Exogenous Gibberellic Acid Advances Reproductive Phenology and Increases Early-Season Yield in Subtropical Blackberry Production

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Abstract: Inadequate winter chill causes poor and erratic budbreak in blackberry (Rubus L. subgenus Rubus Watson), limiting the commercial production in subtropical climates. We examined the effects of exogenous gibberellic acid (GA₃) on the reproductive phenology, fruit number, yield, and fruit quality of three blackberry cultivars (‘Natchez’, ‘Navaho’, and ‘Ouachita’) grown under subtropical climatic conditions in two consecutive growing seasons. A single spray application of GA₃ at 0 or 49 g·ha⁻¹ was performed when plants were dormant in late December to late January. Exogenous GA₃ advanced the onset of budbreak by 12 to 82 days, flowering by four to 20 days, and fruit ripening by 0 to 15 days. When pooling across the cultivars, it also increased early-season yield by 83% to 276% in two consecutive growing seasons and total-season yield by 60% in the second growing season. Among the cultivars, the yield responses to GA₃ were most consistent in ‘Ouachita’, with early-season yield increasing by up to 499%. The average berry weight and soluble solids concentration were slightly reduced by GA₃, but these reductions were not consistent in the two growing seasons and the impact on overall fruit marketability was small. These results suggest that exogenous GA₃ is an effective bud dormancy breaking compound for blackberry, and it could be an important adaptation tool for subtropical blackberry production.

Keywords: budbreak; caneberry; chilling requirement; dormancy; plant growth regulator; Rubus

1. Introduction

The global blackberry (Rubus L. subgenus Rubus Watson) industry has experienced significant growth since the 1990s, which is driven by increased consumer demand, year-round product availability, improved cultivars, and advanced production methods [1]. In the United States, blackberry is the fourth most economically important berry crop, accounting for $549 million in retail sales during 2016 [2]. Fresh-market blackberry production rapidly increased in California in the early 1990s, and it recently expanded to the southeastern United States [3]. In the 2000s, fresh-market blackberry production also showed rapid increases in Mexico and Guatemala along with several countries in Europe, such as Serbia and Hungary [3,4]. Currently, most commercial blackberry production occurs in temperate climates. Commercial blackberry production is limited in subtropical climates, because current blackberry cultivars require relatively high amounts of winter chill for successful flower budbreak.

Budbreak is an important phenological event for temperate fruit crops, as its rate, uniformity, and timing determine the potential crop yield, quality, and profitability [5]. In blackberry plants, flower buds develop on primocanes in late fall [6]. Buds will stay dormant during winter, and budbreak will be induced by warm spring temperatures. In general, bud dormancy has two stages. The first stage is endodormancy, during which buds cannot sprout, even under optimal conditions until they are
exposed to a certain amount of winter chill [7]. The second stage is ecodormancy, in which budbreak is induced by a certain period of warm temperatures [7]. The minimum period of winter chill required to break endodormancy is referred to as the chilling requirement, and it varies considerably among fruit crop species and cultivars. Current floricane-fruiting blackberry cultivars require 300 to 900 h of winter chill (below 7.2 °C) [8–10].

In subtropical climates, inadequate winter chill can result in poor and erratic budbreak, prolonged flowering and fruit set, and ultimately low fruit yield of temperate fruit crops [11–15]. Furthermore, hot and humid summers adversely affect fruit quality. In Florida, for example, the average rainy season runs from late May through October, overlapping with the blackberry harvest window, which occurs between early May and late June. Excessive heat and rain can increase disease damage or induce physiological disorders, such as white drupelets, sunscald, leaky fruit, and red drupelet reversion [16–18]. Therefore, fruit earliness is critical in minimizing fruit quality loss by avoiding unfavorable weather conditions.

Plant hormones play important roles in the regulation of bud dormancy [19]. For example, gibberellins (GAs) act as a signal to induce budbreak in many perennial crops [20]. The major forms of bioactive GAs include GA1, GA3, GA4, and GA7, among which GA3 is one of the most active and widely used GAs in agriculture [21,22]. In sweet cherry and Japanese apricot, the endogenous concentration of GA3 remains low during bud dormancy, whereas it increases during the release of bud dormancy [23,24]. Several fruit and nut crops, such as peach, almond, and pistachio, report the efficacy of exogenous GA3 in inducing budbreak [25–28]. For example, Elsabagh [28] reported that the spray application of GA3 at 500 mg L⁻¹ increased the percentage of vegetative and floral budbreak of almond by 95% and 83%, respectively. To our knowledge, only one study has examined the budbreak induction effects of exogenous GA3 in blackberry. Galindo-Reyes et al. [29] reported that the spray application of GA3 at 100 mg L⁻¹ and thidiazuron at 250 mg L⁻¹ increased the percentage of budbreak from 46% to 81%, advanced fruit earliness by 10 to 15 days, and increased yield by 421% in ‘Comanche’ blackberry. However, this study was conducted under cool climatic conditions at 2400 m above sea level in Mexico, and the tested cultivar is an old floricane-fruiting cultivar, which is not grown commercially in other countries. Furthermore, the tested treatment was a combination of GA3 and thidiazuron, making it difficult to assess the effects of exogenous GA3.

Global warming has the potential to reduce yield of fruit crops that have high chilling requirements [5,30]. Luedeling et al. [31] modeled winter chill in California while using 18 different future scenarios and projected that climatic changes could lead to 50% to 75% reductions in the area with adequate winter chill for many temperate fruit crops by the mid-21st century. Therefore, artificial budbreak induction could be an important adaptation tool not only for subtropical blackberry production, but also for temperate blackberry production to cope with global warming. Although the efficacy of GA3 as a budbreak induction compound is reported in several temperate fruit crops, it has not been fully investigated in blackberry.

The objective of this study was to examine the effects of exogenous GA3 on the reproductive phenology, fruit number, yield, and fruit quality of commercial blackberry cultivars under subtropical climatic conditions. We hypothesized that exogenous GA3 is an effective budbreak induction compound for blackberry, and it can improve its fruit earliness and yield under subtropical climatic conditions.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The field experiments were conducted at the University of Florida’s Gulf Coast Research and Education Center in Balm, Florida, the United States during the 2015–2016 and 2016–2017 seasons. In central Florida, winter chill occurs between November and March. Cumulative chilling hours (0 to 7.2 °C) that were recorded during this period were 165 and 130 h in the 2015–2016 and 2016–2017 seasons, respectively (Table S1). The growing degree days (GDD) recorded between March to June...
were 1659 and 1583 GDD in the 2015–2016 and 2016–2017 seasons, respectively (Figure S1). Three erect, floricanec-fruiting cultivars, ‘Natchez’, ‘Navaho’, and ‘Ouachita’, were used. The estimated chilling requirements of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ are 300, 800 to 900, and 400 to 500 h, respectively [8–10,32]. The 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 April 2013 and six plants were planted in a wooden planter box. The plants were spaced at 0.61 m within a planter box and 1.83 m between planter boxes, and they were grown under a 40% black shade cloth in order to reduce the fruit damage caused by excessive heat and rain during the late harvest. All of the plots were irrigated through drip tapes. The drip tapes had emitters spaced 60 cm apart with a flow rate per emitter of 0.91 L h⁻¹ (Netafim, Fresno, CA, USA). A three-wire T-trellis system was used for trailing blackberry canes, with upper, middle, and lower wires positioned at 1.5, 1.1, and 0.7 m from the ground, respectively. Floricanes were pruned in August after harvesting was completed. Newly emerged primocanes were pruned to five canes per plant and tipped when they reached the top wire. We adopted some management practices used by local growers, such as fertilization and disease control, in the experiment set up, because the optimum commercial blackberry production system has not been established in Florida. Pest management practices that were recommended for blackberry production in the southeast region were followed [33].

2.2. GA₃ Treatment

A commercial GA₃ product with 5.7% active ingredient (a.i.) that was registered for use on various fruit and vegetable crops (ProGibb LV Plus; Valent Biosciences, Libertyville, IL, USA) was used in this study. Plants were treated with GA₃ at 0 or 49 g·ha⁻¹ a.i. (53 mg·L⁻¹ a.i.) via spray application with a spray volume of 935 L·ha⁻¹ (≈104 mL/plant) on 24 December 2015 in the 2015–2016 season and on 27 January 2017 in the 2016–2017 season. All of the spray treatments were performed between 9:00 and 10:00 a.m. using a CO₂-pressured backpack sprayer (model T; Bellspray, Opelousas, LA, USA) equipped with two flat nozzle tips (XR8002; TeeJet Technologies, Wheaton, IL, USA) spaced 0.46 m apart on the spray boom.

2.3. Phenology, Fruit Yield, and Fruit Quality Measurements

The onset of budbreak, flowering, and harvest was individually recorded for all six plants in each experimental unit. We defined budbreak as the appearance of green tissue emerging from a bud, and flowering as the stage when a flower is fully open. Berries were harvested once they were fully ripe. The dates of the phenological events recorded from six plants were averaged for each plot. Harvesting was performed weekly between 4 May and 7 July 2016 in the 2015–2016 season, and between 5 May and 10 July 2017 in the 2016–2017 season. We classified the harvests into early-season yield (harvested before June) and late-season yield (harvested from June to July). We graded berries based on the U.S. Department of Agriculture (USDA) grade standards [34]: the marketable berries are firm, well colored, not misshapen, and not overripe, which are free from mold decay and damage. At each harvest, we recorded total marketable fruit number and weight to determine the fruit number (fruit number per plant) and the average berry weight. Additionally, the four largest marketable berries (by weight) per plot were selected in order to measure soluble solids concentration (SSC) while using a digital refractometer (PAL-1, ATAGO, Tokyo, Japan).

2.4. Regression Analysis

We fitted each data set to the linear regression model by using SigmaPlot in order to describe the relationship between two yield variables (all combinations of average berry weight, fruit set, or total-season yield) (version 14.0; Systat Software Inc., San Jose, CA, USA).
2.5. Experiment Design and Statistical Analysis

There were six treatments, which consisted of three cultivars (’Natchez’, ’Navaho’, and ’Ouachita’) and two GA$_3$ application rates (0 and 49 g·ha$^{-1}$) in a factorial combination. Each treatment had four replicated plots, which were arranged in a split-plot design with cultivar as the main-plot factor and GA$_3$ application rate as the sub-plot factor. Each experimental unit (plot) consisted of six plants.

All of the data were analyzed by using the generalized linear mixed model procedure (PROC GLIMMIX) of the SAS statistical software (SAS 9.4; SAS Institute Inc., Cary, NC, USA). Cultivar, GA$_3$ rate, and cultivar × GA$_3$ rate interaction effects were considered as fixed effects, whereas the replication and replication × cultivar interaction effects were considered as random effects. Continuous data (yield and average berry weight) were modeled with the lognormal distribution (DIST = LOGNORMAL) and continuous percentage data (SSC) were modeled with the beta distribution (DIST = BETAS). For model parameter estimation, boundary constraints on covariance were removed (NOBOUND), and the degrees of freedom for the fixed effects were adjusted by using the Kenward-Roger degrees of freedom approximation (DDFM = KR). The count data (fruit number) were modeled with the negative binomial distribution (DIST = NEGBIN). Model parameters were estimated by using maximum likelihood estimation with quadrature approximation (METHOD = QUAD) and default bias-corrected sandwich estimators (EMPIRICAL = MBN) [35]. For continuous data, data were then back-transformed by exponentiating the sum of the least square mean and the correction factor [36]. For count data and continuous percentage data, the data were rescaled to the original scale by using the inverse link option (ILINK) in the LSMEANS statement. Multiple comparisons of least squares means were performed using the Tukey–Kramer test. Unless otherwise noted, $p$ values less than 0.05 were considered to be statistically significant. Back-transformed or rescaled data are reported in this study.

3. Results

3.1. Phenology

Without GA$_3$ treatment, natural budbreak of all tested cultivars occurred between 11 March and 24 March 2016 in the 2015–2016 season, and between 16 March and 3 April 2017 in the 2016–2017 season (Table 1), during which chill accumulation was maximized and temperatures started to rise rapidly [37]. In the 2015–2016 season, ’Natchez’ had the earliest budbreak, followed by ’Ouachita’ and ’Navaho’. In the 2016–2017 season, natural budbreak of ’Natchez’, ’Navaho’, and ’Ouachita’ delayed by three to 17 days as compared with the 2015–2016 season.

Table 1. Onset of budbreak, flowering, and harvest of ’Natchez’, ’Navaho’, and ’Ouachita’ blackberry grown under subtropical climatic conditions as affected by gibberellic acid (GA$_3$) treatment in the 2015–2016 season and 2016–2017 season.

| Cultivar | GA$_3$ (g·ha$^{-1}$) | 2015–2016 Season | 2016–2017 Season |
|----------|---------------------|------------------|------------------|
|          |                     | Budbreak | Flowering | Harvest | Budbreak | Flowering | Harvest |
| Natchez  | 0                   | 11 March 2016 | NA        | 16 May 2016 | NA        | 16 March 2017 | 26 March 2017 | 13 May 2017 |
|          | 49                  | 31 December 2015 | NA | 16 May 2016 | 4 March 2017 | 22 March 2017 | 13 May 2017 |
| Navaho   | 0                   | 24 March 2016 | NA        | 3 June 2016 | 27 March 2017 | 23 April 2017 | 10 June 2017 |
|          | 49                  | 2 January 2016 | NA        | 12 June 2016 | 2 March 2017 | 3 April 2017 | 26 May 2017 |
| Ouachita | 0                   | 17 March 2016 | NA        | 7 June 2016 | 3 April 2017 | 28 April 2017 | 11 June 2017 |
|          | 49                  | 31 December 2015 | NA | 2 June 2016 | 4 March 2017 | 10 April 2017 | 26 May 2017 |

*Plants were treated with GA$_3$ at 0 or 49 g·ha$^{-1}$ (53 mg·L$^{-1}$) via spray application with a spray volume of 935 L·ha$^{-1}$ on 24 December 2015 in the 2015–2016 season and on 27 January 2017 in the 2016–2017 season. NA = not available.*
The timing of first flowering was only recorded in the 2016–2017 season. Flowering occurred earlier in ‘Natchez’ than in the other two cultivars. In the untreated control, flowering of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ occurred at 10, 27, and 25 days after budbreak, respectively. Consequently, ‘Natchez’ ripened berries earlier than the other two cultivars. In the untreated control, the harvesting of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ started at 58, 75, and 69 days after budbreak, respectively.

The spray application of GA$_3$ advanced the timing of budbreak and flowering in all cultivars. In the 2015–2016 season, budbreak of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ was advanced by the GA$_3$ treatment by 71, 82, and 77 days, respectively, when compared with the respective untreated controls. In the 2016–2017 season, budbreak of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ was advanced by the GA$_3$ treatment by 12, 25, and 30 days, respectively, as compared with the respective untreated controls. The flowering of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ was advanced by the GA$_3$ treatment by 4, 20, and 18 days, respectively, when compared with the respective untreated controls. However, fruit earliness was differently affected by the GA$_3$ treatment among the tested cultivars. In the 2015–2016 season, although the GA$_3$ treatment advanced the first harvest date of ‘Ouachita’ by five days, it did not change the first harvest date of ‘Natchez’ and delayed the first harvest date of ‘Navaho’ by nine days. In the 2016–2017 season, although the GA$_3$ treatment advanced the first harvest date of ‘Navaho’ and ‘Ouachita’ by 15 and 16 days, respectively, it did not change the first harvest date of ‘Natchez’.

### 3.2. Marketable Yield (2015–2016 Season)

Early-season yield was significantly affected by the cultivar × GA$_3$ interaction (Table 2). When compared with the respective untreated controls, the GA$_3$ treatment increased early-season yield by 9% in ‘Natchez’ (244 vs. 265 g/plant) and by 499% in ‘Ouachita’ (7 vs. 41 g/plant), whereas it decreased the marketable yield by 8% in ‘Navaho’ (3.6 vs. 3.3 g/plant).

| Cultivar | GA$_3$ (g·ha$^{-1}$) | Marketable Yield (g/plant) | Early | Late | Total |
|----------|-----------------------|-----------------------------|-------|------|-------|
|          |                       |                             |       |      |       |
| Natchez  | 0                     | 243.5 a w                   | 174.7 ab | 372.5 a |
|          | 49                    | 264.9 a                    | 321.4 a | 538.9 a |
| Navaho   | 0                     | 3.6 c                       | 39.3 de | 43.0 cd |
|          | 49                    | 3.3 c                       | 27.9 e | 29.9 d |
| Ouachita | 0                     | 6.9 c                       | 54.1 cd | 61.2 c |
|          | 49                    | 41.3 b                      | 93.6 bc | 129.7 b |
|          |                       |                             |       |      |       |
| Pooled data |                    |                             |       |      |       |
| Natchez  |                       | 254.0 a                    | 236.9 a | 448.0 a |
| Navaho   |                       | 3.4 c                      | 33.1 c | 35.8 c |
| Ouachita |                       | 16.8 b                    | 71.1 b | 89.1 b |
|          | 0                     | 18.1 b                    | 71.9 b | 99.3 |
|          | 49                    | 33.1 a                    | 94.3 a | 127.8 |

| p value |
|---------|
| Cultivar | 0.0001 |
| GA$_3$   | 0.0923 |
| Cultivar × GA$_3$ | 0.0738 |

$^z$ Plants were treated with GA$_3$ at 0 or 49 g·ha$^{-1}$ (53 mg·L$^{-1}$) via spray application with a spray volume of 935 L·ha$^{-1}$ on 24 December 2015 in the 2015–2016 season. $^x$ Harvesting was performed four times between 4 May and 24 May 2016. $^y$ Harvesting was performed six times between 2 June and 7 July 2016. $^w$ All treatment means ($n = 4$) or pooled data of each main effect in a column with the same letter are not significantly different (Tukey–Kramer test, $p < 0.1$).
The late-season yield was significantly affected by the cultivar × GA₃ interaction (Table 2). When compared with the respective untreated controls, the GA₃ treatment increased late-season yield by 84% in ‘Natchez’ (175 vs. 321 g/plant) and by 73% in ‘Ouachita’ (54 vs. 94 g/plant), whereas it decreased late-season yield by 29% in ‘Navaho’ (39 vs. 28 g/plant).

Total-season yield was significantly affected by the cultivar × GA₃ interaction (Table 2). When compared with the respective untreated controls, the GA₃ treatment increased total-season yield by 45% in ‘Natchez’ (373 vs. 539 g/plant) and by 112% in ‘Ouachita’ (61 vs. 130 g/plant), whereas it decreased total-season yield by 30% in ‘Navaho’ (43 vs. 30 g/plant).

3.3. Marketable Yield (2016–2017 Season)

Early-, late-, and total-season yield data presented in Table 3 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar × GA₃ interaction. The early-season yield was significantly affected by both cultivars and GA₃ treatment. ‘Natchez’ produced ten (35 vs. 365 g/plant) and twenty-four times (15 vs. 365 g/plant) as much yield as ‘Navaho’ and ‘Ouachita’, respectively, and ‘Ouachita’ produced 56% less yield than ‘Navaho’ (35 vs. 15 g/plant). Averaging across the three cultivars, the GA₃ treatment increased early-season yield by 276% (30 vs. 112 g/plant) when compared with the untreated control.

| Cultivar | GA₃ (g·ha⁻¹) | Marketable Fruit Yield (g/plant) |
|----------|-------------|---------------------------------|
| Natchez  | 0           | a w                            |
|          | 49          | a w                            |
| Navaho   | 0           | a w                            |
|          | 49          | ab                            |
| Ouachita | 0           | ab                            |
|          | 49          | ab                            |

Pooled data

| Cultivar | Marketable Fruit Yield (g/plant) |
|----------|---------------------------------|
| Natchez  | 364.5 a                           |
| Navaho   | 35.0 b                            |
| Ouachita | 15.3 b                            |
|          | 0 29.9 ab                         |
|          | 49 112.4 a                        |

p value

|            | Cultivar | GA₃ | Cultivar × GA₃ |
|------------|----------|-----|--------------|
|            | 0.0064   | 0.0113 | 0.3080 |
|            | 0.0180   | 0.2977 | 0.0052 |

Table 3. Effects of gibberellic acid (GA₃) treatment on early-, late-, and total-season yield of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions in the 2016–2017 season.

Cultivars significantly affected late-season yield: ‘Natchez’ produced approximately three times as much yield as ‘Navaho’ (209 vs. 612 g/plant) and ‘Ouachita’ (180 vs. 612 g/plant), and ‘Ouachita’ produced 14% less yield than ‘Navaho’ (209 vs. 180 g/plant). Averaging across the three cultivars, the GA₃ treatment increased late-season yield by 40% (241 vs. 336 g/plant) as compared with the untreated control.

The total-season yield was significantly affected by both cultivars and GA₃ treatment. ‘Natchez’ produced about four (235 vs. 898 g/plant) and five times (197 vs. 898 g/plant) as much yield as ‘Navaho’
and 'Ouachita', respectively. Averaging across the three cultivars, the GA₃ treatment increased total-season yield by 60% (274 vs. 438 g/plant) when compared with the untreated control.

Although the cultivar × GA₃ interaction was not statistically significant, data indicated a trend of cultivar-dependent responses to the GA₃ treatment. First, according to the Tukey–Kramer test, significant total-season yield differences among the tested cultivars were only found in the untreated control. Second, the GA₃ treatment resulted in numerical reductions of 4% (916 vs. 880 g/plant) in 'Natchez', but numerical increases of 135% (153 vs. 359 g/plant) and 81% (146 vs. 265 g/plant) in 'Navaho' and 'Ouachita', respectively.

3.4. Fruit Number

Fruit number data presented in Table 4 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar × GA₃ interaction. Fruit number was significantly affected by both cultivars and GA₃ treatment in the 2015–2016 season. ‘Natchez’ produced approximately six (12 vs. 70 berries/plant) and three times (24 vs. 70 berries/plant) as many berries as ‘Navaho’ and ‘Ouachita’, respectively, and ‘Ouachita’ produced twice as many berries as ‘Navaho’ (12 vs. 24 berries/plant). Averaging across the three cultivars, the GA₃ treatment increased fruit number by 48% (22 vs. 33 berries/plant) when compared with the untreated control.

Table 4. Effects of gibberellic acid (GA₃) treatment on fruit number, average berry weight (wt.), soluble solids concentration (SSC) of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions in the 2015–2016 and 2016–2017 seasons.

| Cultivar | 2015–2016 Season | 2016–2017 Season |
|----------|------------------|------------------|
|          | Fruit Number     | Avg. Berry wt.   | SSC °Brix | Fruit Number | Avg. Berry wt. | SSC °Brix |
|          | (g/ha⁻¹) (no./plant) | (g) | °Brix | (no./plant) | (g) | °Brix |
| Natchez  | 0 58.8 ab ×   | 6.43 a | 9.99 bc | 180.2 | 4.85 a | 8.46 b |
|          | 49 87.1 a      | 5.89 a | 9.54 c | 214.0 | 4.31 ab | 7.96 b |
| Navaho   | 0 13.5 d       | 3.20 c | 11.50 a | 59.1 | 2.50 d | 11.79 a |
|          | 49 10.2 d      | 2.91 c | 9.83 c | 129.7 | 2.58 cd | 11.17 a |
| Ouachita | 0 14.1 cd      | 4.19 b | 11.65 a | 43.6 | 3.42 bc | 11.88 a |
|          | 49 39.5 bc     | 3.23 c | 10.60 b | 69.9 | 3.57 ab | 11.29 a |
| Pooled data | 70.3 a     | 6.16 a | 9.76 b | 196.4 a | 4.57 a | 8.20 b |
| Navaho   | 11.8 c         | 3.05 c | 10.63 a | 87.5 ab | 2.54 c | 11.47 a |
| Ouachita | 23.6 b         | 3.68 b | 11.11 a | 55.2 b | 3.49 b | 11.58 a |
|          | 0 22.1 b       | 4.42 a | 11.02 a | 77.4 | 3.46 a | 10.58 a |
|          | 49 32.8 a      | 3.81 b | 9.98 b | 124.0 | 3.41 b | 10.01 b |

| p value | Cultivar | 0.0001 | 0.0001 | 0.0001 | 0.0191 | 0.0002 | 0.0001 |
|         | GA₃      | 0.0221 | 0.0054 | 0.0001 | 0.1155 | 0.7792 | 0.0079 |
|         | Cultivar × GA₃ | 0.1012 | 0.1905 | 0.0090 | 0.6087 | 0.3619 | 0.9800 |

* Plants were treated with GA₃ at 0 or 49 g·ha⁻¹ (53 mg·L⁻¹) via spray application with a spray volume of 935 L·ha⁻¹ on 24 December 2015 in the 2015–2016 season and on 27 January 2017 in the 2016–2017 season. ° SSC was measured weekly on four largest, fully ripe berries. The season average data are presented. × All treatment means (n = 4) or pooled data of each main effect in a column with the same letter are not significantly different (Tukey–Kramer test, p < 0.05).

Fruit number was only significantly affected by cultivars in the 2016–2017 season: ‘Natchez’ produced about two (88 vs. 196 berries/plant) and four times (55 vs. 196 berries/plant) as many berries as ‘Navaho’ and ‘Ouachita’, respectively. Although not statistically significant (p = 0.1155), the GA₃ treatment increased fruit number by 60% (77 vs. 124 berries/plant), when compared with the untreated control.
3.5. Average Berry Weight

The average berry weight data that are presented in Table 4 and discussed below were pooled by each main effect, as they were not significantly affected by the cultivar \( \times \) GA\(_3\) interaction. The average berry weight was significantly affected by both cultivars and GA\(_3\) treatment in the 2015–2016 season. ‘Natchez’ produced 102% (3.05 vs. 6.16 g/berry) and 67% (3.68 vs. 6.16 g/berry) heavier berries than ‘Navaho’ and ‘Ouachita’, respectively, and ‘Ouachita’ produced 21% (3.05 vs. 3.68 g/berry) heavier berries than ‘Navaho’. Averaging across the three cultivars, the GA\(_3\) treatment decreased the average berry weight by 14% (4.42 vs. 3.81 g/berry), when compared with the untreated control.

The average berry weight was only significantly affected by cultivar in the 2016–2017 season: ‘Natchez’ produced 80% (2.54 vs. 4.57 g/berry) and 31% heavier berries than ‘Navaho’ and ‘Ouachita’ (3.49 vs. 4.57 g/berry), respectively, and ‘Ouachita’ produced 37% (2.54 vs. 3.49 g/berry) heavier berries than ‘Navaho’. The average berry weight was minimally affected by the GA\(_3\) treatment.

3.6. Regression Analysis

In all cultivars, the average berry weight showed a weak correlation with fruit number in the 2015–2016 season (\( R^2 = 0.00–0.36 \)) and in the 2016–2017 season (\( R^2 = 0.00–0.43 \) ) (Figure 1).

![Figure 1](image)

**Figure 1.** Linear correlation between fruit number and average berry weight of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions in the 2015–2016 season (A) and the 2016–2017 season (B). Plants were treated with gibberellic acid (GA\(_3\)) at 0 or 49 g ha\(^{-1}\) (53 mg L\(^{-1}\)) via spray application with a spray volume of 935 L ha\(^{-1}\) on 24 December 2015 in the 2015–2016 season and on 27 January 2017 in the 2016–2017 season.

In all of the cultivars, the total-season yield showed a high positive correlation with fruit number (\( R^2 = 0.89–0.98 \)), whereas its correlation with the average berry weight was relatively weak (\( R^2 = 0.06–0.47 \) ) (Figure 2A,B). Similar trends were observed in the 2016–2017 season, with a high positive correlation between fruit number and total-season yield (\( R^2 = 0.88–0.96 \)) and a relatively weak correlation between the average berry weight and total-season yield (\( R^2 = 0.12–0.61 \) ) (Figure 2C,D).
4.1. Blackberry Reproductive Phenology in a Subtropical Climate

Phenological behaviors are often important cultivar selection criteria, particularly in new production areas. Phenology data also provide a foundation for developing crop management and marketing strategies. In this study, the reproductive phenology of three floricane-fruiting blackberry cultivars grown in Florida’s subtropical climate was characterized based on the timing of flower budbreak, flowering, and fruit ripening (Table 1). In temperate regions of North America, budbreak, flowering, and fruit ripening of blackberry generally occur from late March to early April, mid-May to mid-June, and early June to late July, respectively [32,38–41]. Our results suggest that these reproductive phenological events can be advanced by two to four weeks in a subtropical climate than in temperate climates. Among the three tested cultivars, ‘Natchez’ was the earliest cultivar, with fruit ripening starting in mid-May, about four weeks earlier than the other two cultivars (Table 1). The superior earliness of ‘Natchez’ is also reported under temperate climatic conditions in Arkansas: fruit ripening starts in early June for ‘Natchez’, but in mid-June for ‘Navaho’ and ‘Ouachita’ [32,40,41].
Blackberry is adapted to temperate climates, and winter chill plays an important role in the reproductive phenology [5]. For example, the chilling requirements of current florican-fruiting blackberry cultivars in the United States range from 300 to 900 h [8–10]. Chilling hours accumulated in this study ranged from 130 to 165 h per season, which were below the minimum chilling requirement for blackberry (Table S1). In central Florida, the typical budbreak of the tested blackberry cultivars does not exceed 30%. Although the percentage of budbreak was not recorded in this study, the observed budbreak was similar with the regular seasons. We also observed limited flower development per cluster (inflorescence). It is reported that ‘Natchez’, ‘Navaho’, and ‘Ouachita’ can produce, on average, seven, six, and six flowers per cluster, respectively, in Arkansas [42–44]. In our observation, the average number of flowers per cluster in ‘Natchez’, ‘Navaho’, and ‘Ouachita’ was five, two, and two, respectively. We speculate that the lack of winter chill may have adversely affected flower differentiation and/or development, thereby limiting fruit number and yield.

Subtropical blackberry production is limited because of the relatively high chilling requirements of commercial blackberry cultivars. Only a few studies have reported blackberry phenology and productivity in subtropical climates. Hussain et al. [45] examined the phenology of ‘Tupy’ blackberry grown at 566 m above sea level under subtropical climatic conditions in southern Brazil (latitude 23°23′ S and longitude 51°11′ W), and they reported that budbreak, flowering, and fruit ripening began from mid-Sep, mid-Oct, and mid-Nov, respectively. Galindo-Reyes et al. [29] reported that ‘Comanche’ blackberry grown at 2400 m above sea level produced 27.9 g/cane in central Mexico (latitude 19°32′ N and longitude 98°46′ W). Although both studies were conducted in subtropical climates, the information is not fully applicable to this study. First, these studies used ‘Tupy’ and ‘Comanche’, both of which are thorny cultivars and not commercially produced in the United States [1,4]. Second, these studies were conducted at much higher elevations (566 to 2400 m) when compared with the experiment site of this study (40 m), likely providing greater chilling accumulation. Therefore, our results suggest that, without artificial budbreak induction, current U.S. blackberry cultivars are not suitable to the commercial production in subtropical climates.

4.2. Exogenous GA3 Advances Blackberry Reproductive Phenology and Increases Early-Season Yield

Although GA3 is not commercially used for budbreak induction, its efficacy has been reported in several temperate fruit crops. In peach, Donoho and Walker [26] found that the spray application of GA3 at 200 to 4000 mg·L⁻¹ increased the percentage of budbreak by 40% to 98%. In almond, Elsabagh [28] reported that the spray application of GA3 at 500 mg·L⁻¹ increased the percentage of floral budbreak from 0% to 83%. In a lab experiment using sweet cherry branch cuttings, 5-µM GA3 treatment increased the percentage of budbreak by about 100% [46]. In this study, although we did not record the number of budbreak, the spray application of GA3 at 49 g·ha⁻¹ (53 mg·L⁻¹) increased fruit number, averaging across cultivars, by 48% to 60% (Table 4), implying that GA3 increased the percentage of budbreak. The increased budbreak by GA3 was also visually evident (Figure S2).

In this study, the efficacy of exogenous GA3 in increasing yield was more pronounced in the early season than in the late season. First, the early-season yield increase was significant in the two consecutive growing seasons, whereas the late-season yield increase was only significant in the first growing season (Tables 2 and 3). Second, averaging across cultivars, exogenous GA3 increased early-season yield by 83% to 276%, but it increased the late-season yield only by 31% to 40%. These results suggest that exogenous GA3 is particularly effective in improving fruit earliness. Similar results have been reported in other crops. In strawberry, GA3 applied at 20 to 80 mg·L⁻¹ at the beginning of winter increased early-season yield by up to 253% without affecting the total-season yield [47]. In coffee, early-season yield of untreated and GA3-treated (100 mg·L⁻¹) buds accounted for approximately 20% and 70% of total yield, respectively [48]. The potential benefits of improving fruit earliness in subtropical climates are discussed below (Section 4.5).

Interestingly, the magnitude of GA3-induced earliness appears to become less pronounced with the phenological progression. Exogenous GA3 advanced the onset of budbreak, flowering, and fruit
ripening by 12 to 82, four to 20, and 0 to 15 days, respectively (Table 1). In temperate fruit crops, it is reported that exogenous GA₃ promotes budbreak, but inhibits subsequent flower development [19,49]. However, GA₃ can be rapidly degraded or deactivated in plant tissue [50]. In this study, because GA₃ was applied two to three months before flowering, the inhibitory effect of residual GA₃ on flower development was likely minimal. Therefore, we speculate that cold weather after budbreak (January and February in the 2015–2016 season) [37] may have suppressed the development of flowering laterals, thereby diminishing the efficacy of GA₃ in advancing reproductive phenology. The results also indicate cultivar-dependent responses, with ‘Navaho’ and ‘Ouachita’ improving earliness to a greater extent than ‘Natchez’. Estimated chilling requirements of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ are 300, 800 to 900, and 400 to 500 h [8–10,32]. It is likely that the cultivar-dependent responses to exogenous GA₃ are associated with the chilling requirements.

4.3. Exogenous GA₃ Improves Fruit Number and Total-Season Yield

To our knowledge, only one study has examined the effect of exogenous GA₃ on blackberry yield. Galindo-Reyes et al. [29] reported that the spray application of GA₃ at 100 mg·L⁻¹ and thidiazuron at 250 mg·L⁻¹ increased fruit yield by 421% 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 49 g·ha⁻¹ (53 mg·L⁻¹) increased the total-season yield per plant by up to 45% for ‘Natchez’, 135% for ‘Navaho’, and 112% for ‘Ouachita’, suggesting that GA₃ alone can increase blackberry yield. Furthermore, the yield increases by GA₃ were likely due to the increased induction of budbreak, as indicated by high correlations that were detected between fruit number and total-season yield (R² = 0.88–0.98) (Figure 2).

In this study, yield was recorded on a per-plant basis. When the yield per plant was converted to the yield per hectare using the commercial standard planting density (2778–4157 plants/ha) [4], the highest total-season yield in this study was 916.2 g/plant (Table 3), which was equal to 2.55–3.81 t·ha⁻¹. The average commercial blackberry yield in Oregon (a major blackberry-producing state located in the Pacific Northwest) from 2007 to 2017 was 10.3 t·ha⁻¹, according to the USDA’s National Agricultural Statistics Service [51]. Based on these values, our highest yield accounts for 24.7% to 37.0% of the average commercial yield. Although blackberry yields in central Florida are limited primarily by inadequate winter chill, the relatively low yields in this study may be partly due to the experiment setup. First, the plant spacing used in this study (0.61 m) was narrower than the commercial standard plant spacing in the United States (0.8–1.2 m) [4], limiting the light interception and canopy growth. Second, because plants were grown in wooden planter boxes in this study, restricted root growth may have limited plant nutrient uptake and, thus, overall plant growth.

4.4. Negative Side Effects of Exogenous GA₃ on Fruit Quality

In raspberry, berry weight is negatively correlated with fruit number [52], and this correlation can be explained by source–sink relationships. In the 2015–2016 season, exogenous GA₃ increased the fruit number by 48%, but reduced the average berry weight by 14% (Table 4). However, correlation analysis revealed no significant association between the two variables (Figure 1). Furthermore, although the 2016–2017 season results showed more pronounced positive effects of exogenous GA₃ on fruit number and total-season yield than the 2015–2016 season, they showed no significant impact on the average berry weight (Table 4). These results suggest that the impact exogenous GA₃ on the average berry weight cannot be explained by source–sink relationships.

Fruit SSC is one of the important fruit quality components in blackberry [53]. In this study, exogenous GA₃ reduced SSC by 5% to 9% (0.6 to 1.0°Brix) over the two seasons (Table 4). Fruit SSC was negatively correlated with fruit number in ‘Ouachita’, but it had no significant correlation with the average berry weight (Figure S3), which suggests that reductions in fruit SSC by exogenous GA₃ can be explained by source–sink relationships rather than “dilution effect”. In addition, fruit SSC generally increases with fruit maturity in blackberry [54,55]. In this study, however, it is unlikely that fruit SSC
was affected by fruit maturity, as only fully ripe berries were harvested. Therefore, the exact cause for fruit SSC reductions by exogenous GA3 is unknown. Nonetheless, the spray application of GA3 before budbreak appears to have only small impacts on SSC in blackberry.

4.5. Practical Implications

A single foliar application of GA3 at 49 g·ha⁻¹ appears to be highly effective in advancing reproductive phenology and increasing early-season and total-season yield of floricane-fruitching blackberry cultivars under inadequate chilling conditions, especially for high-chill cultivars. One of the advantages of this strategy is its relatively low cost. For example, one application of the GA3 product at 49 g·ha⁻¹ is about $50 per hectare, based on the price at a local major supplier of agricultural chemicals. However, precautions should be taken when implementing the use of GA3. First, the anticipated weather after budbreak should be considered when determining the application timing. GA3 may need to be applied when the risk of freeze damage is minimal in order to avoid freeze damage on developing flowers. However, delaying GA3 application could lessen the efficacy of GA3 in improving fruit earliness. Second, adequate primocane growth should be promoted by optimum cane management practices prior to GA3 application. The beneficial effects of budbreak induction by GA3 can be maximized when there are more buds per plant. Finally, the optimum application rate of GA3 should be determined for each cultivar. It is known that GA3 can have phototoxicity effects in many fruit crops (e.g., flower thinning) [49], although they were not observed in this study.

Hot and humid summers characterize subtropical climates. In Florida, the average rainy season runs from late May through October, and it overlaps with the blackberry harvest window, which occurs between early May and late June. Excessive heat and rain can adversely affect fruit quality by increasing disease damage or inducing physiological disorders, such as white drupelets, sunscald, leaky fruit, and red drupelet reversion [16–18]. Therefore, improved fruit earliness by exogenous GA3 may improve not only early-season yield, but also fruit quality and marketability by avoiding unfavorable weather conditions.

Winter chill is one of the important criteria of a location’s suitability for blackberry production. Inadequate winter chill is the major limiting factor for blackberry production in subtropical climates. The results in this study suggest that the expansion of blackberry production into subtropical regions may be feasible with artificial budbreak induction by exogenous GA3. In addition, with ongoing and projected climate change, exogenous GA3 may become an important management practice in temperate climates. It is projected that global warming could lead to an increase in the global mean temperature of between 1.6 and 6.9 °C by the end of the 21st century [56]. In fruit growing regions of central California, Baldocchi and Wong [57] reported that observed trends in winter chill reductions since the 1950s range between 50 to 260 h per decade. They also projected that the reduction rate in winter chill between 1950 and 2100 is about 40 h per decade. Luedeling et al. [31] modeled winter chill in California using 18 different future scenarios and projected that climatic changes could lead to 50% to 75% reductions in the area of safe winter chill for many temperate tree species or cultivars by the mid-21st century. Therefore, GA3 could be an important adaptation tool for temperate blackberry production in order to cope with global warming.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1317/s1, Table S1: Chilling hours and chill portions at the experiment site in central Florida during the 2015–2016 and 2016–2017 season. Figure S1: Growing degree days (GDD) recorded from March to June at the experiment site in central Florida during the 2015–2016 and 2016–2017 season. Figure S2: ‘Ouachita’ blackberry grown under subtropical climatic conditions in the 2016–2017 season: the untreated plants (left) and plants treated with gibberellic acid (GA3; right). Figure S3: Linear correlation between average berry weight or fruit number and fruit soluble solids concentration (SSC) of ‘Natchez’, ‘Navaho’, and ‘Ouachita’ blackberry grown under subtropical climatic conditions in the 2015–2016 season (A,B) and the 2016–2017 seasons (C,D).

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