Influence of Preharvest and Postharvest Applications of Glycine Betaine on Fruit Quality Attributes and Storage Disorders of ‘Lapins’ and ‘Regina’ Cherries

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Abstract. This study aimed to evaluate whether preharvest or postharvest application of glycine betaine (GB) has the potential to improve fruit quality [fruit firmness (FF), size, skin color, soluble solids content (SSC), and titratable acidity (TA)] and susceptibility to storage disorders (peduncle browning, pitting, and decay) in ‘Lapins’ or ‘Regina’ sweet cherries, and to determine whether factors such as application frequency or timing impacted the efficacy of GB spraying. Adding 2 or 4 g L−1 GB to hydro-cooling water (0 °C) as postharvest treatment did not affect fruit size, skin color, SSC, TA, peduncle browning, or pitting development; however, it did result in fruit softening and a low incidence of decay. GB applied preharvest at 2 or 4 g L−1 once at 1 week before harvest (1WBH) was more effective for retaining FF and less peduncle browning and pitting compared with postharvest treatment. Increasing the preharvest GB application frequency from one time (1WBH or pit hardening) to three times (pit hardening, straw color, and 1WBH) enhanced FF and TA levels and resulted in lower pitting. The reduction in fruit size was observed for ‘Regina’, but not for ‘Lapins’. Changes in the contents of phosphorous (P), potassium (K), and magnesium (Mg) were unaffected by GB at harvest, whereas three GB sprays increased the total nitrogen (N) content. Compared with ‘Lapins’, ‘Regina’ allowed more calcium (Ca) uptake by GB and ultimately had firmer flesh. In conclusion, three preharvest applications of 4 g L−1 GB showed great potential to improve quality attributes, to reduce the susceptibility to storage disorders, and to increase the Ca content of ‘Regina’ cherries.

Sweet cherry is a highly perishable fruit with a short storage life. Fruit firmness (FF) is an important quality trait that has an impact on storage potential, disorder resistance, and decay development (Kappel et al., 1996). Poor appearance of soft fruit, such as dull skin color, surface pitting, and peduncle browning after storage or shipping, negatively impacts consumer purchase decisions.

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were harvested 1 d before the commercial harvest date and then packed in commercial polyethylene bags (1 kg) with a 2% perforation ratio. For Expts. 1 and 2 in 2016, 2 and 4 g L⁻¹ GB were applied to hydro-cooling water at 0 °C for ‘Lapins’ or ‘Regina’ cherries or sprayed 1 week before harvest (1WBH) for ‘Lapins’ trees to evaluate the effects of preharvest and postharvest application of GB on fruit response during storage. After 4 weeks of storage at 0 °C, postharvest application of GB resulted in a loss of FF, but not in preharvest treatment. Additionally, preharvest application had additional benefits for reducing peduncle browning and pitting development. Therefore, preharvest application frequency and timing were investigated in Expts. 3 and 4.

Expt. 1. In 2016, ‘Lapins’ and ‘Regina’ cherries free of any visible damage or fungal infection and of uniform size were picked on 22 June. Fruit of each cultivar with intact peduncles were divided into three treatments × three replicates × one box per replicate (= 9 boxes of 10 kg per box). Fruit from each treatment was soaked for 10 min in 12 L of one of the following solutions: hydro-cooling water at 0 °C (control); 2 g L⁻¹ (GB hydro-cooling water with 2 g L⁻¹ GB); or 4 g L⁻¹ GB (hydro-cooling water with 4 g L⁻¹ GB). After treatment, fruit were air-dried with a fan for 15 min and then packed and stored at 0 °C for 4 weeks. After 2 or 4 weeks of storage, a sample of 480 fruit from each treatment was transferred to 20 °C and analyzed after 4 h of temperature equilibration. Fruit with three replicates with 60 fruit per replicate were evaluated for fruit quality attributes (i.e., FF, fruit size, skin color, SSC, and TA). Three replicates with 100 fruit per replicate were evaluated for storage disorders (i.e., peduncle browning, pitting, and decay).

Expt. 2. In 2016, ‘Lapins’ trees were randomly selected and divided into three treatments with three replicates of two trees each and then treated with H₂O with 1 g L⁻¹ nonionic surfactant (control), 2 g L⁻¹ GB, or 4 g L⁻¹ GB. All treatments were applied once on 15 June. ‘Lapins’. Fruit were harvested on 22 June an then loaded into polyethylene bags and stored at 0 °C for up to 4 weeks. Fruit quality attributes and storage disorders were analyzed as described for Expt. 1.

Expt. 3. In 2017, ‘Lapins’ or ‘Regina’ trees were randomly selected and divided into three treatments with three replicates of two trees each and then treated with H₂O with 1 g L⁻¹ nonionic surfactant (control), 2 g L⁻¹ GB, or 4 g L⁻¹ GB. All treatments were applied three times: at pit hardening (20 May for ‘Lapins’; 23 May for ‘Regina’); straw color (9 June for ‘Lapins’; 14 June for ‘Regina’); and 1WBH (27 June for ‘Lapins’; 2 July for ‘Regina’). Fruit quality attributes and disorders were analyzed as described for Expt. 1. A fruit nutrients analysis was performed at harvest to investigate the impact of GB on the total nitrogen (N), phosphorous (P), magnesium (Mg), potassium (K), and calcium (Ca) contents of the fruit.

**Evaluations of peduncle browning, pitting, and decay.** Peduncle browning was evaluated after 2 and 4 weeks of storage and recorded as a percentage of 100 sample fruit peduncles showing browning of more than 30% of the entire surface (Clayton et al., 2003). Pitting was evaluated after evaluating the peduncle browning. Grading was standardized using a 4-point scale (Toivonen et al., 2004): 1, superficial pitting, pit diameter 1 mm or smaller, very shallow depression of the skin with diffuse edges; 2, minimal pitting, pit diameter 1 to 2 mm; 3, moderate pitting, pit diameter 2 to 3 mm, deeper and wider, with clearly distinct edges; and 4, severe pitting, pit diameter 3 mm or larger, very deep, edges of pits sunken into pulp tissue. Pitting was calculated in each replicate as the total number of fruits in each of the four categories multiplied by the four factors (1, 2, 3, and 4), and the total was divided by 100 and expressed as 1 to 4. Decay was expressed as a percentage of 100 fruit samples showing any type of decay; however, the decay organisms were not identified.

**Determination of fruit nutrients.** Thirty fruit per replicate per treatment were collected for the nutrient analysis. Total N was determined using a combustion analyzer, and P, Mg, K, and Ca were determined using a Thermo 6500 duo inductively coupled plasma spectrophotometer (Thermo and Fisher Scientific, Waltham, MA). Samples were washed, oven-dried at 65 °C, and ground to pass through a 2-mm sieve. After digesting in a microwave system (MARS Express CEM, Matthews, NC) by nitric acid and hydrogen peroxide, prepared samples were analyzed and data were expressed based on dry weight as g kg⁻¹.

**Results and Discussion**

**Postharvest application of GB to ‘Lapins’ and ‘Regina’ cherries.** Postharvest application of GB to fruit has been demonstrated to delay quality deterioration, to promote bioactive compound accumulation, and to enhance chilling tolerance (Razavi et al., 2018; Shan et al., 2015, 2016). However, in this study, 2 or 4 g L⁻¹ GB in hydro-cooling water applied postharvest did not affect fruit skin color, SSC, or TA of ‘Lapins’ or ‘Regina’ cherries, but these treatments markedly reduced FF during storage at 0 °C compared with control (Table 1). High FF is strongly preferred for sweet cherry so they are better able to withstand the handling, sorting, packing, storing, and transporting processes (Correia et al., 2017). Loss of FF directly impacts weight loss, off flavors, flesh browning, surface pitting, discoloration of the green stem, and fungal rotting (Kappel et al., 1996; Turner et al., 2005; Zheng et al., 2016). Although postharvest application of GB to ‘Lapins’ or ‘Regina’ cherries was shown to have an adverse effect on FF, GB-treated fruit had equal rates of peduncle browning and pitting relative to untreated fruit. Interestingly, a low incidence of decay was observed for GB-treated fruit, indicating that postharvest application of GB could enhance the tolerance of fruit against environmental stresses and senescence that might contribute to increased activity of antioxidant enzymes and bioactive compounds (Awad et al., 2015; Liu et al., 2011).

**Preharvest application of GB on ‘Lapins’ cherries.** Preharvest GB sprays have previously shown efficacy in alleviating the adverse drought stress and rain-induced cracking (Hansen, 2010; Lahdenperä, 2006). To identify the role of GB in fruit development, GB at 2 or 4 g L⁻¹ was sprayed on ‘Lapins’ trees at 1WBH. Compared with the postharvest application results, spraying GB 1WBH did not affect FF, but it did increase resistance to peduncle browning and pitting development (Table 2). No effect of GB was observed on fruit size, skin color, or SSC accumulation. Although GB did not affect TA during the first 2 weeks of storage, significantly lower levels of TA were
obscured for GB-treated fruit after 4 weeks of storage. In addition, GB applied preharvest showed inhibition of decay that was similar to that for postharvest application. These results indicated that GB applied preharvest was more beneficial for reducing fruit susceptibility to storage disorders than postharvest application, perhaps due to the action of GB as a plant growth regulator of fruit development.

Effects of application frequency and timing of GB on 'Lapins' and 'Regina' cherries. To further confirm the effects of GB on fruit development, application frequency and timing were investigated. In 2017, three GB sprays of 2 and 4 g L⁻¹ significantly increased FF of 'Lapins' cherries compared with untreated fruit. For 'Regina' cherries, when GB was applied at 4 g L⁻¹, increased FF was observed, indicating that GB application at 4 g L⁻¹ was sufficient to improve FF of both cultivars (Table 3). In 2018, a single application of GB at 4 g L⁻¹ once at the pit hardening stage or three times at pit hardening, straw color, and 1WBH for 'Lapins' did not cause high FF at harvest; however, it slowed the reductions of FF after 4 weeks of storage (Table 4). For 'Regina', three applications of GB maintained high FF levels at harvest or during storage. Correa et al. (2019) reported that 1 g L⁻¹ GB applied at 30, 49, and 56 d after full bloom increased the FF of 'Skeena' cherries. Lahdenperä (2006) reported that two or three split applications of GB significantly reduced fruit cracking in 'Garnet' cherries. Furthermore, early split applications of GB at the late green/start of straw color and straw/pink transition stages resulted in reduced fruit cracking compared to two applications of GB spray twice at the straw/pink transition and light red stages. This explained why reducing the GB frequency from three times to one time at the pit hardening stage had little to no effect on FF. Taken together, the 2016 to 2018 preharvest application results suggested that multiple and early applications of GB were an effective way of improving the FF of sweet cherry.

Developing strategies to increase fruit size is of great interest to growers. In this study, regardless of the application concentration, timing, or frequency, GB did not affect the fruit size of 'Lapins', as previously shown for 'Skeena' (Correa et al. 2019). GB did decrease the fruit size of 'Regina' when applied three times or once at pit hardening. Generally, the reduced fruit size may be due to excessive crop loading, deficit irrigation, dwarfing rootstocks, or improper thinning (Edin et al., 1993; Neilsen et al., 2007; Whiting and Lang, 2004; Whiting and Ophardt, 2005). However, 'Regina' typically displays a poor fruit set in Oregon’s Mid-Columbia region, and its light bearing results in relatively large fruit at harvest (Warner, 2013). Declines in the fruit size of 'Regina' may be due to the effects of GB on cell division or enlargement. There are three distinct growth stages during sweet cherry development (Coombe, 1976). Stage I is characterized by rapid enlargement beginning at full bloom; stage II is characterized by slowed pericarp development with endocarp hardening and embryo development; and stage III is characterized by rapid pericarp development before fruit ripening (Tukey, 1936). At the end of stage II, the pit hardening stage, a high rate of cell division that will determine the final cell number within the fruit nears completion (Tukey and Young, 1939). The 2017 to 2018 results assured that a single application of GB at pit hardening decreased the observed fruit size of 'Regina' cherries at harvest. However, it is unclear whether spraying GB after the pit hardening stage would alleviate or diminish the negative effects on fruit size. Fruit skin color is a primary indicator of the reliable prediction of overall quality and
maturity of sweet cherry (Drake and Elfving, 2002; Ingalsbe et al., 1965; Serrano et al., 2009). Consumer purchase decisions regarding fresh cherry are greatly influenced by the darkening of skin color (Crisostomo et al., 2003). GB sprays could result in a deeper red color for both ‘Skeena’ and ‘Sweetheart’ cherries (Correia et al., 2019). However, in this study, skin color of either cultivar was unaffected by GB (Tables 2–4). Therefore, it was difficult to definitively conclude the effects of GB on fruit coloring.

Ragab et al. (2015) reported that foliar application of 5 to 20 mmol L−1 of GB under deficit irrigation promoted high SSC levels in tomato fruit. However, the SSC accumulation and TA level of fruit in response to GB were inconsistent for ‘Lapins’. For example, GB did not affect SSC, but it decreased the TA in 2017; in 2018, low levels of SSC and increased TA levels were observed with GB treatments. For ‘Regina’, GB had a positive effect on increasing the levels of SSC and TA, especially with three application of GB at 4 g L−1 GB. Correia et al. (2019) found that the total sugar levels of ‘Skeena’ and ‘Sweetheart’ cherries were not influenced by GB; the sum of the organic acids in ‘Sweetheart’ significantly increased with GB treatment, but this effect was not seen in ‘Skeena’. Therefore, the responses of SSC and TA to GB in cherry might be cultivar-dependent.

Consumers prefer fresh sweet cherry with fewer physiological disorders, such as pectic browning, flesh browning, surface pitting, bruising, and fungal diseases.
Table 5. Effects of 2018 preharvest spray of GB at 4 g L⁻¹ applied 1 time (pit hardening) or 3 times (pit hardening, straw color, and 1 week before harvest) on the contents of total nitrogen (N), phosphorous (P), magnesium (Mg), potassium (K), and calcium (Ca) of ‘Lapins’ and ‘Regina’ at harvest.

| Preharvest treatment* | N (g kg⁻¹) | P (g kg⁻¹) | K (g kg⁻¹) | Mg (g kg⁻¹) | Ca (g kg⁻¹) |
|-----------------------|------------|------------|------------|-------------|-------------|
| Lapins                |            |            |            |             |             |
| Control               | 6.66 b     | 1.17 a     | 10.47 a    | 0.51 a      | 0.50 a      |
| 4 g L⁻¹ GB 1 time     | 5.93 b     | 1.05 a     | 10.23 a    | 0.47 a      | 0.43 a      |
| 4 g L⁻¹ GB 3 times    | 9.99 a     | 1.87 a     | 10.27 a    | 0.52 a      | 0.44 a      |
| Regina                |            |            |            |             |             |
| Control               | 4.90 b     | 1.18 a     | 9.50 a     | 0.44 a      | 0.57 b      |
| 4 g L⁻¹ GB 1 time     | 4.77 b     | 1.19 a     | 8.63 a     | 0.48 a      | 0.77 a      |
| 4 g L⁻¹ GB 3 times    | 6.80 a     | 1.18 a     | 9.53 a     | 0.49 a      | 0.73 a      |

*GB was applied as the commercial product Bluestim. For one application, GB treatment was applied at the pit hardening stage (end of stage II of development). For three applications, GB treatment was applied at the pit hardening stage, straw color stage (skin color transition from green to straw color), and 1 week before harvest.

Table 5. Effects of 2018 preharvest spray of GB at 4 g L⁻¹ applied 1 time (pit hardening) or 3 times (pit hardening, straw color, and 1 week before harvest) on the contents of total nitrogen (N), phosphorous (P), magnesium (Mg), potassium (K), and calcium (Ca) of ‘Lapins’ and ‘Regina’ at harvest.

**Preharvest treatment**

| N (g kg⁻¹) | P (g kg⁻¹) | K (g kg⁻¹) | Mg (g kg⁻¹) | Ca (g kg⁻¹) |
|------------|------------|------------|-------------|-------------|
| Control    | 6.66 b     | 1.17 a     | 10.47 a     | 0.51 a      | 0.50 a      |
| 4 g L⁻¹ GB 1 time | 5.93 b | 1.05 a     | 10.23 a     | 0.47 a      | 0.43 a      |
| 4 g L⁻¹ GB 3 times | 9.99 a | 1.87 a     | 10.27 a     | 0.52 a      | 0.44 a      |

**Conclusion**

Preharvest GB applications enhanced fruit quality and reduced the incidence of disorders for ‘Lapins’ and ‘Regina’ cherries more than postharvest applications. The efficacy of preharvest GB spraying for improving cherry quality and storability varied by cultivar and application concentration, frequency, and timing. This study found that the optimum application concentration, frequency, and timing for preharvest GB applications to ‘Lapins’ or ‘Regina’ cherries were three applications of 4 g L⁻¹ at pit hardening, straw color, and 1 week before harvest. This protocol effectively maintained relatively high FF, TA, and total N content, as well as reduced the rates of peduncle browning, pitting, and decay during 4 weeks of storage (Tables 2–4). Clearly, the concentration, frequency, and timing of the GB application affected the ability of ‘Lapins’ or ‘Regina’ cherries to resist disorder development.

**Effect of GB on fruit nutrients.** In this study, the total N, P, K, Mg, and Ca of the fruit were examined at harvest. In both cultivars, a single application or three applications of GB at 4 g L⁻¹ did not affect the contents of P, K, and Mg, but three applications of GB significantly increased the total N content (Table 5). Marschner (1995) reported that a higher N content in plants might result in greater nutrient uptake from the soil. Swarts et al. (2017) found that a preharvest application of N increased the N content in fruit, but it had a detrimental effect on FF. Moreover, a high rate of N applied to fruit (i.e., 42 mg L⁻¹) reduced the fruit size and TA, whereas SSC and FF were unaffected (Neilsen et al., 2007). In our study, the increased N content resulting from three applications of GB resulted in high FF. Although smaller fruit were found for GB-treated ‘Regina’ cherries, GB applied once at pit hardening did not cause a significantly higher N level in either cultivar. Therefore, another possibility is that the high N content of fruit might result from GB residues; the GB molecule includes one N atom. Therefore, multiple applications of GB allowed more GB into the fruit. However, this hypothesis requires further investigation.

Our previous study demonstrated that an increase in the Ca content in fruit tissue contributed to firmer cherries (Dong et al., 2019). Interestingly, a significantly higher Ca content was observed in GB-treated ‘Regina’ cherries, which may have resulted in increased FF. It may be that adding exogenous Ca to the GB spray protocol increased the weight of cherries, although no change was found in FF (Correia et al., 2019). Additionally, it is unclear whether Ca sprays could increase the small fruit size of GB-treated ‘Regina’ cherries.

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Supplemental Table 1. Meteorological data from 2016, 2017, and 2018.

| Yr  | Month | Mean temp (°C) | Mean humidity (%) | Mean wind (m·s⁻¹) | Precipitation (mm) |
|-----|-------|----------------|-------------------|-------------------|--------------------|
| 2016| April | 13.9           | 59                | 1.30              | 8.4                |
|     | May   | 16.1           | 57                | 1.47              | 5.1                |
|     | June  | 18.9           | 57                | 1.61              | 1.3                |
|     | July  | 20.6           | 57                | 2.23              | 1.2                |
| 2017| April | 9.4            | 70                | 0.89              | 32.3               |
|     | May   | 15.6           | 60                | 1.39              | 0                  |
|     | June  | 18.9           | 57                | 1.74              | 0                  |
|     | July  | 22.8           | 49                | 2.37              | 0                  |
| 2018| April | 11.1           | 65                | 1.07              | 2.0                |
|     | May   | 17.2           | 56                | 2.10              | 0                  |
|     | June  | 18.3           | 54                | 1.88              | 0.3                |
|     | July  | 23.9           | 43                | 1.74              | 0                  |