Southern Region Small Fruit Consortium

Final Report Research

Title: Evaluation of Gibberellic Acid and Prohexadione Calcium for Cane Management in Novel and Standard Height Blackberries

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Public Abstract

Blackberries have become an increasingly important specialty crop in recent years. They provide consumers with a healthy fruit containing many antioxidants. In the US, blackberry production costs are rising due to the labor-intensive nature of managing and harvesting fresh market fruit. New growing systems are of interest to try to mitigate production costs. This research examined the impact of two plant growth regulators (PGRs), prohexadione calcium (P-Ca) and gibberellic acid (GA_3), on vegetative growth of three blackberry cultivars with standard height, semi-dwarf, and a dwarf plant architecture ('Natchez', 'Sweet-Ark™ Ponca', and 'Baby Cakes®',

respectively). The study was conducted at two locations in Arkansas and North Carolina. Results at both locations indicate P-Ca delays plant height development across all cultivars, though the effects were more dramatic in the standard height cultivar. The effects of GA_3 on plant height were much smaller, and the most notable impact was increased lateral development at the North Carolina site on standard height plants.

Introduction

Blackberry (*Rubus* subgenus *Rubus*) germplasm with 'novel' plant architecture is being developed for landscape/garden and commercial purposes in the University of Arkansas (UA) System Division of Agriculture fruit breeding program (Worthington and Clark, 2020). In this context, novel plant architecture refers to reduced stature or brachytic dwarfism. Plants impacted by brachytic dwarfism have shortened internodes without impact on the size of other organs. The first novel UA cultivar, ''Baby Cakes®', is marketed toward the home garden market in the Bushel and Berry® collection of Star® Roses and Plants (Clark and Boches, 2016).

Blackberries with shortened internodes may also have value in commercial production systems. The new cultivar 'Ponca' has reduced internode length compared to standard cultivars like 'Natchez' and 'Ouachita' with no apparent impact on yield. The shortened internodes on 'Ponca' are believed to reduce labor needed for primocane tipping (Clark, 2020).

Plants with reduced stature and columnar architecture may also be of interest for their potential uses in substrate, long-cane, and other high-density production systems. A maximum height of 1.5 m and uniform canopy are desirable characteristics for ease of management and uniform harvest time in substrate systems (Kempler, 2004). The ideal plant architecture for maximizing yield and minimizing labor costs in field and substrate-based production systems has yet to be determined.

Plant growth regulators in blackberries

Plant growth regulators (PGRs) are used to manage vegetative growth in many fruit crops, particularly apples. Gibberellins are a group of endogenous hormones that contribute to the regulation of terminal shoot growth *(*Camara et al., 2018; Hedden and Thomas, 2012*).* P-Ca reduces terminal growth by inhibiting the synthesis of gibberellins (Rademacher, 2000). Meanwhile, gibberellic acid (GA_3), is also a common PGR with many registered uses including increasing plant height, fruit size, firmness, and yield and altering maturity and reproduction in varied horticultural crops (https://www.valentbiosciences.com/cropenhancement/products/progibb/). Malik and Archbold (1992) observed reduced primocane cane height (up to 48%) with foliar GA_3 treatments but increased lateral branch elongation. Effects on node number, and internode length were not reported.

In blackberry, prohexadione calcium (P-Ca) is being tested as an alternative strategy to manage primocane growth in place of or in addition to tipping. Tipping has been shown to increase lateral branch development and increase yield (Strik et al., 2012). However, the labor costs of tipping are high (~$1,480 USD ha^{-1}), and the tipping wounds increase the risk of cane blight

(*Leptosphaeria coniothyrium*) infection (Brannen and Krewer, 2012). In preliminary trials conducted with plots of ‘Osage’ and ‘Von’ grown in North Carolina during 2018, three applications of 200 ppm P-Ca reduced the primocane height and internode length by 25% and 38%, respectively. P-Ca treatment did not impact yield during the year of application in 2018 (Kon et al., 2020a), but cumulative yields decreased by 22% during the 2019 season (Kon et al., 2020b).

<u>Gibberellins and dwarfism</u>

Mutations in genes related to gibberellic acid (GA) synthesis and metabolism cause dwarfism in many crops, including wheat and rice (Hedden, 2003). It is unknown whether the shortened internodes in novel UA blackberries are caused by impaired GA synthesis, GA insensitivity (i.e. impaired GA metabolism), or some other mechanism. The cause of the shortened internodes in novel blackberries may impact how these cultivars respond to PGRs. If novel blackberries have impaired GA synthesis, then hypothetically internode length would increase in response to exogenous GA_3 application, but would be reduced in response to P-Ca applications (which inhibit GA synthesis). In contrast, if novel blackberries exhibit dwarfism due to GA insensitivity, we might expect that neither P-Ca nor GA_3 applications would impact internode length.

Objectives: (1) Determine effects and interactions of University of Arkansas System Division of Agriculture (UA) blackberry cultivars with varying plant architecture and gibberellic acid (GA_3) rates on vegetative growth responses, and (2) determine effects and interactions of UA blackberry cultivars with varying plant architecture and prohexadione calcium (P-Ca) rates on vegetative growth responses.

Methods

Three cultivars were used in this experiment: ‘Natchez’, a standard plant, ‘Sweet-Ark™ Ponca’, a semi-dwarf plant, and ‘Baby Cakes®’, a dwarf plant. The GA_3 and P-Ca experiments were conducted separately in parallel on two separate trellises for application purposes. Each experiment was organized as a completely randomized design with six replicates per treatment combination. The study was conducted in two locations at the Mountain Horticultural Crops Research and Extension Center in Mills River, NC, and the UA Fruit Research Station in Clarksville AR during the summer of 2021.

Blackberry plants were provided as plugs by Agristarts (Apopka, FL) and Star® Roses (West Grove, PA). Plugs were transplanted to 7.5 L nursery pots filled with Pro-Mix BX General Purpose soil (Premier Tech Horticulture, Quakertown, PA) with all-purpose Osmocote Classic (The Scotts Company, Marysville, OH) added to the soil mixture. Plants were kept in a greenhouse until the frost-free date for each site. Plants were then moved to a trellised and drip-irrigated area for the duration of the experiment.

Chemical treatments began when ‘Natchez’ plants averaged 30 cm in height. GA_3 (GA_3 Solution (13 mg/ml); Phytotech Labs, Lenexa, KS) treatments were applied weekly for five weeks at the North Carolina location and six weeks at the Arkansas location. At both locations, three rates were used for GA_3: 0 (control), 250, and 500 ppm. A soil drench method was used for GA_3 applications. Each treatment including the control also had Silwet™ L-77 (Momentive Performance Materials, Waterford, NY) added as a surfactant at a rate of 0.125% (v/v). Three bi-weekly P-Ca (Kudos 27.5 WDG; Fine Americas, Inc., Walnut Creek, CA) applications were made at both locations. Three rates were also tested for P-Ca: 0 (control), 125, and 250 ppm. Each treatment for P-Ca included a non-ionic surfactant (Regulaid; KALO, Inc., Overland Park, KS) at a rate of 0.125% (v/v) plus a water conditioner (Choice Weather Master; Loveland Products, Inc., Greeley, CO) at a rate of 0.39% (v/v). A foliar spray application method was used for P-Ca with a CO_2 sprayer at 276 kPa (Bellspray, Inc., Opelousas, LA) until the canopy was thoroughly wetted.

Plant height was recorded multiple times throughout the experiment at each site. At the Arkansas location, plant heights were recorded weekly. At the North Carolina location, plant heights were recorded three times throughout the experiment. Upon termination of the experiment, many response variables were measured including: final plant height, number of nodes, number of laterals, length of laterals, cane diameter, and biomass weights grouped by tissue type. An area under height progress curve (AUHPC), change in plant height over the course of the experiment, average internode length, and total leaf dry weight were calculated. The sum of leaf area and root dry weight were only measured at the North Carolina location. In total, 18 variables were measured or calculated for analysis of this experiment (Table 1).

Table 1. A list of response variables examined and the description of how the measurements were taken or calculated.

Response variables	Description of measurement
Area Under Height Progress Curve (AUHPC)	$AUHPC = \sum_{i=1}^{n} \left[\frac{H_{i+1} + H_i}{2}\right] [X_{i+1} - X_i]$
Plant height change (Δheight)	The difference in initial height and final cane height.
Final diameter	The final diameter of the proximal end of the cane.
Number of nodes	The final count of nodes on the main cane.
Internode length	Final plant height divided by the number of nodes.
Number of laterals (initial)	The number of laterals present prior to chemical application.
Number of laterals (final)	The number of laterals present at experiment termination.
Sum of lateral lengths	The sum of the lengths of laterals taken on a per plant basis.
Average lateral length	The mean lateral length taken on a per plant basis.
Cane dry weight	The dry weight of the main cane tissue.
Cane leaf dry weight	The dry weight of the leaves taken from the main cane.
Lateral stem dry weight	The dry weight of lateral stems.
Lateral leaves dry weight	The dry weight of the leaves taken from the laterals.
Total leaf dry weight	The sum of dry weights of main cane leaves and lateral leaves.
Total stem dry weight	The sum of dry weights of the main cane and lateral stems.
Total dry weight	The sum of dry weights for all cane, stem, and leaves.
Root dry weight	The dry weight of the plant's root system.
Sum of leaf area	The sum of total leaf area.

Data for the GA_3 and P-Ca experiments were analyzed separately as completely randomized designs in Proc GLIMMIX in SAS v. 9.4 (Cary, NC) with PGR rate, cultivar, location, and their interactions treated as fixed effects. Many significant interaction effects with location were discovered in the initial analysis, so the North Carolina and Arkansas sites were subsequently analyzed separately. Mean separation was performed using Fisher's Protected Least Square Means at $\alpha = 0.05$. Graphics were generated in RStudio version 4.0.4 (R Core Team, Vienna, Austria) using the package ggplot2.

Results and Discussion

P-Ca

Genotypes were expected to differ for most response variables given that cultivars were specifically chosen for unique plant architecture characteristics. Genotypic differences were significant for every response variable measured except for total dry weight in the North Carolina location. Genotype was the only significant factor affecting final cane diameter, lateral leaf dry weight, and cane leaf dry weight in both locations. In Arkansas, genotype was also the only significant factor affecting total leaf dry weight, total stem dry weight, total dry weight, lateral stem dry weight, sum of lateral lengths, and cane dry weight.

The only variable that was impacted by P-Ca application rate with no significant interaction with genotype in both locations was internode length. Plants assigned to the control group had longer average internodes than the plants assigned to either 125 and 250 ppm rates in both North Carolina and Arkansas. Rate alone was also found to be significant in North Carolina for multiple variables including cane dry tissue weight, lateral dry tissue weight, total stem dry tissue weight, and total dry weight of all tissue types.

Genotype by rate interactions were particularly interesting as these effects indicate that blackberry cultivars with varying plant architecture responded differently to the chemical applications. Five variables had significant interactions between genotype and rate for the P-Ca study at one location: AUHPC, number of nodes, total leaf dry weight, root dry weight, and sum of leaf area (Table 2). Only one variable was found to be significant at both locations, and that was plant height change (Δheight).

AUHPC was utilized to account for total area under the height progress curve. This formula calculates the area of each trapezoid between time intervals and associated heights recorded for those days, then sums that area. This resulted in a standardized way of looking at height progress given that heights were recorded at different time intervals at each location. At both locations, the 'Natchez' control group had a higher AUHPC than all other genotype and P-Ca rate combinations (Table 2, Figure 2). There was no significant difference between the AUHPC in 'Natchez' at the 125 and 250 ppm rates in either location. AUHPC in 'Ponca' and 'Baby Cakes®' was not affected by P-Ca rate in Arkansas. In North Carolina, AUHPC of 'Ponca' and 'Baby Cakes®' plants assigned to the 125 and 250 ppm rates was reduced compared to the control groups.

Similar trends were observed for the change between the initial and final heights of plants. ‘Natchez’ plants not treated with P-Ca had a greatest difference between initial and final height than all other treatment combinations in both locations (Table 2). In Arkansas, there was no difference in change in plant height of ‘Natchez’ plants assigned to the 125 and 250 ppm P-Ca rates, but in North Carolina the ‘Natchez’ plants in the 125 ppm P-Ca group had intermediate change in height compared to the 0 and 250 ppm P-Ca groups. Th ‘Ponca’ plants in the control group had a larger difference between the initial and final height than the plants in the 125 and 250 ppm P-Ca rates in both locations and there was no significant difference between the 125 and 250 ppm rates. In North Carolina the ‘Baby Cakes®’ plants in the control group had a greater change in plant height than the plants in the 125 and 250 ppm groups. However, there was no significant difference in change in plant height of ‘Baby Cakes®’ plants in the 0, 125, and 250 ppm groups in Arkansas.

Leaf dry weight produced distinctly different results at each location. In Arkansas, ‘Ponca’ was found to have the highest leaf tissue dry weight and ‘Baby Cakes®’ the lowest. Dry leaf weight was not affected by P-Ca rate for either cultivar. Leaf dry weight was only impacted by P-Ca rate in ‘Natchez’, had greater total leaf dry weight in the control group than the 250 ppm group. In North Carolina, the ‘Baby Cakes®’ 250 ppm group was found to have a significantly higher leaf tissue dry weight than the 125 ppm group, though there was no significant difference between the 250 ppm and control groups. Leaf dry area in ‘Ponca’ and ‘Natchez’ were similar and no significant differences among P-Ca treatment groups were observed for either cultivar.

Two of the significant response variables were only measured at the North Carolina site: root dry weight and leaf area. Sum of leaf area followed a similar trend to leaf dry tissue weight at the North Carolina site. ‘Baby Cakes®’ at the 250-ppm rate of P-Ca showed significantly higher leaf area than most other treatment combinations, though there was no significant difference between ‘Baby Cakes®’ plants in the control and 250-ppm groups. There was no significant effect of P-Ca on leaf area on ‘Natchez’ and ‘Ponca’ plants. ‘Natchez’ plants in the control group had significantly lower root mass than the plants in the 125 and 250 ppm P-Ca groups. However, the effect of P-Ca rate on root growth was not significant in ‘Ponca’ or ‘Baby Cakes®’. The root weight of ‘Baby Cakes®’ plants was lower than all other treatment combinations except for the ‘Ponca’ control group.

Figure 2. To easily visualize the AUHPC calculation concept, the trapezoid segments can be seen in the following graph. The significant response of 'Natchez' to P-Ca applications is clearly illustrated.

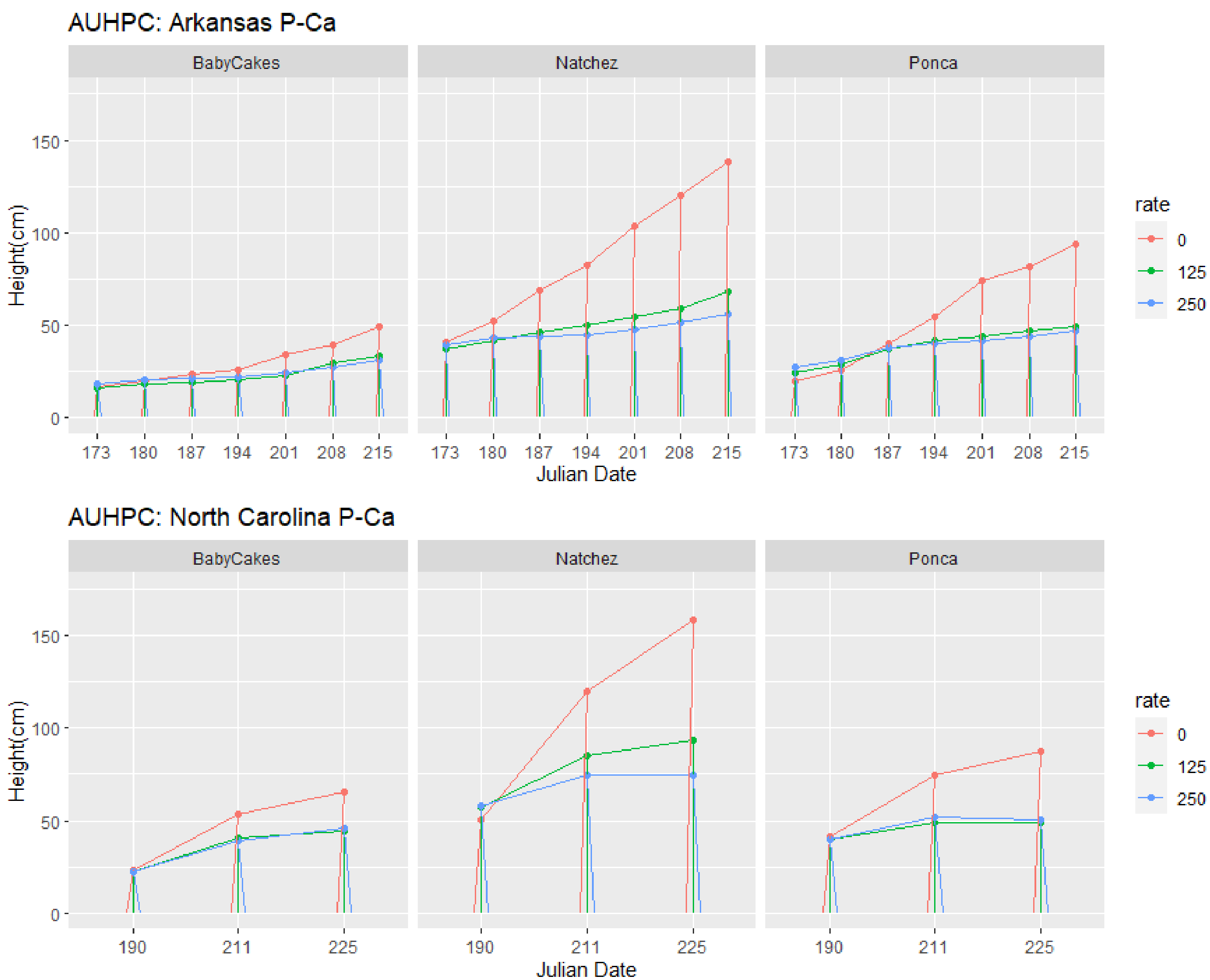


Table 2. Genotype by rate interactions of P-Ca for significant response variables for at least one of the experiment sites.

Genotype by Rate Interactions P-Ca															
			Variable												
Rate	Genotype	Location	AUHPC		Δheight		Number of nodes		Total leaf dry weight		Root dry weight		Sum of leaf area	
Control	Baby Cakes®	AR	1239.00	cd	34.75	c	20.5	e	7.93	cd	na		na	
125	Baby Cakes®	AR	953.17	d	18.00	c	20.3	e	6.21	d	na		na	
250	Baby Cakes®	AR	981.17	d	14.33	c	23.0	de	8.26	cd	na		na	
Control	Natchez	AR	3619.58	a	105.17	a	38.0	a	15.73	b	na		na	
125	Natchez	AR	2133.83	cb	33.33	c	30.0	bc	12.96	bc	na		na	
250	Natchez	AR	1948.33	cb	18.83	c	25.5	cde	9.55	cd	na		na	
Control	Ponca	AR	2333.92	b	79.00	b	33.0	ab	21.46	a	na		na	
125	Ponca	AR	1716.17	bcd	28.67	c	30.5	bc	25.69	a	na		na	
250	Ponca	AR	1626.33	bcd	23.67	c	28.7	bcd	23.36	a	na		na	
Control	Baby Cakes®	NC	1641.33	d	42.60	b	31.0	ab	22.10	ab	8.17	c	2588.52	ab
125	Baby Cakes®	NC	1257.55	e	21.73	cd	26.8	bc	18.25	b	6.98	c	2122.28	bcde
250	Baby Cakes®	NC	1246.00	e	23.42	cd	29.8	ab	24.80	a	6.78	c	2939.89	a
Control	Natchez	NC	3741.62	a	108.27	a	34.3	a	20.72	ab	12.13	bc	2320.68	bc
125	Natchez	NC	2746.51	b	35.90	bc	29.5	ab	21.62	ab	22.03	a	2278.31	bcd
250	Natchez	NC	2438.68	bc	16.72	d	22.8	cd	18.51	b	27.47	a	1923.65	cde
Control	Ponca	NC	2344.77	c	46.63	b	25.7	bc	18.35	b	9.83	bc	1968.17	cde
125	Ponca	NC	1613.38	d	8.82	d	17.8	d	18.09	b	15.23	b	1808.42	de
250	Ponca	NC	1677.55	d	10.07	d	17.8	d	18.40	b	14.07	b	1784.63	e

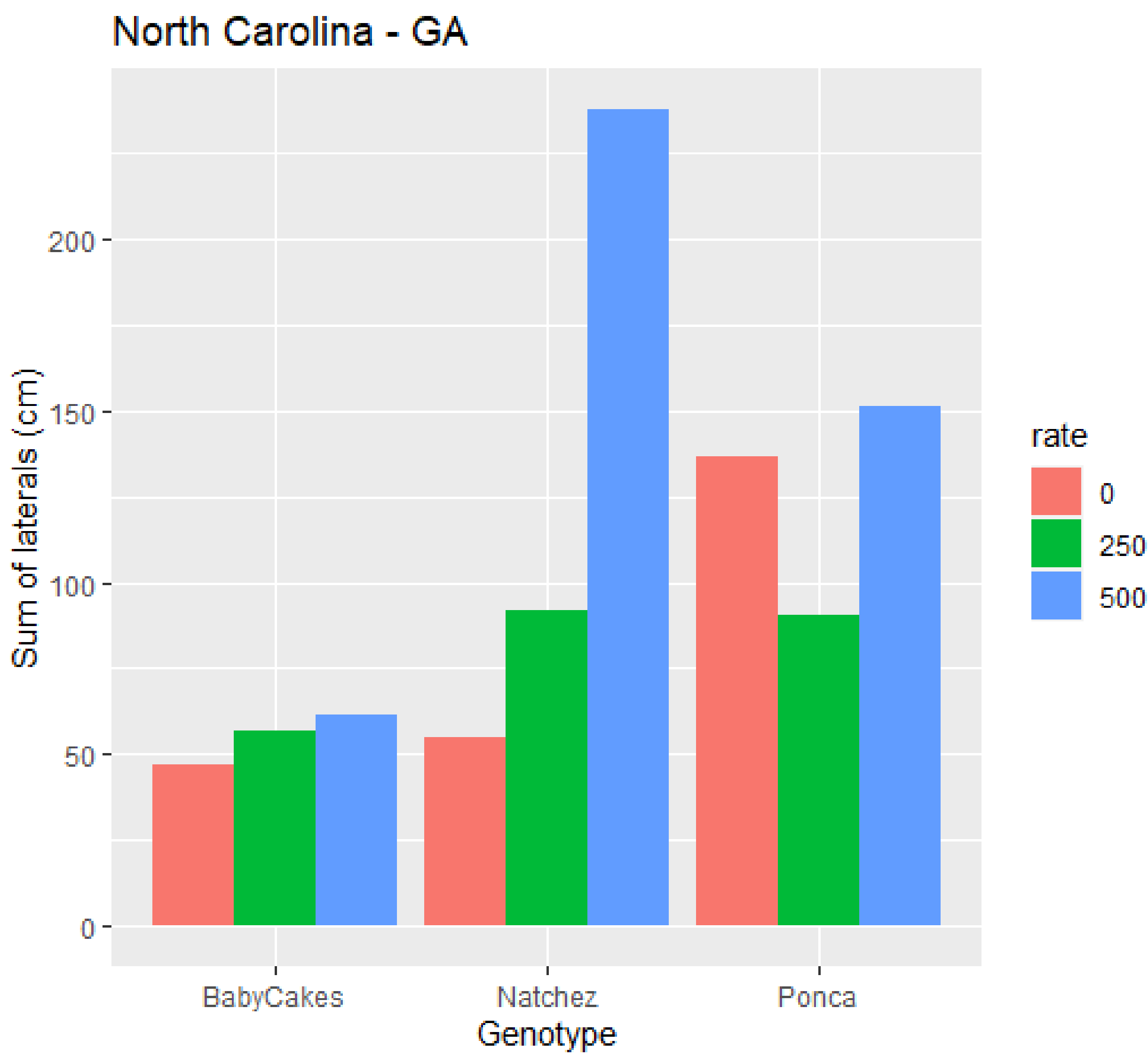
LS-means with the same letter in each column and for each location are not significantly different at $p < 0.05$.
†AUHPC, area under height progress curve; Δ height, change in plant height over the season; na, not measured at AR site.

GA_3

Applications of GA_3 were found to have few effects on vegetative growth in blackberries. Many response variables only distinguished differences between genotypes, with no significant effects of GA_3 rate. The only response variables that were significantly altered by GA_3 applications at both locations were sum of lateral lengths, lateral dry weight, and lateral leaf dry weight. In Arkansas, the lateral leaf dry weight of blackberries in the 250 and 500 ppm treatment groups was higher than the control group. In North Carolina, the 500 ppm treatment group had higher lateral dry weight than the 0 and 250 ppm treatment groups, which were statistically similar. Significant effects of rate at only one location were cane leaf dry weight, total stem dry weight, root dry weight, and sum of leaf area. In Arkansas, total stem dry weight and total dry weight were increased by GA_3 applications. In North Carolina, cane leaf dry weight and root dry weight was reduced in plants assigned to the 500 ppm treatment group compared to the control group, while sum of leaf area was significantly lower in the 250 ppm treatment group compared to the control and 500 ppm treatments.

The only response variable with a significant GA_3 rate interaction was the sum of lateral lengths at the North Carolina location (Fig. 2). Sum of lateral length was significantly higher for the 'Natchez' 500 ppm treatment group, indicating significant elongation of laterals in the standard height cultivar only. Differences in lateral length in 'Baby Cakes ®' plants with different GA_3 rates were not significant. In Arkansas, the lateral lengths of plants in the 250 and 500 ppm treatment groups had longer laterals than the control group and there was no significant interaction with genotype. Previous research has also showed that lateral induction increases with exogenous GA applications (Malik and Archbold, 1992). However, in contrast to the findings of Malik and Archbold (1992), there was no significant effect on overall plant height in this study (Figure 3). This discrepancy may be attributed to mobility of GA_3 and the drench application protocol utilized in the experiment.

Figure 2. A bar graph showing the mean sum of laterals for each genotype and respective rate in North Carolina.



Conclusions
This study concluded that P-Ca application had more obvious impacts on blackberry plant development than GA_3. The most informative results of this study were variables impacted by interactions between genotype and PGR application rates. Since GA_3 and P-Ca applications are not commonly used in blackberry production, this research provides a baseline understanding of the potential these chemicals could have in a cultural management system. Applications of GA_3 could fit into a production system for increasing lateral length given more research to solidify findings of this study. P-Ca applications are effective for reducing plant height progression and could play a role in delaying primocane height development as a cultural management technique for blackberries of varying plant architecture.

Future research to build on this experiment may include examining blackberry plant response to other types of bioactive GAs, as this could increase the observable effects or influence vegetative growth in different ways. In addition, investigation of the different impact that foliar versus drench applications of GA_3 have on blackberry is worth investigating to determine mobility patterns.

Impact statement

This study explores the baseline understanding of how dwarf and semi-dwarf plants respond to two plant growth regulators, GA_3 and P-Ca. Overall, the main effect of GA_3 was longer laterals, and 'Natchez' responded most significantly to treatments. P-Ca was found to stunt plant height progression and reduce vegetative growth notably on all cultivars, although less drastically on 'Ponca' and 'Baby Cakes®'. Results will be presented in full at the Southern Region American Society for Horticultural Science meeting in 2022.

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