Exogenous Gibberellic Acid and Cytokinin Effects on Budbreak, Flowering, and Yield of Blackberry Grown under Subtropical Climatic Conditions

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Abstract. In subtropical climates, inadequate winter chill limits blackberry (Rubus L. subgenus Rubus Watson) production by causing poor and erratic floral budbreak. To compensate for a lack of chilling, bud dormancy-breaking agents must be developed for subtropical blackberry production. Our previous study showed that gibberellic acid (GA3) promotes budbreak in three blackberry cultivars but has potential negative side effects on floral development in ‘Natchez’. 6-benzyladenine (6-BA) is a synthetic cytokinin that can act as an antagonist of gibberellins during floral transition. The objectives of this study were to evaluate cultivar × exogenous GA3 interactions, characterize dose effects of exogenous GA3, and examine synergistic effects of GA3 and 6-BA. Three field experiments were conducted in west central Florida. All spray treatments were applied at the end of the chilling period. In the first experiment, ‘Natchez’, ‘Navaho’, and ‘Ouachita’ were treated with GA3 at 0 or 99 g·ha⁻¹. Budbreak was promoted by exogenous GA3 in all three cultivars (9.9% to 4.5% vs. 42.9% to 69.4%), but yield responses varied considerably. Exogenous GA3 increased the yield of ‘Navaho’ and ‘Ouachita’ by 560% to 931%, whereas it induced flower abortion and caused a 15% yield reduction in ‘Natchez’. In the second experiment, ‘Natchez’ was treated with GA3 at 0, 25, 99, or 198 g·ha⁻¹. Budbreak increased linearly with GA3, but yield decreased exponentially with GA3 because of dose-dependent flower abortion. In the third experiment, ‘Natchez’ was subjected to five treatments: 1) water control; 2) GA3 spray application; 3) 6-BA spray application; 4) combined spray application of GA3 and 6-BA; and 5) sequential spray application of 6-BA at 9 days after GA3 application. Application rates were 99 and 47 g·ha⁻¹ for GA3 and 6-BA, respectively. Exogenous 6-BA suppressed GA3-induced flower abortion only to a limited extent. As a result, GA3-containing treatments caused 65% to 83% yield reductions compared with the control (2382 vs. 410–823 g/plant). These results demonstrate that GA3 is a highly effective bud dormancy-breaking agent for blackberry. However, the drawback of GA3 is cultivar-dependent flower abortion, which cannot be fully mitigated by 6-BA. The use of GA3 can be an important management practice for subtropical blackberry production, but its practical implementation must consider cultivar-dependent responses.

Blackberry (Rubus L. subgenus Rubus Watson) is a deciduous berry crop grown primarily in temperate regions. In recent years, the global blackberry industry has grown significantly, driven by increased consumer demand, year-round product availability, improved cultivars, and advanced production methods (Clark and Finn, 2014). The leading producers include the United States, Serbia, Hungary, Mexico, and China (Strik et al., 2007). In the United States, blackberry is the fourth most economically important berry crop, generating $697 million in retail sales during 2019 (California Strawberry Commission, 2019). Although blackberry production was traditionally concentrated in the West Coast of the United States, it is currently expanding to the Southeastern United States (Clark and Finn, 2014), where the production acreage increased by 52% (996 vs. 1512 ha) from 2007 to 2017 (USDA, 2017).

Adequate winter chill is a prerequisite for successful commercial production of many temperate fruit crops (Atkinson et al., 2013; Luedeling et al., 2011). For blackberry, buds must be exposed to a certain amount of winter chill to break bud dormancy in spring. This so-called chilling requirement (CR), generally calculated as cumulative hours at temperatures below 7.2 °C, varies from 300 to 700 h among current major florican-fruited blackberry cultivars (Carter et al., 2006; Drake and Clark, 2000). In subtropical climates, mild winter temperatures barely satisfy the CR, resulting in incomplete bud development, poor and erratic budbreak, prolonged flowering, nonsynchronous fruit set, and, ultimately, low fruit yield (Fear and Meyer, 1993; Lin and Agehara, 2020). Therefore, commercial blackberry production in Florida is limited mostly to small-scale U-pick operations for the local market (Andersen, 2007). Bud dormancy-breaking agents must be developed to improve the productivity and consistency of subtropical blackberry production.

Bioactive gibberellins (GA), such as GA3 and GA4, have important roles in the regulation of dormancy release in many perennial crops (Horvath et al., 2003; Ionescu et al., 2016). The high efficacy of exogenous GA to induce budbreak of fruit and nut crops has been reported for sweet cherry (Prunus avium L.) (Cai et al., 2019; Vimont et al., 2018), peach (Prunus persica L.) (Chauhan et al., 1961; Donoho and Walker, 1957), pistachio (Pistacia vera L.) (Tzoutzoukou et al., 1998), and Japanese apricot (Prunus mume Sieb. et Zucc.) (Zhuang et al., 2013). For blackberry, however, exogenous GA has been tested by only a few studies. Our previous study found that exogenous GA3 was highly effective for advancing the onset of budbreak and increasing the yield of ‘Ouachita’ blackberry grown in central Florida (Lin and Agehara, 2020). Exogenous GA3 exerts different effects on floral development depending on the development stage (Yamaguchi et al., 2014; Yamaguchi, 2008). GA application during dormancy can induce budbreak, but application during flowering or fruit setting can result in flower or fruit abortion (Southwick and Glozer, 2000). Our previous study found that exogenous GA3 applied at the budbreak initiation stage promoted budbreak but caused a nonsignificant yield reduction in ‘Natchez’ blackberry, suggesting its potential negative side effects on floral development (Lin and Agehara, 2020). Cytokinins act as an antagonist of GA during floral transition in apple (Malus ×domestica Borkh.) by upregulating the expression of GA degradation genes and repressing the GA signaling pathway (Li et al., 2018, 2019). In lupin (Lupinus angustifolius L.), the application of 6-benzyladenine (6-BA), a synthetic cytokinin, at 2 mmx completely prevented flower abortion (Atkins and Pigeaire, 1993). Therefore, combined application of GA3 and cytokinins may provide synergistic effects. In fact, Galindo-Reyes et al. (2004) reported a 421% yield increase in ‘Comanche’ blackberry treated with both GA3 and thidiazuron, which is a cytokinin-like substance. However, individual effects of GA3 and thidiazuron were not evaluated in their study.

The goal of this study was to optimize the application protocol of GA3 as a bud dormancy-breaking method for subtropical blackberry production. We evaluated cultivar × exogenous GA3 interactions, characterized dose effects of exogenous GA3, and examined synergistic effects of GA3 and 6-BA.

Materials and Methods

Experiment sites and plant material. One field experiment was conducted at the...
University of Florida’s Gulf Coast Research and Education Center in Balm, FL (lat. 27°27′ N, long. 82°23′ W; elevation 39 m) during the 2017 to 2018 season (Expt. 1). Three erect, floricanuc-fruited cultivars, Natchez, Navaho, and Ouachita, with estimated CRs of 300, 800 to 900, and 400 to 500 h, respectively, were used (Drake and Clark, 2000; McWhirt, 2016). Plants were established in wooden planter boxes (length 3.7 m × width 0.6 m × height 0.3 m) filled with aged pine bark in Apr. 2013. Each plot (experiment unit) consisted of six plants. Plants were spaced at 0.61 m within a row and 1.83 m between rows, and they were grown under a 40% black shade cloth to reduce fruit damage caused by excessive heat and rain. For trailing blackberry canes, we used a three-wire T-trellis system with the upper, middle, and lower wires positioned at 1.5, 1.1, and 0.7 m from the ground, respectively. We pruned floricanes at the ground level after harvesting was completed. Newly emerged primocanes were pruned to five canes per plant and tipped when they reached the top wire. Laterals were trained and tied on the closest wires. Blackberry production and cane management practices recommended in the southeast region were followed (Fernandez and Krewer, 2008).

Two field experiments were conducted at a commercial blackberry farm located in Plant City, FL (lat. 28°03′ N, long. 82°19′ W; elevation 39 m) during the 2017 to 2018 (Expt. 2) and 2018 to 2019 (Expt. 3) seasons. ‘Natchez’ blackberry tissue culture seedlings (Expt. 2) and 2018 to 2019 (Expt. 3) seasons. Harvested berries were graded based on the U.S. Department of Agriculture (USDA) grade standards (USDA, 2016). Both number and fresh weight of harvested berries were recorded during Expt. 1, whereas only fresh weight was recorded during Expts. 2 and 3.

To determine fruit size and quality, the four largest (by weight) marketable berries were sampled per plot from six peak harvests during Expt. 1 and Expt. 2. For each berry, fresh weight was recorded, and berry length and width were measured across the longest and widest parts, respectively, using a digital caliper. The soluble solids concentration (SSC) was measured using a digital refractometer (PAL-1; ATAGO, Tokyo, Japan) on unfiltered juice. Fruit juice was squeezed from the entire berry with a stainless-steel garlic press.

Statistical analysis. To describe the dose-responses of dependent variables to GA$_3$ application rates during Expt. 2, we fitted each data set to the following four models by using SigmaPlot (version 14.0; Systat Software Inc., San Jose, CA): linear Eq. [1]; quadratic Eq. [2]; exponential plateau Eq. [3]; and exponential plateau Eq. [4]. The best model was selected based on the smallest corrected Akaika information criterion (AICc).

\[ y = a + bx \]  

\[ y = a + bx + cx^2 \]  

\[ y = a + b \exp(-kx) \]  

\[ y = a + b[1 - \exp(-kx)] \]  

Data were analyzed by the generalized linear mixed model procedure (PROC GLIMMIX) in SAS statistical software (SAS 9.4; SAS Institute Inc., Cary, NC). In Expt. 1, cultivar, GA$_3$ rate, and cultivar × GA$_3$ rate interaction were considered fixed effects, and replication and replication × cultivar interaction were considered random effects. In Expt. 2 and Expt. 3, treatments were considered fixed effects and replication was considered a random effect.

Continuous data (yield, berry fresh weight, berry length, and berry width) were modeled using a lognormal distribution (DIS=T=LOGNORMAL). For the model parameter...
estimation, boundary constraints on covariance were removed (NOBOUND), and degrees of freedom for the fixed effects were adjusted by using the Kenward-Roger degrees of freedom approximation (DDFM=KR). Continuous proportion data (budbreak and SSC) were modeled with the beta distribution (DIST=BETA). If budbreak data were 0%, then they were converted to 1% before the subsequent statistical analysis. Flower count data were modeled with the negative binomial distribution (DIST=NEGBIN) in Expt. 1, and with the Poisson distribution (DIST=POISSON) in Expt. 3. Fruit count data (fruit number) were modeled with the Poisson distribution in Expt. 1. The model selection in these tests was performed based on the smallest AICc.

Budbreak and cumulative flower count data were analyzed using a repeated measures analysis because they were collected repeatedly from the same experimental unit. To identify the appropriate covariance structure, model parameters were estimated by using maximum likelihood estimation based on Laplace approximation (METHOD=LAPLACE) with default bias-corrected sandwich estimators (EMPIRICAL=MBN) (Bowley, 2015). The appropriate covariance structure was selected based on the smallest AICc. When the appropriate covariance structure was chosen, model parameters were estimated using the restricted subject pseudo-likelihood method (METHOD=RSPL), and degrees of freedom for the fixed effects were adjusted using Kenward-Roger degrees of freedom approximation (DDFM=KR2) to control the type I error (Stroup, 2018).

For continuous data, the data were back-transformed by exponentiating the least square means. For continuous proportion data and count data, data were rescaled to the original scale by using the inverse link option (ILINK) in the LSMEANS statement. Least square means comparisons were performed using the Tukey-Kramer test. Unless otherwise noted, $P$ values < 0.05 were considered statistically significant. Back-transformed or rescaled data are reported in this study.

### Table 1. Budbreak of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA$_3$) in the 2017–18 season (Expt. 1).^a^

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>GA$_3$ (g ha$^{-1}$)</th>
<th>9 Mar. (10 DAT)</th>
<th>15 Mar. (16 DAT)</th>
<th>23 Mar. (24 DAT)</th>
<th>28 Mar. (29 DAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natchez</td>
<td>0</td>
<td>4.3 a</td>
<td>5.3 bc</td>
<td>4.2 bc</td>
<td>4.5 bc</td>
</tr>
<tr>
<td>Navaho</td>
<td>99</td>
<td>46.6 a</td>
<td>54.2 ab</td>
<td>57.8 a</td>
<td>57.7 ab</td>
</tr>
<tr>
<td>Ouachita</td>
<td>0</td>
<td>0.9 b</td>
<td>0.9 d</td>
<td>0.9 c</td>
<td>0.9 c</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>14.0 b</td>
<td>31.1 c</td>
<td>42.0 b</td>
<td>42.9 b</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.9 b</td>
<td>0.9 d</td>
<td>0.9 c</td>
<td>0.9 c</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>49.5 a</td>
<td>65.1 a</td>
<td>70.5 a</td>
<td>69.4 a</td>
</tr>
</tbody>
</table>

^a^Plants were treated with GA$_3$ at 0 or 99 g ha$^{-1}$ (0 or 106 mg L$^{-1}$) via spray application with a spray volume of 935 L ha$^{-1}$ on 27 Feb. 2018.

### Table 2. Flower number, fruit number, and total marketable fruit yields of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions as affected by gibberellic acid (GA$_3$) treatment in the 2017–18 season (Expt. 1).^a^

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>GA$_3$ (g ha$^{-1}$)</th>
<th>Flower no./cane</th>
<th>Fruit number (no./plant)^b^</th>
<th>Marketable fruit yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natchez</td>
<td>0</td>
<td>16.9 ab</td>
<td>38.6 bc</td>
<td>167 ab</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>13.5 ab</td>
<td>25.5 c</td>
<td>65 bc</td>
</tr>
<tr>
<td>Navaho</td>
<td>0</td>
<td>12.4 b</td>
<td>25.5 c</td>
<td>65 bc</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>14.1 b</td>
<td>151.8 a</td>
<td>429 a</td>
</tr>
<tr>
<td>Ouachita</td>
<td>0</td>
<td>5.0 b</td>
<td>9.9 c</td>
<td>26 c</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>33.9 ab</td>
<td>76.8 ab</td>
<td>268 a</td>
</tr>
</tbody>
</table>

^a^Plants were treated with GA$_3$ at 0 or 99 g ha$^{-1}$ (0 or 106 mg L$^{-1}$) via spray application with a spray volume of 935 L ha$^{-1}$ on 27 Feb. 2018.

### Table 3. Berry size and soluble solids concentration (SSC) of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA$_3$) in the 2017–18 season (Expt. 1).^a^

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>GA$_3$ (g ha$^{-1}$)</th>
<th>Berry FW (g)</th>
<th>Berry length (cm)</th>
<th>Berry width (cm)</th>
<th>SSC (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natchez</td>
<td>6.79 a*</td>
<td>2.56 a</td>
<td>2.15 a</td>
<td>8.9</td>
<td></td>
</tr>
<tr>
<td>Navaho</td>
<td>4.28 b</td>
<td>2.10 b</td>
<td>1.90 b</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Ouachita</td>
<td>4.73 b</td>
<td>2.15 b</td>
<td>1.99 ab</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4.82 b</td>
<td>2.24</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>5.52 a</td>
<td>2.27</td>
<td>10.4</td>
<td></td>
</tr>
</tbody>
</table>

^a^Because cultivar × GA$_3$ interaction was nonsignificant, data were pooled by each main effect. Data were collected for four largest marketable berries per plot from six peak harvests. The season average data are presented.

^b^Plants were treated with GA$_3$ at 0 or 99 g ha$^{-1}$ (0 or 106 mg L$^{-1}$) via spray application with a spray volume of 935 L ha$^{-1}$ on 27 Feb. 2018.

^c^For each main effect, treatment means (n = 4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, $P < 0.05$). FW = fresh weight.
Results

GA3 × cultivar interaction on budbreak (Expt. 1). Budbreak was significantly affected by the cultivar × GA3 interaction (Table 1). The magnitude of GA3-induced budbreak varied considerably among the tested cultivars. Compared with the respective controls, exogenous GA3 increased budbreak 10 to 14 times, 16 to 48 times, and 55 to 78 times in ‘Natchez’, ‘Navaho’, and ‘Ouachita’, respectively (Table 1). At 29 days after treatment (DAT), exogenous GA3 increased budbreak from 4.5% to 57.7% in ‘Natchez’, from 0.9% to 42.9% in ‘Navaho’, and from 0.9% to 69.4% in ‘Ouachita’.

GA3 × cultivar effects on fruit number and yield (Expt. 1). Flower number per cane was significantly affected by the cultivar × GA3 interaction (Table 2). Compared with the respective controls, flower number was increased by exogenous GA3 by 240% (12.4 vs. 42.1 flowers/cane) in ‘Navaho’, whereas exogenous GA3 had no significant effects on ‘Natchez’ and ‘Ouachita’.

Fruit number and marketable fruit yield showed similar responses and were significantly affected by the cultivar × GA3 interaction (Table 2). Compared with the respective controls, exogenous GA3 increased marketable fruit yield by 560% (65 vs. 429 g/plant) in ‘Navaho’ and by 931% (26 vs. 268 g/plant) in ‘Ouachita’; whereas exogenous GA3 had no significant effects on ‘Natchez’ and ‘Ouachita’.

Fruit number and marketable fruit yield showed similar responses and were significantly affected by the cultivar × GA3 interaction (Table 2). Compared with the respective controls, exogenous GA3 increased marketable fruit yield by 560% (65 vs. 429 g/plant) in ‘Navaho’ and by 931% (26 vs. 268 g/plant) in ‘Ouachita’; whereas exogenous GA3 had no significant effects on ‘Natchez’ and ‘Ouachita’.

Berry fresh weight was significantly affected by both cultivars and exogenous GA3 (Table 3). ‘Natchez’ produced 44% to 59% heavier berries than ‘Ouachita’ and ‘Navaho’. Exogenous GA3 increased berry fresh weight by 15% (4.82 vs. 5.52 g/berry) compared with the control. Berry length and width showed trends similar to those of berry fresh weight. Fruit SSC was not significantly affected by cultivars and exogenous GA3.

GA3 application rate effects on budbreak in ‘Natchez’ (Expt. 2). Budbreak was nearly zero when plants were treated with GA3 on 20 Feb. 2018. In the control, the majority of budbreak occurred by 8 DAT: budbreak slowly increased from 24.5% at 8 DAT to 31.1% at 29 DAT (Fig. 1). Budbreak was induced rapidly by exogenous GA3. At 8 DAT, budbreak showed a linear dose-response, increasing from 24.5% to 58.3% with increasing GA3 application rates. At 15 to 29 DAT, the dose-response continued to have a similar linear increase, but the slope decreased gradually from 0.15 to 0.09. This change in slope gradient was due to the budbreak that increased gradually in the control and to the dieback of sprouted buds that occurred only in the GA3 treatments: budbreak at 198 g·ha⁻¹ GA3 decreased from 58.3% at 8 DAT to 50.9% at 29 DAT.

GA3 application rate effects on fruit number and yield in ‘Natchez’ (Expt. 2). Flowering laterals without flowers was increased by exogenous GA3, and this negative side effect was maximized at 198 g·ha⁻¹ (Fig. 2A). The dose-response was described by an exponential plateau model: flowering laterals without flowers increased sharply from 1.7% in the control to 55.5% at 99 g·ha⁻¹ GA3 and then increased gradually to 66.4% at 198 g·ha⁻¹ GA3. According to the exponential plateau model, the estimated minimum (the control) and upper asymptote values were 2.5% and 63.6%, respectively.

Flower abortion was induced by exogenous GA3 in a dose-dependent manner (Fig. 2B). Abortion occurred more severely in nondistal flowers, and some distal flowers remained intact (Fig. 3). The dose-response was described by an exponential decay: flower number per cane decreased sharply in the control from 21.8 to 5.8 at 99 g·ha⁻¹ GA3 and then decreased gradually to 4.2 at 198 g·ha⁻¹ GA3. According to the exponential decay model, the estimated minimum (the control) and lower asymptote values were 21.8 and 4.1, respectively.

Fruit number was decreased by exogenous GA3 as a result of flower abortion (Figs. 2C and 3C). The dose-response was described by an exponential decay: fruit number decreased sharply in the control from 287 to 113 at 99 g·ha⁻¹ GA3 and then decreased gradually to 109 at 198 g·ha⁻¹ GA3. According to the exponential decay model, the estimated maximum (the control) and lower asymptote values were 287 and 110, respectively.

Marketable fruit yield was also decreased by exogenous GA3 in a dose-dependent manner (Fig. 2D). The dose-response was described by an exponential decay: marketable fruit yield decreased sharply in the control from 2041 g/plant to 477 g/plant at 99 g·ha⁻¹ GA3 and then decreased gradually to 341 g/plant at 198 g·ha⁻¹ GA3. According to the exponential decay model, the estimated maximum (the control) and lower asymptote values were 2037 and 378 g/plant, respectively.

GA3 application rate effects on fruit size and quality in ‘Natchez’ (Expt. 2). Berry fresh weight showed a linear dose-response, decreasing from 13.2 to 10.4 g/berry with increasing GA3 application rates (Fig. 4A). No significant tested regression model was...
found to describe the relationship between berry length and the GAs treatments (Fig. 4B). On average, berry lengths were 3.45, 3.21, 3.40, and 3.36 cm at 0, 25, 99, and 198 g·ha⁻¹ GAs, respectively. Berry width showed a linear dose-response, decreasing from 2.66 to 2.36 cm with increasing GAs application rates (Fig. 4C). In contrast, fruit SSC increased linearly from 11.8 to 12.7 °Brix with increasing GAs application rates (Fig. 4D).

**GA₃ and 6-BA effects on budbreak in 'Natchez' (Expt. 3).** At 1 d before treatment (DBT), the budbreak was minimal, ranging from 1.4% to 3.9% in all treatments (Table 4). In the control, budbreak occurred gradually and only partially; budbreak increased from 16.4% at 9 DAT to 37.7% at 24 DAT. All GA₃-containing treatments rapidly induced budbreak to a similar extent. At 9 DAT, the GA₃-containing treatments showed significantly higher percentages of budbreak (83.2% to 86.6%) than the control (16.4%). Similar trends were observed at 16 and 24 DAT. In contrast to the GA₃-containing treatments, the 6-BA treatment had minimal effects on budbreak.

**Discussion**

Exogenous GA₃ induces budbreak in floricane-fruiter blackberry cultivars. Blackberry is adapted to temperate climates, and the GA₃-containing treatments had 65% to 83% lower marketable yield than the control (2382 vs. 410–823 g/plant). Marketable fruit yield also varied between the two treatments containing both GA₃ and 6-BA: the GA₃ + 6-BA treatment had a 101% higher marketable fruit yield than the GA₃ treatment (410 vs. 823 g/plant).

![Fig. 3. Budbreak and flowering of 'Natchez' blackberry grown under subtropical climatic conditions as affected by spray application of GA₃ in the 2017–18 season (Expt. 2).](image)

![Fig. 4. Dose-responses of berry size and soluble solids concentration (SSC) to spray application of GA₃ in 'Natchez' blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2).](image)
winter chill has an important role in breaking bud dormancy. Consequently, inadequate winter chill is a major limiting factor for subtropical blackberry production (Lin and Agehara, 2020). GA are phytohormones that act as a signal to break bud dormancy in many perennial crops (Horvath et al., 2003). Major bioactive GA are GA$_4$, GA$_5$, GA$_6$, and GA$_7$, among which GA$_3$ is one of the widely used plant growth regulators in horticultural production (Rodrigues et al., 2012; Yamauchi, 2008). To our knowledge, only one study has examined budbreak induction effects of exogenous GA$_3$ in blackberry. Galindo-Reyes et al. (2004) reported that the combined spray application of GA$_3$ at 100 mg·L$^{-1}$ and thidiazuron at 250 mg·L$^{-1}$ increased budbreak from 46% to 81% in ‘Comanche’ blackberry. However, the tested treatment was a combination of GA$_3$ and thidiazuron, making it difficult to assess the effects of exogenous GA$_3$. In this study, a single application of GA$_3$ at 99 g·ha$^{-1}$ (106 mg·L$^{-1}$) increased budbreak from 4.5% to 57.7% in ‘Natchez’, from 0.9% to 42.9% in ‘Navaho’, and from 0.9% to 69.4% in ‘Ouachita’ (Table 1), suggesting that GA$_3$ alone can effectively induce budbreak in floricanie-fruited blackberry. A linear dose-response relationship between GA$_3$ and budbreak was observed in ‘Natchez’ (Fig. 1) also suggests that budbreak can be further improved by optimizing the application rate.

Budbreak induction by exogenous GA$_3$ may be associated with antagonistic interactions between GA$_3$ and abscisic acid (ABA) (Horvath et al., 2003; Ionescu et al., 2016). In many Rosaceae fruit crops, GA production is downregulated at the onset of bud dormancy and upregulated during budbreak, whereas the exact opposite trend occurs in ABA production (Ito et al., 2019; Wen et al., 2016). Such dynamics of endogenous GA and ABA levels can be triggered by exogenous GA$_3$ (Yue et al., 2018; Zhang et al., 2016). In addition, exogenous GA$_3$ could promote budbreak indirectly by stimulating oxidative stress responses (Beauvieux et al., 2018). Zhuang et al. (2013) found that application of 100 μM GA$_3$ to Japanese apricot increased budbreak from 20% to 60% and stimulated the production of proteins involved in oxidation-reduction responses.

Cultivar-dependent effects of exogenous GA$_3$ on flowering and fruit production. In ‘Natchez’, despite significantly increased budbreak, exogenous GA$_3$ decreased marketable fruit yield by causing severe flower abortion (Figs. 2B and 3C). Inhibitory effects of GA$_3$ on floral development are well-recognized in many woody perennial crops (Engin et al., 2014; Facteau et al., 1989; Hoad, 1984; Muñoz-Fambuena et al., 2012). In fact, many studies have reported the high efficacy of GA$_3$ as a flower or fruit thinning agent for several perennial fruit crops, including apricot (Prunus armeniaca L.), Japanese plum (Prunus salicina Lindl.), nectarine (Prunus persica var. nucipersica Schneid.), and peach (García-Pallas et al., 2001; González-Rossia et al., 2006; Southwick et al., 1997; Southwick and Glazer, 2000). It is important to note, however, that GA$_3$ was applied at the flowering or fruit setting stage in these previous studies. In this study, flower abortion occurred even though GA$_3$ was applied at the budbreak initiation stage, which was at least 20 d before flowering. This observation suggests that residual GA$_3$ can remain above the optimum level in the tissue for an extended period, or that exogenous GA$_3$ can induce the upregulation of GA$_3$ production at an excessive level.

Interestingly, aborted flowers in the GA$_3$ treatments were mostly nondistal flowers (Fig. 3). This selective GA$_3$-induced flower abortion may indicate that absorbed GA$_3$ is subjected to differential translocation between distal and nondistal flowers, or that the sensitivity to GA$_3$ differs between distal and nondistal flowers. It is also important to note that GA$_3$-induced flower abortion was not observed in other cultivars, suggesting that this phytotoxicity is a cultivar-dependent response. In our previous study, we found that reproductive phenology of floricanie-fruited blackberry varies considerably among the three cultivars. The most remarkable difference is that ‘Natchez’ has a much shorter interval between budbreak and flowering than ‘Navaho’ and ‘Ouachita’ (10 vs. 25–27 d) (Lin and Agehara, 2020). This phenological difference may explain why GA$_3$-induced flower abortion occurred only in ‘Natchez’.

In ‘Natchez’, GA$_3$-induced flower abortion at 99 g·ha$^{-1}$ resulted in 15%, 77%, and 74% yield reductions in Expts. 1, 2, and 3, respectively. The variable yield reductions by

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**Table 4. Budbreak of ‘Natchez’ blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA$_3$) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Budbreak (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18 Feb. (1 DBT)</td>
</tr>
<tr>
<td>Control</td>
<td>2.6</td>
</tr>
<tr>
<td>GA$_3$</td>
<td>1.8</td>
</tr>
<tr>
<td>6-BA</td>
<td>3.9</td>
</tr>
<tr>
<td>GA$_3$ + 6-BA</td>
<td>1.4</td>
</tr>
<tr>
<td>GA$_3$ → 6-BA</td>
<td>2.4</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.9797</td>
</tr>
</tbody>
</table>

*Means (n = 3–4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, $P < 0.05$).

DBT = days before treatment; DAT = days after treatment.

**Table 5. Cumulative flower number and marketable fruit yield of ‘Natchez’ blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA$_3$) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative flower no./cane</th>
<th>Marketable fruit yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69</td>
<td>4.73</td>
</tr>
<tr>
<td>GA$_3$</td>
<td>0.76</td>
<td>2.20</td>
</tr>
<tr>
<td>6-BA</td>
<td>0.50</td>
<td>2.55</td>
</tr>
<tr>
<td>GA$_3$ + 6-BA</td>
<td>0.88</td>
<td>2.96</td>
</tr>
<tr>
<td>GA$_3$ → 6-BA</td>
<td>0.57</td>
<td>2.08</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.9796</td>
<td>0.4855</td>
</tr>
</tbody>
</table>

*Means (n = 3–4) in a column with the same letter or no letter are not significantly different (Tukey-Kramer test, $P < 0.05$).

DAT = days after treatment.
exogenous GA₃ may be due to the different plant performances among the three experiments. Marketable fruit yield of the control in Expt. 1 was only 8% to 10% compared with Expts. 2 and 3. Therefore, in Expt. 1, the negative effects of GA₃ may have appeared less pronounced because of other yield constraints. Planting density was 9870 plants/ha in Expt. 1, but 5237 plants/ha in Expts. 2 and 3. With the relatively high planting density, plant performance could have been limited because of increased competition for resources, thereby reducing marketable fruit yield on a per-plant basis. Plants were established in 2013 in Expt. 1, but in 2015 in Expts. 2 and 3. In Florida, the productivity of ‘Natchez’ is reported to decline relatively quickly compared with other cultivars. In fact, the marketable yield of ‘Natchez’ declined by 47% to 78% from the previous two seasons in Expt. 1, but yield decline was not observed in Expts. 2 and 3 (data not shown).

In contrast to ‘Natchez’, ‘Navaho’ and ‘Ouachita’ had increased marketable yield in response to exogenous GA₃ by 560% and 931%, respectively. Estimated CRs of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ are 300, 800 to 900, and 400 to 500 h (Drake and Clark, 2000; McWhirt, 2016). It appears that the beneficial effects of exogenous GA₃ are more pronounced in high-chill cultivars. Similar results were obtained in our previous study: ‘Ouachita’ showed the highest yield increase in response to exogenous GA₃ among the same three cultivars (Lin and Agehara, 2020). These results suggest that CRs and potential phototropism are important criteria for determining the suitability of the tested cultivar for exogenous GA₃.

Exogenous 6-BA does not alleviate GA₃-induced flower abortion. Exogenous GA₃ treatment could be an important adaptation tool for temperate blackberry production to cope with global warming. It is important to note that precautions should be taken when implementing the use of GA₃. First, GA₃ treatment has cultivar-specific phototropism. Because of severe flower abortion, GA₃ treatment is not recommended for ‘Natchez’.

**Practical implications for two floricaule blackberry cultivars: Navaho and Ouachita.** Our results demonstrate that spray application of GA₃ at 99 g·ha⁻¹ is highly effective for increasing both budbreak and marketable fruit yield under subtropical climatic conditions. Exogenous GA₃ has several key features that enable successful commercial implementation. First, according to the manufacturer, it has a favorable safety profile. Second, its application cost is inexpensive. Based on the price at a local major supplier of agricultural chemicals, one application of the GA₃ product at 99 g·ha⁻¹ costs ~$100 per hectare. Third, it has no negative effects on fruit development and quality. Currently, commercial blackberry production is extremely limited in subtropical climates because of inadequate winter chill. Therefore, GA₃ treatment could be an important management practice for improving the adaptability of current major blackberry cultivars to subtropical climates. With the projected loss of winter chill in temperate fruit production areas (Betts et al., 2011; Luedeling et al., 2009), GA₃ treatment could also become an important adaptation tool for temperate blackberry production to cope with global warming.

**Literature Cited**


Fear, C.D. and M.-D.L. Meyer. 1993. Breeding and development. For example, a high cytokinin-to-GA ratio induces floral bud formation, but a low cytokinin-to-GA ratio promotes the development of vegetative buds in *Li* et al., 2018; Xing et al., 2016. Therefore, the limited efficacy of 6-BA to mitigate GA₃-induced flower abortion in this study may be due to the inadequate application rate of 6-BA relative to GA₃. Further research is needed to test combined applications of 6-BA and GA₃ over a wide range of rates or ratios.


