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

Syuan-You Lin and Shinsuke Agehara

Gulf Coast Research and Education Center, Institute of Food and Agricultural Sciences, University of Florida, 14625 CR 672, Wimauma, FL 33598

Additional index words. Bramble, dormancy, flower abortion, plant growth regulator, *Rubus*

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 (0.9% 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 900 h among current major floriferous fruiting 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 mM 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°76'N, long. 82°23'W; elevation 39 m) during the 2017 to 2018 season (Expt. 1). Three erect, floricanes-fruiting 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 were transplanted in raised beds in the open field in 2015. Each plot consisted of four and five plants for Expt. 2 and Expt. 3, respectively. Plants were spaced at 0.9 m within rows and 3.6 m between rows. Beds were covered with black landscape fabric and north-south-oriented with a planting density of 3237 plants/ha. Plants were trained to a three-wire vertical trellis with the upper, middle, and lower wires positioned at 1.4, 0.9, and 0.4 m from the ground, respectively. Primocanes were tipped to encourage lateral

growth when they reached the lowest wire. Primocanes were then tied to the trellis and trained to loop down when they reached the top wire. Floricanes were pruned in early July, immediately after the final harvest.

Treatments and experimental design. All GA₃ and 6-BA treatments in this study were prepared using ProGibb LV Plus with 5.7% active ingredient (a.i.) (Valent Biosciences, Libertyville, IL) and MaxCel with 1.9% a.i. (Valent Biosciences). Immediately before application, treatment solutions were prepared by adding each chemical in water at the concentrations described. All treatments were applied between 9:00 and 10:00 AM using a CO₂-pressured backpack sprayer (model T; Bellspray, Opelousas, LA) equipped with two flat nozzle tips (XR8002; TeeJet Technologies, Wheaton, IL) spaced 0.46 m apart on the spray boom. The spray pressure was 296 kPa and the spray volume was 935 L·ha⁻¹.

In Expt. 1, there were six treatments, including three cultivars (Natchez, Navaho, and Ouachita) and two GA₃ application rates (0 and 99 g·ha⁻¹ a.i. or 0 and 106 mg·L⁻¹ a.i.) in a factorial treatment structure. There were four replicated plots for each treatment that were arranged in a split-plot design with cultivar as the main plot factor and GA₃ application rate as the subplot factor. All treatments were applied on 27 Feb. 2018. The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2017 through 27 Feb. 2018 was 199 h.

In Expt. 2, treatments comprised four spray application rates of GA₃ (0, 25, 99, and 198 g·ha⁻¹ a.i. or 0, 27, 106, and 212 mg·L⁻¹ a.i.). There were five replicated plots for each treatment that were arranged in a randomized complete block design. All treatments were applied on 20 Feb. 2018. The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2017 through 20 Feb. 2018 was 227 h.

In Expt. 3, there were five treatments: water control; GA₃ (spray application of GA₃); 6-BA (spray application of 6-BA); GA₃ + 6-BA (combined spray application of GA₃ and 6-BA); and GA₃ → 6-BA (sequential spray application of 6-BA at 9 d after GA₃ application). Application rates were 99 and 47 g·ha⁻¹ a.i. for GA₃ and 6-BA, respectively. There were four replicated plots for the control and three replicated plots for the other treatments that were arranged in a randomized complete block design. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the GA₃ → 6-BA treatment, which was applied on 28 Feb. 2019. All treatments including the water control were supplemented with a nonionic surfactant (Regulaid; Kalo, Inc., Overland Park, KS) at 0.1% (v/v). The number of accumulated chilling hours at temperatures below 7.2 °C recorded from 1 Nov. 2018 through 19 Feb. 2019 was 201 h.

Floral budbreak and flower number. We selected five representative floricanes per plot before treatments. For each cane, a section containing 20 nodes, starting with

the third node from the cane tip, was labeled to monitor budbreak. We counted the number of nodes with budbreak on a mostly weekly basis. Budbreak was defined as the stage at which a green tip was visible. The percentage of budbreak was calculated by dividing the number of nodes with budbreak by 20 and multiplying by 100. Flowers at or above the popcorn stage were counted as flowers (Fernandez, 2013; Hussain et al., 2016).

Fruit yield, size, and quality. Harvesting was performed weekly between 9 May and 5 July 2018 during Expt. 1, between 7 May and 15 June 2018 during Expt. 2, and between 22 Apr. and 14 June 2019 during Expt. 3. 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 GA₃ 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 decay Eq. [3]; and exponential plateau Eq. [4]. The best model was selected based on the smallest corrected Akaike information criterion (AICc).

$$y = a + bx \quad [1]$$

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

$$y = a + b \exp(-kx) \quad [3]$$

$$y = a + b[1 - \exp(-kx)] \quad [4]$$

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, GA₃ rate, and cultivar × GA₃ 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 (DIST=LOGNORMAL). For the model parameter

Received for publication 17 Aug. 2020. Accepted for publication 24 Sept. 2020.

Published online 29 October 2020.

This study was supported in part by the Florida Specialty Crop Block Grant Program (16SCBGPFL0041). We thank Dustin Groom for allowing us to conduct experiments on his farm and for his cooperation. We thank all members of the Horticultural Crop Physiology Lab at the Gulf Coast Research and Education Center for their technical assistance. This article was presented in the Krome memorial section at the 2020 Florida State Horticultural Society Annual Meeting.

The use of brand names and any mention or listing of commercial products or services in the publication does not imply endorsement by University of Florida nor discrimination against similar products or services not mentioned.

S.A. is the corresponding author. E-mail: sagehara@ufl.edu.

This is an open access article distributed under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

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 pseudolikelihood 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 (GA₃) in the 2017–18 season (Expt. 1).^z

Cultivar	GA ₃ (g·ha ⁻¹)	Budbreak (%)			
		9 Mar. (10 DAT)	15 Mar. (16 DAT)	23 Mar. (24 DAT)	28 Mar. (29 DAT)
Natchez	0	4.3 ab ^y	5.3 bcd	4.2 bc	4.5 bc
	99	46.6 a	54.2 ab	57.8 a	57.7 ab
Navaho	0	0.9 b	0.9 d	0.9 c	0.9 c
	99	14.0 b	31.1 c	42.0 b	42.9 b
Ouachita	0	0.9 b	0.9 d	0.9 c	0.9 c
	99	49.5 a	65.1 a	70.5 a	69.4 a
		<i>P</i> value			
Cultivar		0.0304	0.0743	0.0883	0.1835
GA ₃		0.0001	0.0001	0.0001	0.0001
Cultivar × GA ₃		0.0001	0.0001	0.0001	0.0001

^zPlants were treated with GA₃ 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.

^yTreatment means or pooled data of each main effect (n = 4) in a column with the same letter are not significantly different (Tukey-Kramer test, *P* < 0.05). DAT = days after treatment.

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 (GA₃) treatment in the 2017–18 season (Expt. 1).^z

Cultivar	GA ₃ (g·ha ⁻¹)	Flower no./cane	Fruit number (no./plant) ^y	Marketable fruit yield (g/plant)
Natchez	0	16.9 ab ^x	47.7 bc	197 ab
	99	13.5 ab	38.6 bc	167 ab
Navaho	0	12.4 b	25.5 c	65 bc
	99	42.1 a	151.8 a	429 a
Ouachita	0	5.0 b	9.9 c	26 c
	99	33.9 ab	76.8 ab	268 a
		<i>P</i> value		
Cultivar		0.3515	0.0213	0.0701
GA ₃		0.0103	0.0001	0.0001
Cultivar × GA ₃		0.0089	0.0001	0.0006

^zPlants were treated with GA₃ 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.

^yHarvest was performed nine times between 9 May and 5 July 2018.

^xTreatment means or pooled data of each main effect (n = 4) in a column with the same letter are not significantly different (Tukey-Kramer test, *P* < 0.05).

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 (GA₃) in the 2017–18 season (Expt. 1).^z

Cultivar	GA ₃ ^y (g·ha ⁻¹)	Berry FW (g)	Berry length (cm)	Berry width (cm)	SSC (°Brix)
Natchez		6.79 a ^x	2.56 a	2.15 a	8.9
Navaho		4.28 b	2.10 b	1.90 b	11.2
Ouachita		4.73 b	2.15 b	1.99 ab	10.7
	0	4.82 b	2.24	1.98	10.0
	99	5.52 a	2.27	2.04	10.4
		<i>P</i> value			
Cultivar		0.0014	0.0007	0.0083	0.1356
GA ₃		0.0493	0.6176	0.0879	0.4808
Cultivar × GA ₃		0.3546	0.7922	0.1169	0.1192

^zBecause cultivar × GA₃ interaction was nonsignificant, data were pooled by each main effect. Data were collected for four largest marketable berries per plot sampled from six peak harvests. The season average data are presented.

^yPlants were treated with GA₃ 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.

^xFor 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 effects 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’; however, it caused a nonsignificant 15% yield reduction in ‘Natchez’ (197 vs. 167 g/plant).

GA₃ × cultivar effects on fruit size and quality (Expt. 1). The data presented in Table 3 and discussed here were pooled by each main effect because they were not significantly affected by the cultivar × GA₃ interaction.

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 maximum (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 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 esti-

mated 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

Fig. 1. Linear dose-responses of budbreak to gibberellic acid (GA₃) in ‘Natchez’ blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2). Plants were treated with GA₃ at 0, 25, 99, or 198 g·ha⁻¹ (0, 27, 106, or 212 mg·L⁻¹) via spray application with a spray volume of 935 L·ha⁻¹ on 20 Feb. 2018. Budbreak was nearly zero at the time of GA₃ treatment.

Fig. 2. Dose-responses of flowering, fruit number, and yield to spray application of GA₃ in ‘Natchez’ blackberry grown under subtropical climatic conditions in the 2017–18 season (Expt. 2). (A) Percentage of flowering laterals without flowers. (B) Flower number per cane recorded at the end of flowering period. (C) Fruit number. (D) Marketable fruit yield. Plants were treated with GA₃ at 0, 25, 99, or 198 g·ha⁻¹ (0, 27, 106, or 212 mg·L⁻¹) via spray application with a spray volume of 935 L·ha⁻¹ on 20 Feb. 2018.

found to describe the relationship between berry length and the GA₃ 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 GA₃, respectively. Berry width showed a linear dose-response, decreasing from 2.66 to 2.36 cm with increasing GA₃ application rates (Fig. 4C). In contrast, fruit SSC increased linearly from 11.8 to 12.7 °Brix with increasing GA₃ 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.

GA₃ and 6-BA effects on flowering and yield in 'Natchez' (Expt. 3). Plants started flowering on 15 Mar. (24 DAT) in all treatments (Table 5). The cumulative flower number per cane initially showed no significant difference among the treatments, ranging from 0.50 to 0.88 and from 2.08 to 4.73 at 24 DAT and 32 DAT, respectively. Thereafter, the control produced flowers steadily: the cumulative flower number increased from 12.20 at 38 DAT to 27.08 at 70 DAT. In contrast, no new flowering occurred in all GA₃-containing treatments. As a result, flower number measured from 52 to 70 DAT was significantly lower in the GA₃-containing treatments than in the control and 6-BA treatment. At the end of the flowering period (70 DAT), the GA₃-containing treatments produced 87% to 92% fewer flowers than the control (27.08 vs. 2.20–3.53 flowers/cane).

Marketable fruit yield was significantly decreased by the GA₃-containing treatments regardless of the 6-BA treatment (Table 5). The plants produced the highest marketable fruit yield in the control, and a comparable yield was obtained in the 6-BA treatment.

The GA₃-containing treatments had 65% to 83% lower marketable fruit 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 → 6-BA treatment (410 vs. 823 g/plant).

Discussion

Exogenous GA₃ induces budbreak in florican-fruited blackberry cultivars. Blackberry is adapted to temperate climates, and

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). (A) Budbreak in the control at 15 d after treatment (DAT). (B) Budbreak in the GA₃ treatment at 198 g·ha⁻¹ at 15 DAT. (C) Flowering laterals at 29 DAT. Plants were treated with GA₃ at 0, 25, 99, or 198 g·ha⁻¹ (0, 27, 106, or 212 mg·L⁻¹) via spray application with a spray volume of 935 L·ha⁻¹ on 20 Feb. 2018.

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). (A) Berry fresh weight (FW). (B) Berry length. (C) Berry width. (D) SSC. Plants were treated with GA₃ at 0, 25, 99, or 198 g·ha⁻¹ (0, 27, 106, or 212 mg·L⁻¹) via spray application with a spray volume of 935 L·ha⁻¹ on 20 Feb. 2018. Data were collected for four largest marketable berries per plot sampled from six peak harvests. The season average data are presented.

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 GA₁, GA₃, GA₄, and GA₇, among which GA₃ is one of the widely used plant growth regulators in horticultural production (Rodriguez et al., 2012; Yamaguchi, 2008). To our knowledge, only one study has examined budbreak induction effects of exogenous GA₃ in blackberry. Galindo-Reyes et al. (2004) reported that the combined spray application of GA₃ 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 GA₃ and thidiazuron, making it difficult to assess the effects of exogenous GA₃. In this study, a single application of GA₃ 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 GA₃ alone can effectively induce budbreak in floricanefruiting blackberry. A linear dose-response relationship between GA₃ and budbreak observed in ‘Natchez’ (Fig. 1) also suggests that budbreak could be further improved by optimizing the application rate.

Budbreak induction by exogenous GA₃ may be associated with antagonistic interactions between GA₃ 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 GA₃ (Yue et al., 2018; Zhang et al., 2016). In addition, exogenous GA could promote budbreak indirectly by stimulating oxidative stress responses (Beauvieux et al., 2018). Zhuang et al. (2013) found that application of 100 μM GA₄ 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 GA₃ on flowering and fruit production. In ‘Natchez’, despite significantly increased budbreak, exogenous GA₃ decreased marketable fruit yield by causing severe flower abortion (Figs. 2B and 3C). Inhibitory effects of GA₃ on floral development are well-recognized in many woody perennial crops (Engin et al., 2014; Facticeau et al., 1989; Hoad, 1984; Muñoz-Fambuena et al., 2012). In fact, many studies have reported the high efficacy of GA₃ 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 Glozer, 2000). It is important to note, however, that

GA₃ was applied at the flowering or fruit setting stage in these previous studies. In this study, flower abortion occurred even though GA₃ was applied at the budbreak initiation stage, which was at least 20 d before flowering. This observation suggests that residual GA₃ can remain above the optimum level in the tissue for an extended period, or that exogenous GA₃ can induce the upregulation of GA₃ production at an excessive level.

Interestingly, aborted flowers in the GA₃ treatments were mostly nondistal flowers (Fig. 3). This selective GA₃-induced flower abortion may indicate that absorbed GA₃ is subjected to differential translocation between distal and nondistal flowers, or that the sensitivity to GA₃ differs between distal and nondistal flowers.

It is also important to note that GA₃-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 floricanefruiting 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 GA₃-induced flower abortion occurred only in ‘Natchez’.

In ‘Natchez’, GA₃-induced flower abortion at 99 g·ha⁻¹ resulted in 15%, 77%, and 74% yield reductions in Expt. 1, 2, and 3, respectively. The variable yield reductions by

Table 4. Budbreak of ‘Natchez’ blackberry grown under subtropical climatic conditions as affected by spray application of gibberellic acid (GA₃) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).

Treatment ^z	Budbreak (%)			
	18 Feb. (1 DBT)	28 Feb. (9 DAT)	7 Mar. (16 DAT)	15 Mar. (24 DAT)
Control	2.6	16.4 b ^y	33.7	37.7
GA ₃	1.8	86.6 a	88.0	84.2
6-BA	3.9	31.5 ab	40.5	41.3
GA ₃ + 6-BA	1.4	83.2 a	84.5	79.3
GA ₃ → 6-BA	2.4	85.0 a	80.4	80.7
P value	0.9797	0.0001	0.0010	0.0055

^zTreatments were as follows: control, GA₃ (spray application of GA₃), 6-BA (spray application of 6-BA), GA₃ + 6-BA (combined spray application of GA₃ and 6-BA), and GA₃ → 6-BA (sequential spray application of 6-BA at 9 d after GA₃ application). Application rates were 99 and 47 g·ha⁻¹ for GA₃ and 6-BA, respectively, with a spray volume of 935 L·ha⁻¹. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the GA₃ → 6-BA treatment, which was applied on 28 Feb. 2019.

^yMeans (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 (GA₃) and 6-benzyladenine (6-BA) in the 2018–19 season (Expt. 3).

Treatment ^z	Cumulative flower no./cane					Marketable fruit yield (g/plant)
	15 Mar. (24 DAT)	23 Mar. (32 DAT)	29 Mar. (38 DAT)	12 Apr. (52 DAT)	30 Apr. (70 DAT)	
Control	0.69	4.73	12.20	22.62 a ^y	27.08 a	2382 a
GA ₃	0.76	2.20	2.33	2.33 b	2.33 c	615 bc
6-BA	0.50	2.58	5.35	10.20 ab	12.66 ab	2330 a
GA ₃ + 6-BA	0.88	2.96	3.53	3.53 b	3.53 bc	823 b
GA ₃ → 6-BA	0.57	2.08	2.20	2.20 b	2.20 c	410 c
P value	0.9796	0.4855	0.0015	0.0001	0.0001	0.0001

^zTreatments were as follows: control, GA₃ (spray application of GA₃), 6-BA (spray application of 6-BA), GA₃ + 6-BA (combined spray application of GA₃ and 6-BA), and GA₃ → 6-BA (sequential spray application of 6-BA at 9 d after GA₃ application). Application rates were 99 and 47 g·ha⁻¹ for GA₃ and 6-BA, respectively, with a spray volume of 935 L·ha⁻¹. All treatments were applied on 19 Feb. 2019, except for the 6-BA application in the GA₃ → 6-BA treatment, which was applied on 28 Feb. 2019.

^yMeans (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 8970 plants/ha in Expt. 1, but 3237 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 phytotoxicity are important criteria for determining the suitability of the tested cultivar for exogenous GA₃.

Exogenous 6-BA does not alleviate GA₃-induced flower abortion in 'Natchez'. Antagonistic interactions between cytokinins and GA occur at biosynthesis and during catabolism and have effects throughout the signaling levels during many developmental processes (Weiss and Ori, 2007). It is hypothesized that GA inhibits flowering by antagonizing the inductive effect of cytokinins on flowering in apple (Li et al., 2019; Zhang et al., 2019). During floral bud formation, cytokinin production is upregulated but GA production is downregulated in apple (Li et al., 2019), Japanese pear (*Pyrus serotina* Rehd.) (Banno et al., 1985), mango (*Mangifera indica* L.) (Burondkar et al., 2016), and lychee (*Litchi chinensis* Sonn.) (Chen, 1990). In lupin, exogenous 6-BA at 2 mM during the flowering stage completely prevented flower abortion (Atkins and Pigeaire, 1993). In this study, mitigation of GA₃-induced flower abortion by exogenous 6-BA was observed to a limited extent. Despite having similar percentages of budbreak, the GA₃ + 6-BA treatment produced 101% higher marketable fruit yield than the GA₃ → 6-BA treatment (Table 5). The GA₃ + 6-BA treatment also had a 34% higher marketable fruit yield than the GA₃ treatment, although this difference was not statistically significant. In the GA₃ → 6-BA treatment, 6-BA was applied 9 d after

GA₃, which was likely too late to counteract GA₃-induced flower abortion.

The ratio of cytokinin to GA has an important role in the regulation of bud 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 apple (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.

Practical implications for two floricanefruiting 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.

It is important to note that precautions should be taken when implementing the use of GA₃. First, GA₃ treatment has cultivar-specific phytotoxicity. Because of severe flower abortion, GA₃ treatment is not recommended for 'Natchez'. Before testing GA₃ treatment on new cultivars, the optimum application rate should be determined based on budbreak induction and potential phytotoxicity. Second, to avoid freeze damage to developing flowers, GA₃ should be applied when the maximum chill accumulation is achieved and the risk of freeze damage is minimal.

Literature Cited

- Andersen, P.C. 2007. The blackberry. University of Florida/Institute of Food and Agricultural Sciences, Gainesville, FL. 14 Aug. 2020. <<https://edis.ifas.ufl.edu/hs104>>.
- Atkins, C. and A. Pigeaire. 1993. Application of cytokinins to flowers to increase pod set in *Lupinus angustifolius* L. Austral. J. Agr. Res. 44:1799, doi: 10.1071/AR9931799.
- Atkinson, C.J., R.M. Brennan, and H.G. Jones. 2013. Declining chilling and its impact on

- temperate perennial crops. Environ. Expt. Bot. 91:48–62, doi: 10.1016/j.envexpbot.2013.02.004.
- Banno, K., S. Hayashi, and K. Tanabe. 1985. Relationships between flower bud formation and endogenous growth regulators in Japanese Pear cultivars (*Pyrus serotina* Rehd.). J. Jpn. Soc. Hort. Sci. 54:15–25, doi: 10.2503/jjshs.54.15.
- Beauvieux, R., B. Wenden, and E. Dirlwanger. 2018. Bud dormancy in perennial fruit tree species: A pivotal role for oxidative cues. Front. Plant Sci. 9:657, doi: 10.3389/fpls.2018.00657.
- Betts, R.A., M. Collins, D.L. Hemming, C.D. Jones, J.A. Lowe, and M.G. Sanderson. 2011. When could global warming reach 4°C? Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 369:67–84, doi: 10.1098/rsta.2010.0292.
- Bowley, S.R. 2015. Factorial experiments, p. 163–194. In: Plant et al. (eds.). Hitchhiker's guide to statistics in biology. GLMM ed. Kincardine, Ontario, Canada.
- Burondkar, M.M., K.K. Upreti, A.R. Ambavane, S. Rajan, S.G. Mahadik, and S.G. Bhavne. 2016. Hormonal changes during flowering in response to paclobutrazol application in mango cv. Alphonso under Konkan conditions. Indian J. Plant. Physiol. 21:306–311, doi: 10.1007/s40502-016-0236-1.
- Cai, B., H. Wang, T. Liu, W. Zhuang, Z. Wang, S. Qu, and Y. Qin. 2019. Effects of gibberellins A4 on budbreak, antioxidant enzymes' activity and proline content of flower buds in sweet cherry (*Prunus avium*). Acta Physiol. Plant. 41:88, doi: 10.1007/s11738-019-2876-z.
- California Strawberry Commission. 2019. Retail category trends report. 21 Sept. 2020. <<https://www.calstrawberry.com/en-us/market-data/retail-category-trends>>.
- Carter, P.M., J.R. Clark, C.D. Particka, and D.Y. Crowne. 2006. Chilling response of Arkansas blackberry cultivars. J. Amer. Pomol. Soc. 60:187–197.
- Chauhan, K.S., R.H. Biggs, and J.W. Sites. 1961. Influence of gibberellic acid, naphthaleneacetic acid, indole-3-acetic acid and maleic hydrazide on peach bud dormancy. Proc. Annu. Meet. Fla. State Hort. Soc. 74:374–377.
- Chen, W.-S. 1990. Endogenous growth substances in xylem and shoot tip diffusate of lychee in relation to flowering. HortScience 25:314–315, doi: 10.21273/HORTSCI.25.3.314.
- Clark, J.R. and C.E. Finn. 2014. Blackberry cultivation in the world. Rev. Bras. Frutic. 36:46–57, doi: 10.1590/0100-2945-445/13.
- Donoho, C.W. and D.R. Walker. 1957. Effect of gibberellic acid on breaking of rest period in Elberta peach. Science 126:1178–1179.
- Drake, C.A. and J.R. Clark. 2000. Determination of the chilling requirement of Arkansas thornless blackberry cultivars. Student J. Dale Bump. Coll. Agr. Food Life Sci. 1:30–32.
- Engin, H., Z. Gökbayrak, A. Akçal, and E. Gür. 2014. Gibberellic acid inhibits floral formation and delays flower differentiation in '0900 Ziraat' sweet cherry cultivar. Eur. J. Hort. Sci. 79:260–266.
- Facteau, T.J., K.E. Rowe, and N.E. Chestnut. 1989. Flowering in sweet cherry in response to application of gibberellic acid. Scientia Hort. 38:239–245, doi: 10.1016/0304-4238(89)90071-X.
- Fear, C.D. and M.-D.L. Meyer. 1993. Breeding and variation in *Rubus* germplasm for low winter chill requirement. Acta Hort. 352:295–304, doi: 10.17660/ActaHortic.1993.352.42.
- Fernandez, G.E. and G. Krewer. 2008. 2008 Southeast bramble production. North Carolina Coop.

- Extension. 28 Apr. 2015. <<http://content.ces.ncsu.edu/southeast-regional-caneberry-production-guide/>>.
- Fernandez, G.E. 2013. Buds are tight but not for long. Coop. Extension. North Carolina Coop. Extension. 30 May 2013. <<http://teamrhubus.blogspot.com/2013/04/buds-are-tight-but-not-for-long.html>>.
- Galindo-Reyes, M.A., V.A. González-Hernández, A. Muratalla-Lúa, R.M. Soto-Hernández, and M. Livera-Muñoz. 2004. Forced production of blackberry 'Comanche' through growth regulators. *Rev. Chapingo Ser. Hort.* 10:205–209. (in Spanish with English abstract).
- García-Pallas, I., J. Val, and A. Blanco. 2001. The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA₃ as an alternative to hand thinning. *Scientia Hort.* 90:265–278, doi: 10.1016/S0304-4238(01)00229-1.
- González-Rossia, D., M. Juan, C. Reig, and M. Agustí. 2006. The inhibition of flowering by means of gibberellic acid application reduces the cost of hand thinning in Japanese plums (*Prunus salicina* Lindl.). *Scientia Hort.* 110:319–323, doi: 10.1016/j.scienta.2006.07.022.
- Hoad, G.V. 1984. Hormonal regulation of fruit-bud formation in fruit trees. *Acta Hort.* 149:13–24, doi: 10.17660/ActaHortic.1984.149.1.
- Horvath, D.P., J.V. Anderson, W.S. Chao, and M.E. Foley. 2003. Knowing when to grow: Signals regulating bud dormancy. *Trends Plant Sci.* 8:534–540, doi: 10.1016/j.tplants.2003.09.013.
- Hussain, L., S.R. Roberto, I.C.B. Fonseca, A.M. de Assis, R. Koyama, and L.E.C. Antunes. 2016. Phenology of 'Tupy' and 'Xavante' blackberries grown in a subtropical area. *Scientia Hort.* 201:78–83, doi: 10.1016/j.scienta.2016.01.036.
- Ionescu, I.A., B.L. Möller, and R. Sánchez-Pérez. 2016. Chemical control of flowering time. *J. Expt. Bot.* 68:369–382, doi: 10.1093/jxb/erw427.
- Ito, A., P.A. Tuan, T. Saito, S. Bai, M. Kita, and T. Moriguchi. 2019. Changes in phytohormone content and associated gene expression throughout the stages of pear (*Pyrus pyrifolia* Nakai) Dormancy. *Tree Physiol.* tpz101, doi: 10.1093/treephys/tpz101.
- Li, Y., D. Zhang, X. Zhang, L. Xing, S. Fan, J. Ma, C. Zhao, L. Du, and M. Han. 2018. A transcriptome analysis of two apple (*Malus × domestica*) cultivars with different flowering abilities reveals a gene network module associated with floral transitions. *Scientia Hort.* 239:269–281, doi: 10.1016/j.scienta.2018.04.048.
- Li, Y., D. Zhang, N. An, S. Fan, X. Zuo, X. Zhang, L. Zhang, C. Gao, M. Han, and L. Xing. 2019. Transcriptomic analysis reveals the regulatory module of apple (*Malus × domestica*) floral transition in response to 6-BA. *BMC Plant Biol.* 19:93, doi: 10.1186/s12870-019-1695-0.
- Lin, S.-Y. and S. Agehara. 2020. Exogenous gibberellic acid advances reproductive phenology and increases early-season yield in sub-tropical blackberry production. *Agronomy* 10:1317, doi: 10.3390/agronomy10091317.
- Luedeling, E., M. Zhang, and E.H. Girvetz. 2009. Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950–2009. *PLoS One* 4:e6166, doi: 10.1371/journal.pone.0006166.
- Luedeling, E., E.H. Girvetz, M.A. Semenov, and P.H. Brown. 2011. Climate change affects winter chill for temperate fruit and nut trees. *PLoS One* 6:e20155, doi: 10.1371/journal.pone.0020155.
- McWhirt, A. 2016. Blackberry variety selection. 29 Jan. 2018. <http://extension.missouri.edu/greene/documents/Horticulture/Blackberry/Blackberry-Cultivars%2C%20McWhirt%20Nov_15%2C2016.pdf>.
- Muñoz-Fambuena, N., C. Mesejo, M.C. González-Mas, D.J. Iglesias, E. Primo-Millo, and M. Agustí. 2012. Gibberellic acid reduces flowering intensity in sweet orange [*Citrus sinensis* (L.) Osbeck] by repressing *CiFT* gene expression. *J. Plant Growth Regul.* 31:529–536, doi: 10.1007/s00344-012-9263-y.
- Rodrigues, C., L.P. de S. Vandenberghe, J. de Oliveira, and C.R. Socol. 2012. New perspectives of gibberellic acid production: A review. *Crit. Rev. Biotechnol.* 32:263–273, doi: 10.3109/07388551.2011.615297.
- Southwick, S.M., J.T. Yeager, and K.G. Weis. 1997. Use of gibberellins on 'Patterson' apricot (*Prunus armeniaca*) to reduce hand thinning and improve fruit size and firmness: Effects over three seasons. *J. Hort. Sci. Biotechnol.* 72:645–652, doi: 10.1080/14620316.1997.11515554.
- Southwick, S.M. and K. Glozer. 2000. Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. *HortTechnology* 10:744–751, doi: 10.21273/HORTTECH.10.4.744.
- Strik, B.C., J.R. Clark, C.E. Finn, and M.P. Ban. 2007. Comprehensive crop reports worldwide blackberry production. *HortTechnology* 17:205–213, doi: 10.21273/HORTTECH.17.2.205.
- Stroup, W.W. 2018. Analysis of non-gaussian data, p. 449–509. In: B. Glaz and K.M. Yeaters (eds.). *Applied statistics in agricultural, biological, and environmental sciences*. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI. <https://doi.org/10.2134/appliedstatistics.2015.0081.c16>.
- Tzoutzoukou, C.G., C.A. Pontikis, and A. Tolia-Marioli. 1998. Effects of gibberellic acid on bloom advancement in female pistachio (*Pistacia vera* L.). *J. Hort. Sci. Biotechnol.* 73:517–526, doi: 10.1080/14620316.1998.11511008.
- U.S. Department of Agriculture. 2016. United States standards for grades of dewberries and blackberries. 25 Apr. 2017. <www.ams.usda.gov/sites/default/files/media/DewberriesBlackberriesStandard.pdf>.
- U.S. Department of Agriculture. 2017. USDA/NASS Quickstats Ad-Hoc Query Tool. 8 July 2019. <<https://quickstats.nass.usda.gov/results/08D195DC-7B67-32E7-9167-4314A69E6E69>>.
- Vimont, N., A. Schwarzenberg, M. Domijan, R. Beauvieux, M. Arkoun, J.-C. Yvin, S. Cortijo, P.A. Wigge, E. Dirlewanger, and B. Wenden. 2018. Hormonal balance finely tunes dormancy status in sweet cherry flower buds. *bioRxiv* 423871, doi: 10.1101/423871.
- Weiss, D. and N. Ori. 2007. Mechanisms of cross talk between gibberellin and other hormones. *Plant Physiol.* 144:1240–1246, doi: 10.1104/pp.107.100370.
- Wen, L.H., W.J. Zhong, X.M. Huo, W.B. Zhuang, Z.J. Ni, and Z.H. Gao. 2016. Expression analysis of ABA- and GA-related genes during four stages of bud dormancy in Japanese apricot (*Prunus mume* Sieb. et Zucc.). *J. Hort. Sci. Biotechnol.* 91:362–369, doi: 10.1080/14620316.2016.1160546.
- Xing, L., D. Zhang, C. Zhao, Y. Li, J. Ma, N. An, and M. Han. 2016. Shoot bending promotes flower bud formation by miRNA-mediated regulation in apple (*Malus domestica* Borkh.). *Plant Biotechnol. J.* 14:749–770, doi: 10.1111/pbi.12425.
- Yamaguchi, N., C.M. Winter, M.F. Wu, Y. Kanno, A. Yamaguchi, M. Seo, and D. Wagner. 2014. Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis. *Science* 344:638–641, doi: 10.1126/science.1250498.
- Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* 59:225–251, doi: 10.1146/annurev.arplant.59.032607.092804.
- Yue, C., H. Cao, X. Hao, J. Zeng, W. Qian, Y. Guo, N. Ye, Y. Yang, and X. Wang. 2018. Differential expression of gibberellin- and abscisic acid-related genes implies their roles in the bud activity-dormancy transition of tea plants. *Plant Cell Rpt.* 37:425–441, doi: 10.1007/s00299-017-2238-5.
- Zhang, S., D. Zhang, S. Fan, L. Du, Y. Shen, L. Xing, Y. Li, J. Ma, and M. Han. 2016. Effect of exogenous GA₃ and its inhibitor paclobutrazol on floral formation, endogenous hormones, and flowering-associated genes in 'Fuji' apple (*Malus domestica* Borkh.). *Plant Physiol. Biochem.* 107:178–186, doi: 10.1016/j.plaphy.2016.06.005.
- Zhang, S., C. Gottschalk, and S. van Nocker. 2019. Genetic mechanisms in the repression of flowering by gibberellins in apple (*Malus × domestica* Borkh.). *BMC Genomics* 20:1–15, doi: 10.1186/s12864-019-6090-6.
- Zhuang, W., Z. Gao, L. Wang, W. Zhong, Z. Ni, and Z. Zhang. 2013. Comparative proteomic and transcriptomic approaches to address the active role of GA₄ in Japanese apricot flower bud dormancy release. *J. Expt. Bot.* 64:4953–4966, doi: 10.1093/jxb/ert284.