

## Article

# Canopy Spraying of Absciscic Acid to Improve Fruit Quality of Different Sweet Cherry Cultivars

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**Abstract:** Absciscic acid (ABA) plays a major role in promoting ripening in sweet cherry, a non-climateric fruit. Exogenous application of ABA has been performed to study fruit ripening and cracking, but this growth regulator is not used for commercial production. To determine the potential of this growth regulator to improve sweet cherry fruit quality, ABA canopy spraying was assayed in four cultivars. Canopy spraying of S-ABA significantly: 1) enhanced sweet cherry fruit color in 'Glenred', 'Lapins' and 'Bing' cultivars, but not in 'Royal Rainier' (a bi-colored cultivar), and 2) decreased fruit size and firmness in 'Lapins', 'Bing' and 'Royal Rainier'. Seasonally reproducible effects were seen in 'Lapins' (mid/late-maturing) but not in 'Glenred' (early-maturing). Canopy spraying of nordihydroguaiaretic acid (NDGA) decreased color and increased fruit size in 'Lapins', but not in 'Glenred'. Direct application of ABA on fruits attached to the tree, without application to the foliage, increased Lapins' fruit color without reducing size. These results suggest a localized fruit response to exogenous ABA application on fruit color development, but that a decrease in fruit size may be due to the effects of exogenous ABA on the tree canopy foliage.

**Keywords:** growth regulator; ABA; NDGA; *Prunus avium*; fruit color

## 1. Introduction

Chilean sweet cherry production and exportation have increased dramatically within the past decade, placing sweet cherry as the third most relevant Chilean fruit crop in terms of the volume of fruit production and the number of hectares planted (28,057 hectares in 2020 and 59,000 hectares predicted for 2023) <sup>1,2</sup>. This increase has led to Chile becoming the top sweet cherry fruit exporter worldwide <sup>3</sup>. Chile is also the top southern hemisphere sweet cherry fruit exporter, totaling 94% of all sweet cherry fruit exports between October and March <sup>1</sup>. During the 2019-2020 season, 75% of the Chilean sweet cherries exported corresponded to only four cultivars: 'Lapins' (31%), Santina (18%), Regina (13%), and 'Bing' <sup>1</sup>. Despite distribution problems and a decrease in the price of Chilean sweet cherries at the end of the sweet cherry season (prolonged postharvest storage of shipped fruits due to the COVID-19 pandemic), the 2020-2021 sweet cherry season was still the most successful historically, with an estimated record revenue (US\$1.800 MM FOB) <sup>1</sup>.

Fruit size and maturity (harvest) timing, as well as other sweet cherry quality trait parameters (i.e., firmness, color, soluble solids content), need to be optimized to ensure

that the sweet cherry crop production is economically and environmentally sustainable over time. Towards this end, in addition to the selection of the optimal cultivar and rootstock, traditional agronomic techniques associated with pruning, training, and fruit thinning are the most reliable methods to influence canopy architecture and leaf-to-fruit ratios, in order to achieve and maintain the desirable tree structures and fruit quality <sup>4,5</sup>. Commercial sweet cherry production also uses plant growth regulators as tools to “fine-tune” fruit production and fruit quality parameters (maturity timing, size, weight, firmness, soluble solids, acidity, color, accumulation of health-promoting compounds such as polyphenolics, resistance to cracking, and resistance to pitting).

Sweet cherry is a member of the *Prunus* genus (drupe or “stone fruit”) which are indehiscent fruits with fleshy outer pericarp layers (exocarp and mesocarp) and a hard inner pericarp layer (endocarp) surrounding the seed <sup>6</sup>. However, unlike most fruits of the *Prunus* genus, sweet cherry is a non-climacteric fruit <sup>7,8</sup>. Several phytohormones are involved in triggering ripening in non-climacteric fruits <sup>7,8</sup>. Among these hormones, abscisic acid (ABA) seems to have an active role in promoting the ripening, since endogenous levels of this phytohormone increase when softening begins and color starts to change; moreover, ABA treatment influences the ripening-related parameters such as sugar and anthocyanin accumulation in other non-climacteric fruit models <sup>9-12</sup> and in sweet cherry <sup>13-17</sup>. Other hormones participate possibly through complex networks, with many of them interacting with the ABA pathway <sup>17,18</sup>.

Exogenous application of ABA for improving sweet cherry fruit quality is not a common agronomic practice, with only a few field trials reported using this plant growth regulator in sweet cherry <sup>17,19</sup>. Usually, ABA exogenous treatment has been used to unravel molecular mechanisms that underlie fruit maturity, but these applications are performed in excised shoots or harvested fruits <sup>13-16</sup>, and in early-maturing cultivars such as ‘Satonishiki’ and ‘Hong Deng’. Since ABA has been reported to modify fruit quality parameters, the objective of this work was in the first place to assess the effect of canopy spraying of ABA on four phenotypically contrasting sweet cherry cultivars (‘Glenred’, ‘Lapins’, ‘Bing’ and ‘Royal Rainier’) that differ on color, blooming time, and maturity time. The effect of NDGA, an inhibitor of ABA synthesis, was also explored in the early-maturing (‘Glenred’) and mid/late-maturing (‘Lapins’) sweet cherry cultivars. Additionally, the direct application of ABA on fruits attached to the tree (“on tree” dipping, without application to the foliage) was analyzed in the ‘Lapins’ cultivar since it showed the most consistent effects between seasons in response to canopy spraying of ABA.

## 2. Materials and Methods

### 2.1. Plant material and exogenous application of ABA and NDGA

Sweet cherry (*Prunus avium* L.) adult grown trees (> 15 years) were used from a commercial orchard located in Rengo, Chile, Lon: 070°43'6.78" Lat: S34°27'16.92" during the 2017-2018, 2018-2019 and 2019-2020 seasons to perform the analyses. The trees were grown under similar conditions and maintained using standardized Chilean sweet cherry industry agronomical practices. Trees were manually thinned to 4-5 fruits per apex, resulting in similar fruit loads in all trees. In the canopy spraying assay, eight trees with similar growth status and vigor were selected. In 2017, the cultivars selected were ‘Glenred’, ‘Lapins’, ‘Bing’, and ‘Royal Rainier’, which are common commercial cultivars in Chile <sup>20</sup>. A table with the specifications of these cultivar is shown in Table S1. Four trees were treated with 400 mg<sup>-1</sup>L<sup>-1</sup> of S-ABA (‘Protone ®’) by spraying the tree canopy using a backpack sprayer until runoff, while four trees were left untreated. Canopy ABA spraying was performed when the fruits were transitioning from the straw-yellow stage to the pink stage <sup>16</sup>. In 2018, the ‘Glenred’ and ‘Lapins’ cultivars were selected to repeat the ABA canopy spraying assay. Additionally, during the 2018 season, eight trees selected for each cultivar (‘Glenred’ and ‘Lapins’) were used for the canopy NDGA spraying assay. Four trees of each cultivar were treated with 10 mg<sup>-1</sup>L<sup>-1</sup> of NDGA (Sigma Aldrich ®), applying

it in a similar manner to the canopy S-ABA spraying assay. In 2018 and 2019, an “on-tree” fruit dipping assay was performed on the ‘Lapins’ cultivar. Eight major branches with similar growth status and direction per tree were selected. Fruits of four selected branches were treated with 400 mg·L<sup>-1</sup> of S-ABA (‘Protone ®’) by dipping the fruit directly in the ABA (‘Protone ®’) solution for 30 seconds, whereas control fruits on four additional branches were dipped in water. All treatments were performed between 10 and 11 am (UTC-3).

## 2.2. Sample collection and fruit quality parameters analysis

For the growth curves, the equatorial diameter from the suture of the fruits was measured with a caliper every 3-7 days (20 fruits randomly selected from each of the four trees). For fruit quality parameters evaluation, fruits were harvested when ripe based on commercial harvest criteria. The harvest date was defined as when fruits reached a 3-4 CTIFL (France) skin color value <sup>21</sup> and SSC value of 15-18% <sup>20</sup>. In the case of Royal Rainier (bicolored cultivar), CTIFL was not used. Fruit quality parameters were measured according to the standardized sweet cherry phenotyping protocol <sup>22</sup>. A total of 25 representative fruits per tree were harvested for further analyses <sup>22</sup>. The fruit quality parameters evaluated were fruit weight (FW), external fruit color (CTIFL scale recording 1 to 4), IAD (Index of Absorbance difference between 640 nm, 560 nm, and 750 nm) using a VIS/NIF device Cherry Meter, T.R. ® Turoni, Italy, and fruit firmness using a Durometer (Durofel T.R. ® Turoni, Italy). For total soluble solids content (TSSC, Brix degree) and total acidity (TA, as malic acid content), a subset of five representative fruits per tree were measured, according to Chavoshi *et al.* <sup>22</sup>, using a PAL-BX|ACID Pocket Sugar and Acidity Meter (ATAGO USA, Inc.), respectively. The percentage changes in fruit quality parameters in response to ABA treatment were calculated as follow:

$$\frac{(var_{ABA} - var_{Control})}{var_{Control}} \times 100$$
, where *var* is the parameter analyzed in the ABA or Control treatment.

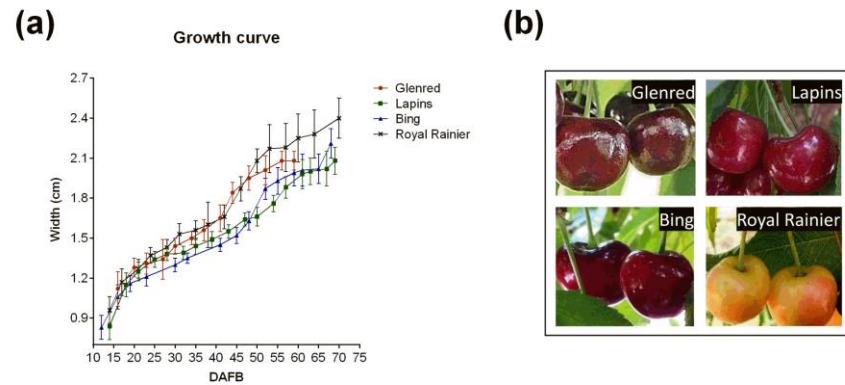
Data were analyzed and graphed with GraphPad Prism version 9.0.0 for Mac OS X, GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com). An “Unpaired t-test” for comparing treatments and statistical significance was assessed with a p-value <0.05.

## 3. Results

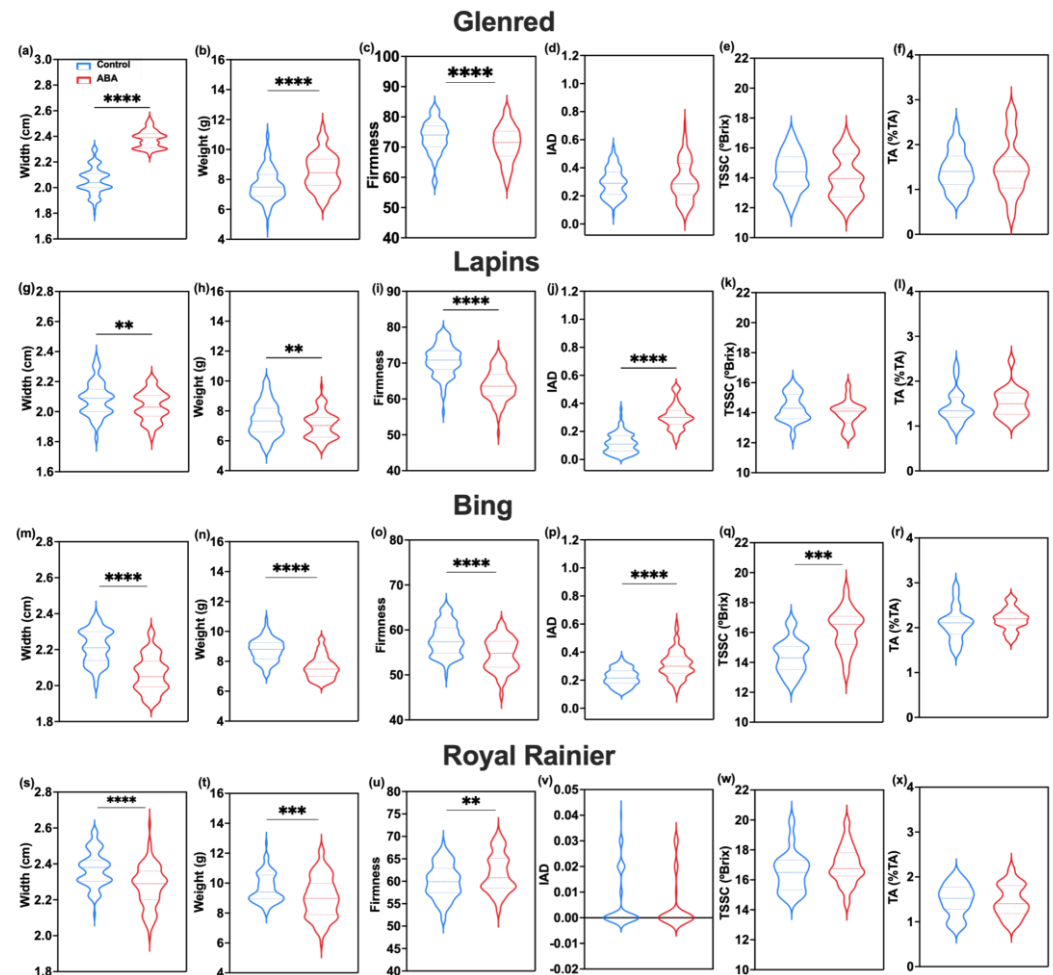
### 3.1. The effect of the ABA canopy spraying on sweet cherry fruit quality parameters of the ‘Glenred’, ‘Lapins’, ‘Bing’ and ‘Royal Rainier’ cultivars during the 2017 season

During the 2017 season, four sweet cherry cultivars were used for the ABA canopy spraying assay. The cultivars selected were ‘Glenred’, ‘Lapins’, ‘Bing’, and ‘Royal Rainier’. These cultivars have variations in their growth curves and maturity timing (**Fig. 1a**) (Gibeaut *et al.*, 2017). ‘Glenred’ is an early-maturing cultivar, while ‘Lapins’ and ‘Bing’ are mid/late-maturing cultivars (**Fig. 1a**), and ‘Royal Rainier’ is an early to mid/late-maturing cultivar with bi-colored fruits (**Fig. 1b**) (**Table S1**).

The application of the ABA growth regulator on the tree canopy of ‘Glenred’ resulted in differences in several fruit quality parameters (**Figure 2a - 2f**). Statistically significant increases in the mean diameter and weight (**Fig. 2a-b**) were detected in response to the exogenous application of ABA to the tree canopy, whereas a statistically significant decrease was detected in the mean firmness (**Fig. 2c**). A difference in the range of distribution of diameter was also observed (**Fig. 2a**). Furthermore, there was an increase in the number of fruits classified as very dark (CTIFL 4) in response to canopy ABA treatment (**Fig. 3a**). No significant differences were detected in the mean IAD, TSSC, and TA levels (**Fig. 2d-f**).



**Figure 1. Growth curves of sweet cherry cultivars used for the ABA canopy spraying assay.** Four sweet cherry cultivars were used to evaluate the effect of ABA canopy spraying application in quality parameters at harvest during the 2017 season. **(a)** Growth curves of 'Glenred', 'Lapins', 'Bing' and 'Royal Rainier' cultivars. **(b)** Representative photographs of sweet cherry cultivars at a mature stage. For the growth curves, measurements of the equatorial diameter from the suture were taken every 3-7 days with a caliper. Data represent the mean  $\pm$  SD. Measurements were from 80 fruits randomly selected from  $n = 4$  trees (20 fruits per tree). DAFB: days after full bloom.

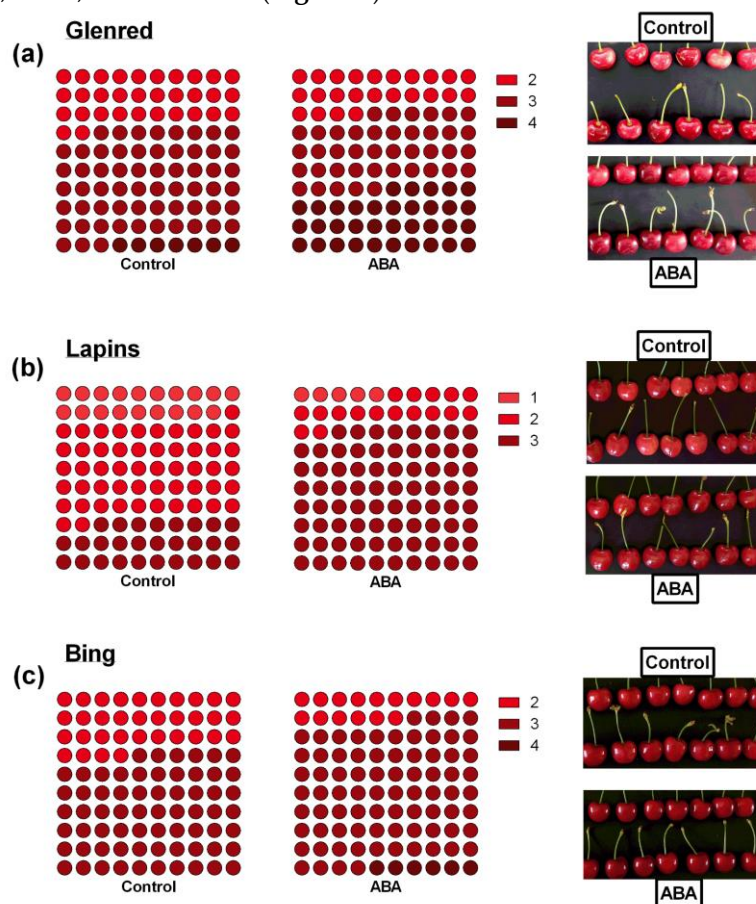


**Figure 2. Sweet cherry fruit quality parameters of ABA canopy sprayed trees during the 2017 season.** (a) - (f) Glenred, (g) - (l) Lapins, (m) - (r) Bing, and (s) - (x) Royal Rainier. Violin plots represent the Kernel density probability distribution of the data, and inner lines indicate the quartile division. 100 fruits from four biological replicates ( $n = 4$  trees, 25 fruits per tree) were used (detailed information in material and methods). Significant differences between ABA treatment and control treatment were performed by an "Unpaired t-test". Asterisks indicate  $p$ -value at: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, and \*\*\*\* < 0.0001.



Regarding 'Lapins', statistically significant decreases in the mean diameter, weight, and firmness (Fig. 2g - 2i) were detected in response to exogenous application of ABA to the tree canopy; whereas a statistically significant increase was detected in the mean IAD of the fruits, and a difference in the range of distribution was also observed (Fig. 2j). Furthermore, there was an increase in the number of fruits classified as dark (CTIFL 3) in response to canopy ABA treatment (Fig. 3b). No significant differences were detected in mean TSSC and TA levels (Fig. 2k-l). In 'Bing', statistically significant decreases in the mean diameter, weight, and firmness (Fig. 2m-o), as well as a statistically significant increase in the mean IAD and TSSC (Fig. 2p-q) of the fruits were detected in response to exogenous application of ABA to the tree canopy. Furthermore, there was an increase in the number of fruits classified as dark and very dark (CTIFL3 and CTIFL 4) in response to canopy ABA treatment (Fig. 3c). No significant differences were detected in mean TA levels (Fig. 2r).

Exogenous application of ABA to the tree canopy of 'Royal Rainier' also showed statistically significant decreases in the mean diameter and weight (Fig. 2s-t); but the mean firmness was significantly increased (Fig. 2w). No significant differences were detected in the mean IAD, TSSC, and TA levels (Fig. 2v-x).



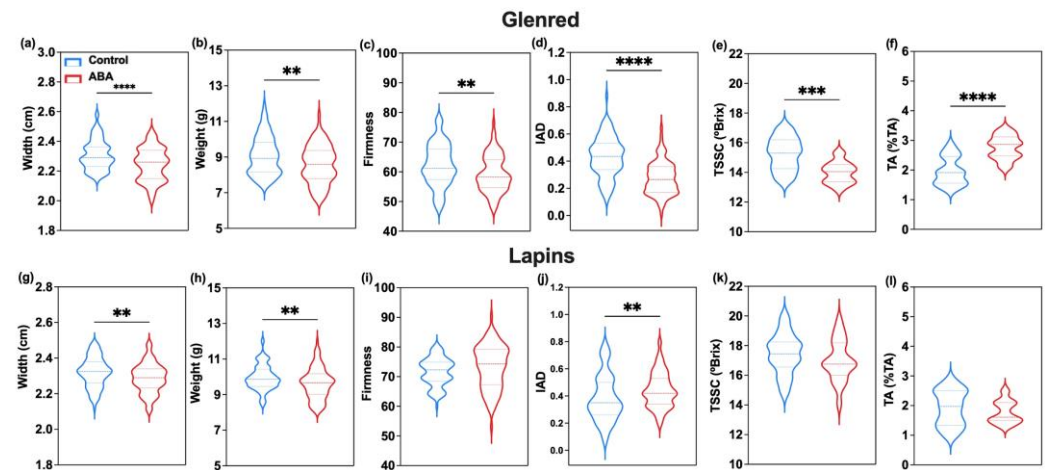
**Figure 3.** ABA canopy spraying effect on sweet cherry fruit CTIFL scale of cultivars 'Glenred', 'Lapins', and 'Bing' at harvest during 2017 season. (a) 'Glenred', (b) 'Lapins' and (c) 'Bing'. The sweet cherry color chart (CTIFL, France) was used to identify changes in color distribution at harvest in response to ABA exogenous canopy spraying treatment. The number 1 indicates the lightest and 4 the darkest red. 100 fruits from n=4 trees were used (25 fruits per tree). A reference photo at harvest for control and ABA-treated fruits is shown.

### 3.2. The effect of ABA and NDGA canopy spraying on sweet cherry fruit quality parameters of cultivars 'Glenred' and 'Lapins' during the 2018 season

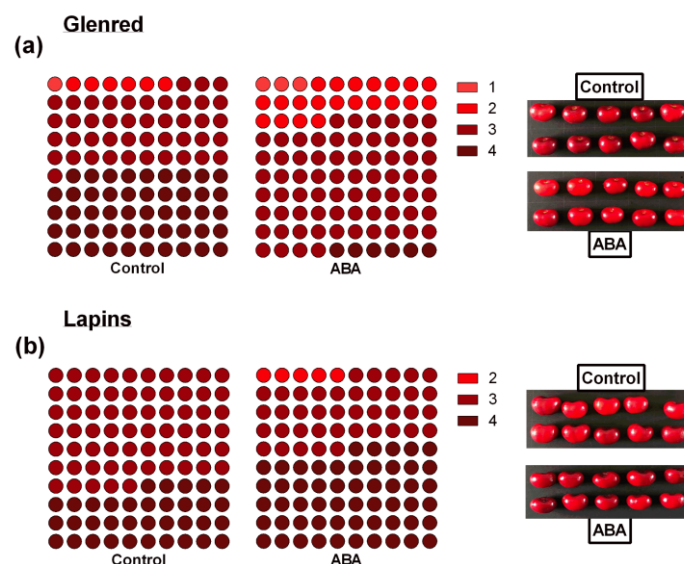
Since 'Glenred' presented a different response to the ABA canopy treatment in 2017 in size and weight when compared to 'Lapins', 'Bing' and 'Royal Rainier', 'Glenred' and

'Lapins' cultivars (cultivars with contrasting responses to ABA canopy treatment) were repeated during the 2018 season.

During the 2018 season, a statistically significant decrease in fruit diameter, weight, firmness, IAD and TSSC were detected in response to exogenous application of ABA to the tree canopy of the 'Glenred' cultivar (**Fig. 4a-e**); whereas the mean TA of the fruits increased significantly (**Fig. 4f**). A difference in the range of distribution of TSSC and TA parameters was also observed (**Fig. 4e-f**). Additionally, there were fewer fruits classified as very dark (CTIFL 4) in response to canopy ABA treatment during the 2018 season (**Fig. 5a**).



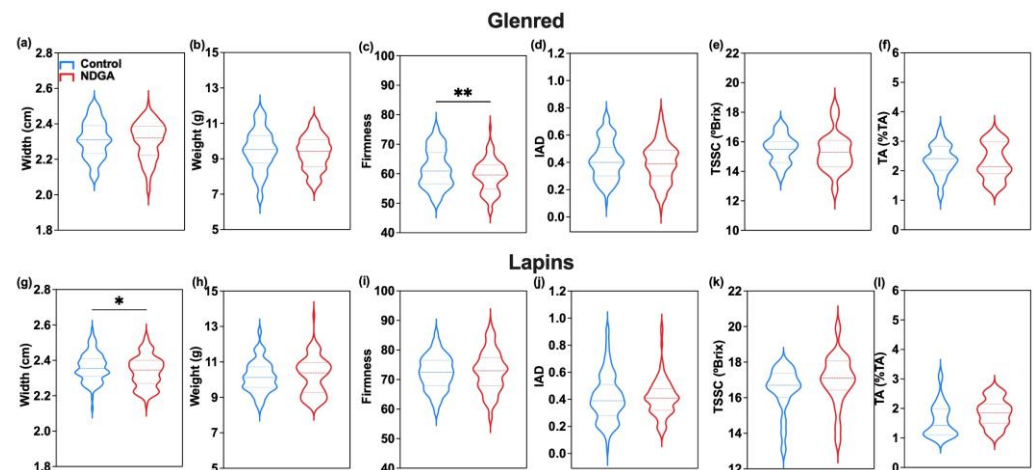
**Figure 4. Canopy spraying effect of ABA on sweet cherry fruit quality parameters in the cultivars 'Glenred' and 'Lapins' during the 2018 season.** (a) - (f) 'Glenred' with canopy ABA treatment and (g) - (l) 'Lapins' with canopy ABA treatment. Violin plots represent the Kernel density probability distribution of the data, and inner lines indicate the quartile division. 100 fruits from four biological replicates ( $n=4$  trees, 25 fruits per tree) were used (detailed information in material and methods section). Significant differences between ABA and control treatment were performed by an "Unpaired t-test". Asterisks indicate  $p$ -value at: \*  $< 0.05$ , \*\*  $< 0.01$ , \*\*\*  $< 0.001$ , and \*\*\*\*  $< 0.0001$ .



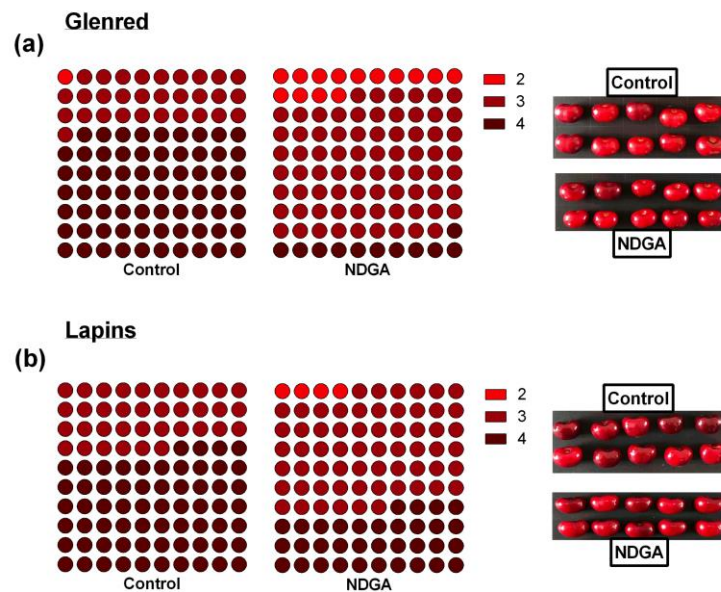
**Figure 5. ABA canopy spraying effect on sweet cherry fruit CTIFL scale of cultivars 'Glenred' and 'Lapins' at harvest during 2018 season.** (a) 'Glenred' and (b) 'Lapins'. The sweet cherry color chart (CTIFL, France) was used to identify changes in color distribution at harvest in response to ABA exogenous canopy spraying treatment. The number 1 indicates the lightest and 4 the darkest red. 100 fruits from  $n=4$  trees were used (25 fruits per tree). A reference photo at harvest for control and ABA-treated fruits is shown.

Similar to what was seen in the 2017 season, during the 2018 season, the effects of this application on the cultivar 'Lapins' led to statistically significant decreases in the mean diameter and weight (Fig. 4a-f) and a statistically significant increase in the mean IAD (Fig. 4d) of the fruits. Additionally, there was also an increase in the number of fruits classified as dark and very dark (CTIFL3 and CTIFL 4) in response to canopy ABA treatment (Fig. 5b). In contrast, no statistical differences were detected in the mean of firmness (Fig. 4c) and in the mean of TSSC or TA (Fig. 4e-f). The effects of canopy spraying of the ABA biosynthesis inhibitor NDGA were also analyzed during the 2018 season on the 'Glenred' and 'Lapins' cultivars. The application of the NDGA to 'Glenred' resulted in a statistically significant decrease in the mean firmness (Fig. 6c). In contrast, no significant differences were detected in mean diameter, weight, IAD, TSSC, and TA levels (Fig. 6a-b, 6d-f). Although IAD had no differences, a decrease in the number of darkest fruits in response to NDGA treatment was found (Fig. 7a).

In the 'Lapins' cultivar, the application of the NDGA on the tree canopy only resulted in a statistically significant decrease in the mean diameter (Fig. 6g). Additionally, there was a decrease in the number of very dark fruits (CTIFL 4) in response to canopy application of NDGA treatment (Fig. 7b). No significant differences were detected in the other parameters (Fig. 6h-l).



**Figure 6.** Canopy spraying effect of NDGA on sweet cherry fruit quality parameters in the cultivars 'Glenred' and 'Lapins' during the 2018 season. ((a) - (f) 'Glenred' with canopy NDGA treatment and (g) - (l) 'Lapins' with canopy NDGA treatment. Violin plots represent the Kernel density probability distribution of the data, and inner lines indicate the quartile division. 100 fruits from four biological replicates ( $n=4$  trees, 25 fruits per tree) were used (detailed information in material and methods section). Significant differences between NDGA and control treatment were performed by an "Unpaired t-test". Asterisks indicate  $p$ -value at: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, and \*\*\*\* < 0.0001.



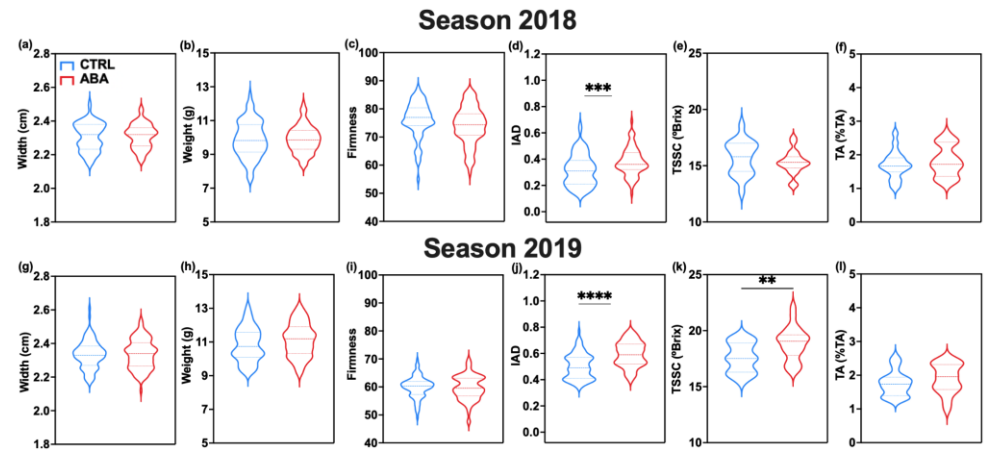
**Figure 7. NDGA canopy spraying effect on sweet cherry fruit CTIFL scale of cultivars 'Glenred' and 'Lapins' at harvest during 2018 season. (a) 'Glenred' and (b) 'Lapins'.** The sweet cherry color chart (CTIFL, France) was used to identify changes in color distribution at harvest in response to NDGA exogenous canopy spraying treatment. The number 1 indicates the lightest and 4 the darkest red. 100 fruits from  $n=4$  trees were used (25 fruits per tree). A reference photo at harvest for control and NDGA-treated fruits is shown.

### 3.3. The effect of “on-tree” ABA fruit dipping on sweet cherry fruit quality parameters of cultivar 'Lapins'

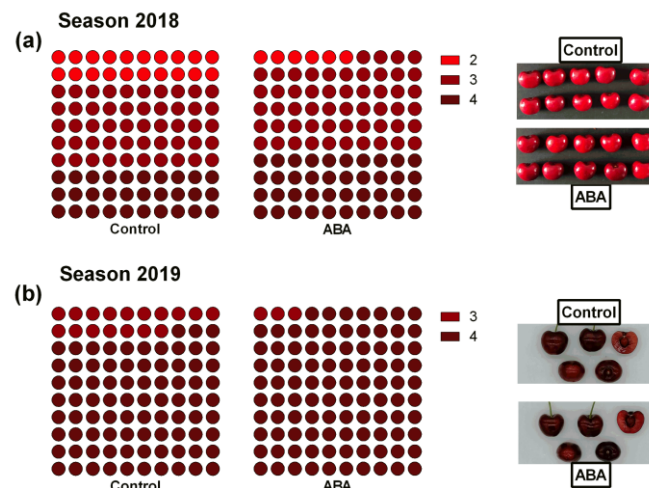
The 'Lapins' cultivar presented the most consistent effects between seasons in response to canopy spraying of ABA. These effects include increases in IAD and color, as well as decreases in width and weight. To determine if these effects are due to an ABA response locally on the fruit or due to an ABA response on the leaves that affect the fruits, a “fruit dipping” assay was used to analyze the effect to ABA without foliar exposition in this cultivar.

Applying ABA by fruit dipping at the transition from the straw-yellow stage to the pink stage also showed differences in fruit quality parameters at harvest (**Fig. 8**). Statistically significant increases in the mean of IAD (**Fig. 8d and 8j**) and TSSC (**Fig. 8k**) were detected at harvest in fruits that had been dipped in ABA. In contrast, width, weight, firmness, and TA parameters did not show statistically significant variations (**Fig. 8**). Also, an increase in the number of darkest fruits in response to ABA dipping treatment was found in both seasons analyzed (**Fig. 9**).





**Figure 8.** Effects of ABA treatment by “on-tree” dipping on the “Lapins” cultivar. (a) - (f) Season 2018, and (g) - (l) Season 2019. Violin plots represent the Kernel density probability distribution of the data, and inner lines indicate the quartile division. 100 fruits from four biological replicates (n= 4 trees, 25 fruits per tree) were used (detailed information in material and methods section). Significant differences between ABA treatment and control treatment were performed by an “Unpaired t-test”. Asterisks indicate  $p$ -value at: \* < 0.05, \*\* < 0.01, \*\*\* < 0.001, and \*\*\*\* < 0.0001.



**Figure 9.** ABA dipping effect on sweet cherry fruit CTIFL scale of “Lapins” cultivar at harvest during seasons 2018 and 2019. (a) Season 2018, and (b) Season 2019. The sweet cherry color chart (CTIFL, France) was used to identify changes in color distribution at harvest in response to ABA “on-tree” fruit dipping treatment. The number 1 indicates the lightest and 4 the darkest red. 100 fruits from n=4 trees were used (25 fruits per tree). A reference photo at harvest for control and ABA-treated fruits is shown.

#### 4. Discussion

The exogenous application of ABA in sweet cherry fruits had been studied nearly 30 years ago<sup>13</sup>. Previously the role of ABA on sweet cherry fruit maturity has usually been studied in early-maturing cultivars (Satonishiki, Hongdeng, Prime Giant)<sup>13,16,23</sup>, with only a few experiments in other mid-maturing cultivars such as 'Bing'<sup>19</sup> and 'Lapins'<sup>17</sup>. Regardless of this, the exogenous application is not a common agronomic practice in sweet cherry in comparison to other growth regulators such as gibberellin (GA<sub>3</sub>)<sup>18,24–30</sup>. Furthermore, the genotypic effect on the ABA response has not been fully addressed. For this reason, in this work we assessed the effect of canopy spraying of ABA on fruit quality parameters at harvest in four genotypically and phenotypically different cultivars ('Glenred', 'Lapins', 'Bing' and 'Royal Rainier') to determine if ABA canopy spraying may be a suitable agronomic practice. Furthermore, the effect of canopy spraying of NDGA,

an inhibitor of ABA synthesis, was also explored in the early-maturing ('Glenred') and mid/late-maturing ('Lapins') sweet cherry cultivars. Finally, to determine if the effect that canopy spraying of ABA had on sweet cherry fruit quality, the direct application of ABA on fruits attached to the tree ("on tree" dipping, without application to the foliage) was analyzed in the most important cultivar ('Lapins').

#### 4.1 Cultivar dependent effect of ABA canopy spraying of sweet cherry trees on fruit quality parameters

To further explore the potential use ABA for improving sweet cherry fruit quality, the effect of exogenous ABA application (canopy spraying) on fruit quality parameters at harvest in four commercial sweet cherry cultivars ('Glenred', 'Lapins', 'Bing' and 'Royal Rainier') was analyzed during 2017 season. As stated before, these cultivars differed in maturity timing, and in the case of 'Royal Rainier' - in color (bicolored cultivar). In this work, our results show that canopy spraying of the tree with ABA affected all four cultivars in terms of width (size) and weight. However, the direction of this effect was not consistent in all cultivars. During the same season (2017), ABA canopy spraying reduced fruit width and weight at harvest in the 'Lapins', 'Bing' and 'Royal Rainier' cultivars. On the other hand, the early-season cultivar 'Glenred' had the opposite effect, whereby the ABA canopy spraying increased fruit width and weight at harvest. However, the effects of ABA canopy spraying of the Glenred cultivar seems to be associated with seasonal effects, possibly different environmental factors, since during the 2018 season, the width and weight of the Glenred fruits at harvest were less than the control trees, similar to what was seen in 'Lapins' during this same season. It is important to note that this seasonal variability of the effect of ABA canopy spraying on fruit width and weight was only detected in the 'Glenred' cultivar, the 'Lapins' cultivar showed a significant consistent decrease in fruit width and weight during both seasons analyzed.

In previous works, there is not much agreement about the effect of ABA on size and weight. The phenomenon of ABA increasing weight was reported previously by Luo et al., 2014<sup>16</sup>, but the evaluation occurred at seven days after treatment (DAT) for the early-maturing cultivar 'Satonishiki'. The mid-maturing cultivar 'Bing' has been reported to not affect fruit size at harvest<sup>19</sup>. In another non-climacteric fruit, *Vitis vinifera*, it has been reported that exogenous ABA treatment produces smaller fruits (reviewed in Kuhn et al., 2013)<sup>11</sup>. Also, an increase in ABA content due to drought stress is correlated with fruit size reduction at harvest (reviewed in Kuhn et al., 2013)<sup>11</sup>. In sweet cherry, the fluridone (an inhibitor of ABA biosynthesis) treatment produces fruits with higher weights<sup>29</sup>, in accordance with the results of this work where NDGA treatment (another ABA biosynthesis inhibitor) increased fruit size in the 'Lapins' cultivar, and ABA treatment (canopy spraying) consistently decreased size and weight in this cultivar in both seasons analyzed (2017 and 2018). These findings support a model in which ABA is a negative regulator of fruit growth during ripening. Interestingly, NDGA treatment during the 2018 season had no effect on size or weight in the "Glenred" cultivar, and the ABA effect was different in both seasons, showing that the effect in these parameters for an early-maturing cultivar needs further investigation to identify environmental factors that may be influencing this seasonal variation in the ABA response. The difference in the consistent decrease in fruit size (width and weight) in ABA canopy sprayed 'Lapins' sweet cherry fruits at harvest, versus the seasonal variations associated with the 'Glenred' cultivar, may be associated with the differential response that early- and mid-maturing cultivars have to additional phytohormones such as gibberellic acid. Recently we have reported the GA<sub>3</sub> modifies the transcript abundance of ABA pathway orthologs and modulates sweet cherry fruit ripening in early- and mid-maturing cultivars<sup>18</sup> and that there is a differential transcriptional response between these cultivars treated with GA<sub>3</sub><sup>30</sup>.

In the case of ripening parameters such as firmness, TSSC and TA, we consistently observed a decrease in fruit firmness at harvest during both seasons (2017 and 2018) in

the 'Glenred' and 'Lapins' ABA canopy sprayed trees, as well as in 'Bing' during the 2017 season. These results are consistent with what has been reported previously<sup>15,16</sup>. On the other hand, it has been reported that ABA treatment increases TSSC levels and decreases TA levels<sup>13,15,16,19</sup>, but we did not detect a consistent significant variation in these parameters in response to the ABA canopy spraying. An increase in TSSC was only observed in the 'Bing' cultivar, whereas TSSC was unaffected by ABA canopy spraying in the 'Lapins' and 'Royal Rainier' cultivars. As previous reports have shown this effect in early-maturing cultivars, it could be presumed that this effect is genotype dependent. Nonetheless, 'Glenred' (an early-maturing cultivar) had the opposite response to ABA canopy spraying, a decrease in TSSC during two seasons (2017 and 2018) and an increase in TA in the 2018 season. The differences between our finding and what has been reported previously, may be due to the method in which the fruits were treated or the stage of fruit development in which the ABA was applied. Kondo and Gemma<sup>13</sup> treated fruits with ABA by dipping the fruits at 30 DAFB (before coloring) or 36 DAFB (Immediately before coloring) stage, meanwhile Ren et al. and Luo et al.<sup>15,16</sup> performed the application on mature harvested fruits. Since our application was at an earlier stage of fruit development (transition from the straw-yellow to the pink stage) and the ABA canopy spraying was an "on tree" assay, our results represent the effects of this growth regulator application to the sweet cherry tree canopy and the subsequent effects it has on fruit quality parameters at harvest.

Regarding color parameter, the color of the fruits at harvest were determined using the IAD index<sup>31</sup> and sweet cherry color chart (CTIFL, France). It is well known that ABA increases color due to an increase in the accumulation of anthocyanins in sweet cherry<sup>13,15,16</sup> and in other non-climacteric fruits such as grapes<sup>10</sup> and strawberry<sup>9,12</sup>. We have also seen fruit color changes in the 'Lapins' cultivar when the tree canopy was sprayed with ABA<sup>17</sup>, and similar results were found in the seasons that we report in this paper. However, here we have also compared this response in 'Lapins' with other cultivars during the same season. The ABA canopy spraying of 'Lapins' and 'Bing' cultivars increased the IAD index and the number of darkest fruits at harvest, showing also a change in the range of distribution of this parameter. This effect was reproducible during two seasons for these cultivars. However, during these same seasons, the response of the 'Glenred' cultivar was not reproducible. During the 2017 season, there was not a significant change in fruit color according to the IAD level, but there was an increase in the number of darkest fruits (CTIFL) at harvest. In contrast, during the 2018 season, the ABA canopy spraying of the 'Glenred' cultivar decreased the fruit IAD index and the number of darkest fruits (CTIFL) at harvest, suggesting that other variables (e.g. environmental factors) may influence the reproducibility of the effect that ABA canopy spraying has on this early-maturing cultivar. More investigation is needed to identify these variables.

It has been reported previously that exogenous application of fluridone decreases the color of fruits in the 'Bing' cultivar<sup>29</sup>. Using the ABA biosynthesis inhibitor NDGA we detected a similar decrease in fruit color measured with CTIFL at harvest in the 'Glenred' and 'Lapins' cultivars, but no significant change was detected using IAD to measure fruit color at harvest in both cultivars. Since IAD makes the absorbance difference between 560 nm, 640 nm and 750 nm wavelengths, it is not only measuring anthocyanins but also UV-interfering compounds with chlorophyll absorbance<sup>31,32</sup>. Therefore, this difference between IAD results and the CTIFL color chart is most likely due to differential levels of compounds other than the anthocyanins. Recently we have reported a difference in the phenolic accumulation dynamics between non-anthocyanin phenols and anthocyanins during sweet cherry fruit development in these two cultivars<sup>33</sup>.

In the case of 'Royal Rainier', there was no effect in IAD index and due it is a bicolored cultivar, it is not suitable for CTIFL color chart analyses. Nonetheless, we did not observe visual differences between control and treated fruits (data not shown). 'Royal Rainier' is a cultivar whose parents are 'Stella'  $\times$  o.p.<sup>34,35</sup>. As a bicolored cultivar, it is presumably that its color production is associated with the allelic variant *PavMYB10.b*<sup>36</sup>. As ABA had no

effect on color in this bicolored cultivar, further investigations are needed to elucidate if this cultivar does not respond to color because of this possible genetic difference or by other factors. Nonetheless, it has been shown in this cultivar that melatonin treatment at post-harvest increases color and the levels of *PaNCED1* (putative gene of the ABA biosynthetic pathway)<sup>37</sup>, suggesting that ABA may play a role in controlling color in this cultivar. The fact that Royal Rainier does not respond in color to ABA is interesting because in *Vitis vinifera* (cultivar Crimson Seedless) it has been shown that exogenous treatment with ABA cultivars enhances the homogenous red color<sup>38,39</sup>, confirming that the ABA canopy spraying effect is different in sweet cherries, and depends on cultivar.

#### 4.2 ABA effect is different between canopy spraying and dipping treatment

Another form of exogenous ABA application (“on-tree” fruit dipping) was analyzed in the ‘Lapins’ cultivar to see differences in fruit quality parameters at harvest. The ‘Lapins’ cultivar was selected because it presented consistent results after ABA canopy spraying treatment between seasons. As we mentioned before, ABA exogenous treatment has usually been performed in harvested fruits of early-maturing cultivars<sup>15,16</sup>. Previously reported “on tree” dipping assay have been reported, but the effect on fruit size was not determined<sup>13</sup>. On the other hand, the spraying effect on size has been shown for ‘Bing’ with no statistically significant difference<sup>19</sup>. Here we show that ABA canopy spraying affects size in ‘Lapins’ consistently decreases fruit size in both seasons analyzed.

In contrast, a decrease in fruit size and weight at harvest was not observed at harvest in the ‘Lapins’ cultivar in the “on tree” dipping assay during both seasons analyzed. Regarding color, IAD and the number of darkest fruits at harvest were increased at harvest in response to “on-tree” dipping in a similar way to the ABA canopy spraying. A summary of the response of the cultivars to ABA canopy spray and “on-tree” dipping during the same season are presented in **Figure 10**. IAD levels were similar between control and both ABA application methods. However, differences were observed in firmness, diameter, and weight. ABA canopy spraying resulted in a statistically significant decrease in fruit size (width and weight), but “on-tree” fruit dipping of ABA without exposing the foliar organs to the ABA had no effect on these parameters. Canopy spraying positively affected firmness and TSSC/TA, whereas dipping negatively affected these parameters. The increase in IAD was also slightly higher in the ABA canopy spraying treatment, in accordance with the effect on TSSC/TA maturity index.

The difference in the effects of ABA application to fruit size, between foliar spraying of ABA on the tree canopy versus the fruit dipping of ABA (without foliage treatment) may be related to the sink-source balance, sugar transport into the fruit, and processes occurring in the leaves that are controlled by ABA<sup>7-8</sup>. However, this does not seem to be the case for fruit color or the ripening process. Commercially, if it would be possible to avoid excessive foliar exposure to ABA spraying, the fruit color may be improved without reducing fruit size. However, this is technically difficult in a commercial setting. It is for that reason that further studies on the effect of ABA on sweet cherry “source” leaves and “sink” fruits may provide insight into the factors that are negatively influencing fruit size, while optimizing the positive effect that ABA displays on fruit color.

Exogenous applications of ABA have been studied in different non-climacteric fruit species to modify fruit quality parameters associated with fruit quality and ripening, especially grapes<sup>40</sup>. Exogenous ABA application is used in table grapes orchards to induce homogenous color accumulation in the exocarp, thereby improving fruit quality without significantly affecting fruit size<sup>38</sup>, but this application is performed several days after *veraison*. Perhaps by applying ABA canopy spraying in sweet cherry at a later stage of development, the color acquisition may be optimized without negatively affecting fruit size. Another option is the use of other plant growth regulators that could counterbalance the negative effect that the ABA canopy spraying has on fruit size. For instance, we observed that NDGA spraying treatment increased fruit width in ‘Lapins’. Furthermore,



others have reported that GA<sub>3</sub> treatment<sup>17,18,29</sup> and fluridone treatment increase the size of sweet cherry fruits<sup>29</sup>.

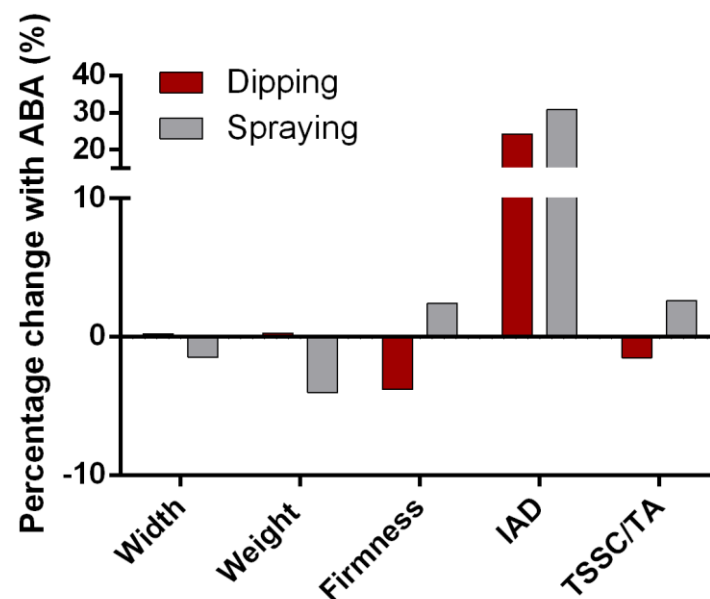


Figure 10. Percentage change of the fruit quality parameters evaluated at harvest in response to ABA treatment for spraying and dipping in 2018 season. Percentage change was calculated as the difference in the parameter between control and treatment (for detailed information see materials and methods section).

A summary of the effects of the different ABA treatments in cultivars per season is shown in **Table 1**. Considering the set of results and analysis, we have shown the ability of exogenous canopy spraying applications of ABA to modify parameters associated with the ripening of the fruit in sweet cherry at harvest, mainly associated with an improvement of the color, in a suitable way for agronomic purposes. We have also shown that the exogenous application of ABA on sugar content and fruit firmness is variable and may depend upon cultivar specificity<sup>41</sup>, ABA concentration, time of application, and environmental conditions<sup>42,43</sup>. Identification of the environmental factors/seasonal variations are involved in this variable response, and the factors from the tree canopy that subsequently alter fruit size are interesting topics for future investigation.

Table 1. Summary of the statistical variations in the physiological parameters of sweet cherry fruits post-treatment at harvest.

Season - Treatment	Statistical variations in the physiological parameters of sweet cherry fruits post-treatment at harvest							
	Cultivar	Width	Weight	Firmness	IAD	TSSC	TA	TSSC/TA
2017 - ABA canopy spraying	'Glenred'	↑	↑	↓	-	-	-	-
	'Lapins'	↓	↓	↓	↑	-	-	-
	'Bing'	↓	↓	↓	↑	↑	-	-
	'R. Rainier'	↓	↓	↑	-	-	-	-
2018 - ABA canopy spraying	'Glenred'	↓	↓	↓	↓	↓	↑	↓
	'Lapins'	↓	↓	-	↑	-	-	-
2018 - NDGA canopy spraying	'Glenred'	-	-	↓	-	-	-	-
	'Lapins'	↓	-	-	-	-	-	-
2018 - ABA fruit dipping	'Lapins'	-	-	↓	↑	-	-	-
2019 - ABA fruit dipping	'Lapins'	-	-	-	↑	↑	-	-

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