweet cherry ripening, thereby impacting the natural ripening process of the fruit [35]. GA also influences the transcript levels of certain genes involved in ABA homeostasis and signalling while also affecting various other pathways. The regulation of GA appears to differ based on whether the sweet cherry variety is early or mid-season. In this study, mid-season varieties were used, indicating that ripening control may occur through the regulation of PP2C gene expression [44,45]. Notably, in climacteric fruits, exogenous GA can also influence ripening and senescence by regulating ethylene-related pathways [46]. In terms of postharvest preservation, combining preharvest GA applications with modified atmosphere packaging (MAP) technology has proven effective in minimising storage losses and maintaining fruit quality during cold storage [47].

The increasing fruit size explains the main effect of GA on 'Lapins', where similar results have been reported for the cv. 'Skeena', 'Sweetheart' and 'Staccato' [29] and in 'Sweetheart' [48]. In 'Bing', however, GA did not increase fruit size, which may be explained by the naturally high crop load in the 'Bing'/'Gisela 12' combination. Similar results were found by Zhang and Whiting [49] but not by Facteau et al. [26], where there was a significant increase in weight. The GA treatments did not increase the already high soluble solids contents (23%) found in the controls.

Other changes, such as the content of crude cell wall extract, quantified here as AIR, achieved similar values among the GA treatments at colour 3; however, in 'Bing', the GA treatment T60 increased the concentration by 38%. This increase has also been observed in other trials, in which it has also been correlated with lower incidences of surface disorders [50,51]. An increase in firmness has been associated with high levels of AIR [52] in cv. 'Kordia', and a slight positive correlation has been observed between firmness and AIR content [53].

Rheological properties, such as the modulus of elasticity, the strain at bioyield and the maximum stress, were characterised at harvest during storage and ripening. Applications of GA increased the modulus of elasticity evaluated at the elastic mode of the tissue at harvest as a result of increased tissue stress since no effect was observed on strain. Hence, high values of the modulus of elasticity are related to a more rigid fruit. The main effect of GA treatment was found in tissue stress at the maximum point, where the GA-treated fruit were more resistant (higher stress values) than the control, without significant differences among the various GA treatments. 'Lapins' fruit treated with GA showed more uniform effects than 'Bing', where high variability was found among the treatments. A single application at the pit-hardening or straw-colour stages, as well as applications in both phenological states, also increased the modulus of elasticity and stress at the maximum point in cv. 'Bing' and 'Sweetheart' [36]. Applications with calcium in Stage I have increased the modulus of elasticity, as reported by Matteo et al. [22], rendering the fruit more resistant to mechanical damage.

The evaluation of these characteristics after 35 days of storage at 0 °C demonstrates that fruit of both cultivars, when treated with GA, maintain a higher resistance (stress at maximum point) and modulus of elasticity; however, fruit had lower values of deformability (strain at bioyield point) compared with control fruit. This reduction by GA treatment reinforces the rigidity of the tissue, so it remains firm under tension and thus should be more sensitive to skin fracturing. This postharvest behaviour can be explained by the conditions of the fruit inside the MAP bag since it was a water-saturated environment, and the fruit will have been under maximum stress. However, fruit softening in sweet cherry under saturated conditions is controversial, with the manipulation of water status not having demonstrated an effect on fruit pressure [54]. Tapia García et al. [14] observed that sweet cherry firmness increases during storage under MAP. On the other hand, it is known that fruit temperature also influences the rheological variables and the sensitivity to mechanical damage [7,37,55]; for this reason, in the development of this experiment, the tests were carried out at a constant pulp temperature of 15 °C.

After 3 days of shelf-life, the fruit modulus of elasticity was slightly lower and stress at the maximum point slightly higher than at the time of removal from cold storage at 0 °C, maintaining significant differences at all times between the GA treatments with higher values than the control. The strain at bioyield increased slightly compared with removal from cold storage at 0 °C, except in the case of the 'Bing' control, which decreased, resulting in a less deformable fruit than that of the GA treatments. In general terms, these changes in rheological variables may be related to increases in temperature in this phase, which increases fruit metabolism and, therefore, increases respiration [55]; also, transpiration due to a greater vapour pressure deficit causes fruit to lose water. Trials on sweet cherry have confirmed that increasing fruit temperature decreased the modulus of elasticity and the fracture pressure [7].

The application of GA reduced the incidence and severity of bruising in both the cultivars examined here. The effect of reduced severity has also been observed in sweet cherries with a single application of 10 or 20 ppm GA and with a double application of 10 ppm [56]. Param and Zoffoli [9] showed that the increase in stress and strain in sweet cherry tissue makes the fruit more resistant to mechanical damage. This trial shows how GA applications are able to increase the stress, making the fruit more resistant to bruising.

The GA treatments did not show significant differences from the controls for the compression and impact tests, but there were differences between the different degrees of maturity, with the more mature fruit, represented by colour 3.5, having the lowest damage index value. The greater resistance to tissue damage has mainly been related to the increase in strain at bioyield due to the natural ripening process, causing the fruit to be more deformable. Lidster et al. [50] observed that the ripest mahogany-coloured fruit appeared to have a maximum resistance to the different forms of impact damage compared with the earlier ripening states. Pitting rating decreased as fruit colour increased, so as maturity progressed, the fruit became less susceptible to pitting [18].

Sweet cherry tissue exhibits viscoelastic properties when deformed [5]. Consequently, when slow loading rates are applied to the fruit, the cherry matrix can deform and flow without rupturing cells, which is associated with its inherent capacity for deformation. In contrast, less mature tissue is stiffer; when subjected to a load, it compresses the parenchyma cells, leading to cell wall fractures and surface pitting.

During the earlier stages of ripening, the cell wall structure undergoes changes that affect both the mechanical strength of the cell walls and cell-to-cell adhesion [57]. This alteration results in different capacities to withstand external mechanical forces. Viscoelastic tissue should ideally strike a balance between deformation and cell wall resistance. As the fruit ripens, it transitions from a stage of high resistance with low deformation to one of weaker resistance, which increases sensitivity to mechanical damage. Therefore, identifying and prioritising the optimal stage of harvest that balances high resistance with sufficient deformation capacity is crucial for minimising damage and ensuring fruit quality.

5. Conclusions

Gibberellic acid is a phytohormone widely used by cherry producers. Determining the best application rate and timing is a difficult decision for farmers and agronomists, so this work contributes by providing more knowledge on this topic. In fact, it can be concluded that GA treatments delayed the harvest date for 2 to 4 days in both cultivars; increased crop yield in 'Lapins' due to enhanced fruit weight and size; and led to fruit being more resistant in both cultivars. In addition, the effects of GA treatments on making the fruit more rigid were maintained after 35 days of postharvest storage at 0 °C. Therefore, GA treatment increased resistance without increasing tissue deformability and even reduced it, making the fruit stiffer during storage at high moisture conditions, which could render other problems, such as in-box fruit cracking, that deserve further research. Moreover, it was found that as fruit maturity advances, sensitivity to mechanical damage (induced impact and compression injury) is reduced as a result of increased fruit deformability (strain at bioyield point). Furthermore, this work provides additional information on the behaviour of rheological variables with respect to mechanical damage depending on the state of maturity of the fruit, which may lead to future research in the field.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112738/s1, Table S1. Effect of gibberellic acid (GA) treatments on induced mechanical damage (fruit damage index) by compression and impact tests on 'Bing' and 'Lapins' sweet cherry cultivars.

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