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# Genetic Control of Prickles and Plant Height in Blackberry

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# Genetic Control of Prickles and Plant Height in Blackberry

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by

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This thesis is approved for recommendation to the Graduate Council.

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# ABSTRACT

The fresh-market blackberry (*Rubus* L. subgenus *Rubus* Watson) industry has been expanding for the past two decades. Blackberry market growth can be attributed to many factors including expanded production regions, improved production systems, and release of superior cultivars. Blackberry breeding is a time- and labor-intensive process which would benefit from implementation of molecular markers for highly heritable, important traits. Next generation sequencing, a high-quality reference genome, and software capable of analyzing this complex genome were applied in a genome-wide association study (GWAS) to reveal maker-trait associations related to prickles and plant height variation in fresh-market blackberry. In 2020 and 2021, 374 and 267 blackberry breeding selections and released cultivars of blackberry were surveyed for prickles and plant height, respectively. A single locus was found for prickles on chromosome four at 30.48 to 34.31 Mb. Two QTL associated with plant height were discovered on chromosomes four and six and physical locations were at 25.95 to 26.53 Mb and 13.94 to 22.40 Mb, correspondingly. Possible candidate genes were determined using locus-specific linkage disequilibrium calculations 5 Mb around the peak marker and searches for genes in pathways related to the traits of interest. Five possible candidate genes were found for prickles: a squamosa promoter-binding protein-like domain 6 (*SPL6*), a MYB domain protein 16 (*MYB16*), an agamous-like MADS-box protein 30 (*AGL30*), a homeobox-leucine zipper protein IV (*HOX3*), and a trichome birefringence-like 27 protein (*TBL27*). Three possible candidate genes were found for plant height near the QTL on chromosome four which were a gibberellin 2-oxidase 6 (*GA2ox6*) and two DELLA protein repressor-of-GA-like 1 (*RGL1*). On chromosome six, two 2-oxoglutarate-dependent dioxygenase and Fe(II)-dependent oxygenase (*GA20ox1*) genes were found within the QTL region. A project on vegetative responses of three blackberry

cultivars with unique architecture attributes to exogenous gibberellic acid ($GA_3$) and prohexadione calcium (P-Ca) applications was conducted to complement the plant height portion of the GWAS. The plant growth regulators (PGRs) were applied to 'Natchez' (typical height, typical internodes), 'Sweet-Ark® Ponca' (typical height, shortened internodes), and Baby Cakes® (reduced-stature and shortened internodes). The experiment was conducted in parallel at two locations (Arkansas and North Carolina) in the summer of 2021. Results of this study were complex. Cultivar main effects were common, and supported the varying architectural attributes of cultivars used in the experiment. The most consistent effect of $GA_3$ observed in this study was an increase in the number and size of lateral branches. In Arkansas all cultivars responded similarly to $GA_3$ treatments with increased number, length, and biomass of laterals as well as increased total stem biomass and total biomass of all tissue types. In North Carolina the lateral biomass of all cultivars was increased by $GA_3$ treatments, but only 'Natchez' had increased lateral number and only 'Natchez' and 'Ponca' had longer laterals on $GA_3$ treated plants. P-Ca applications reduced internode length and stunted primocane height at both locations and on all cultivars. However, in instances where cultivar by P-Ca rate interactions were significant, the effect of P-Ca was strongest in 'Natchez' and weakest in 'Baby Cakes'. Exogenous $GA_3$ and P-Ca may have applications on managing plant height in dwarf and semi-dwarf blackberries, but these results suggest that their responses to PGRs will be subtler than in standard height cultivars. Results indicated that P-Ca is indeed effective for managing vigor of blackberry plants, although effects on yield are unclear and must be researched in-depth to determine viability. Exogenous $GA_3$ applications indicated that UA dwarf blackberry plants are likely somewhat GA-insensitive because applications did not fully recover height in the dwarf cultivar tested. The results of the PGR study also suggest that the positive effect of $GA_3$ on lateral growth and development may be useful in mitigating unintended effects of P-Ca that previous research has indicated could be limiting.

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# DEDICATION

To my parents, Roger and Margie Johns, who have been models for work ethic, resilience, and stability. To Mom, Aunt Barbara, and both of my grandmothers, the women who sparked my interest in plants and shaped the woman I am today. Finally, to my best friend, Melanie, who has been a vital origin of strength, wisdom, and unwavering support through many life experiences. This thesis is dedicated to you all.

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# LITERATURE REVIEW

## Blackberry: History and Botanical Overview

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is a member of the Rosaceae family. The Rosaceae family includes many important fruit crops and ornamental species including peaches (*Prunus persica* L. Batsch), strawberries (*Fragaria* x *annanassa* Duch.), roses (*Rosa* spp. L.), red raspberries (*Rubus idaeus* L.), black raspberries (*Rubus occidentalis* L.), apples (*Malus* spp. Mill.) and almonds (*Prunus dulcis* Mill.). Blackberry belongs to the genus *Rubus*, which is a diverse genus containing approximately 700 species. Other berries included in this genus are raspberries and dewberries. Cultivated blackberries lack a specific epithet due to hybridization among different species of the genus (Clark and Finn, 2011).

Blackberry plants exhibit variability in growth habit, chill requirement, fruit quality and size, as well as several other characteristics (Clark et al., 2007; Clark and Finn, 2011; Hummer, 2018). Blackberry plants consist of perennial crowns and roots and produce biennial canes. The first year's growth commonly produces only vegetative matter, and these canes are referred to as primocanes. When the dormancy requirement is fulfilled, canes are referred to as floricanes, which yield reproductive organs. Some cultivars bear fruit on first year growth, but this a relatively new trait that has been identified in blackberries (Clark and Finn, 2011). Floral bud development takes place between late fall and mid-winter, depending on the environment in which they are grown (Clark et al., 2007). Flowers are perfect and self-fertile and contain up to 250 pistils and multiple anthers. Blackberry fruit are aggregate, meaning they are composed of many juice and fiber-filled sacs which each contain a pyrene (Clark et al., 2007; Hummer, 2018).

Growth habit and architecture vary greatly among different species of blackberry. Blackberries can be found in three basic forms: trailing, semi-erect, and erect (Clark et al., 2007;

Strik and Stanton, 2017). Growth habit dictates which production system a cultivar is suitable for. Plants that have trailing growth habit are predominantly utilized in the processing market and are generally machine-harvested (Clark et al., 2007; Clark and Finn, 2011; Strik et al., 2007). Erect and semi-erect cultivars are well-matched for hand-harvesting, which is required to maintain the integrity of these delicate fruits intended for the fresh market.

Blackberry is native to every continent except Antarctica (Hummer and Janick, 2007). This plant and its fruit were used by ancient cultures, such as the Greeks and Romans (Hummer and Janick, 2007). The oldest food remnants of blackberries were found near Bend, OR, and were radiocarbon dated giving an approximate age of 8,000 BCE (Hummer and Janick, 2007). Blackberry plants were initially used for protective barriers around human settlements, then shifted towards a food interest (Clark et al., 2007; Hummer, 2018). Once the value of the plant was realized for consumption, ancient civilizations cultivated the plant for its medicinal properties and nutritional value. Medicinal applications mentioned in ancient texts include controlling shingles, healing mouth sores, callous treatment, hemorrhoids, as well as various heart and stomach ailments (Hummer, 2010). Recent research in the field of food science has indicated that the health benefits of blackberries are associated with anthocyanin and vitamin content (Glover and Martin, 2012; Hummer, 2010; Wang and Lin, 2000).

Anthocyanins exist as a variety of pigments found in flowers, leaves, and fruit of some plants. These pigments exist on a spectrum which ranges from red to violet, present in varying degrees of intensity (Glover and Martin, 2012). The role of these pigments depends on their chemical structure. The high antioxidant content in blackberries may benefit cardiovascular health, have antiproliferative effects on certain types of cancer, and reduce loss of neurological function with progressive Alzheimer's disease (Glover and Martin, 2012; Hummer, 2010; Wang and Lin, 2000).

### Economic Value and Potential

Blackberries are an economically relevant berry crop (California Strawberry Commission, 2022). There is increasing interest in this crop for the fresh market due to the trending interest in their value as an antioxidant-rich food, increased availability of shippable cultivars, and development of efficient production systems (Clark et al., 2007). The economic value of cultivated blackberry is difficult to quantify. There is little international data available for blackberry production, as it qualifies as a specialty crop in the worldwide market (Strik et al., 2007). The production area of cultivated blackberries worldwide was about 20,000 ha in 2005. This approximation did not account for wild plants that were harvested, which was estimated to be about 8,000 ha (Strik et al., 2007).

Much like international data available, the Agricultural Marketing Resource Center (AgMRC) and United States Department of Agriculture-Agricultural Marketing Service (USDA-AMS) also have little data available on the production of blackberries in the United States. The AgMRC reported only Oregon production data and estimated that the production for 2017 was valued at $31.1 million. Of the total Oregon production value, $25.7 million was generated from production of processing cultivars, and the other $5.4 million was generated from fresh market production (AgMRC, 2021). A large portion of U.S. blackberry production takes place in California. Three counties in California contribute the majority of U.S.-grown fresh-market blackberries, and production value for these counties was approximately $125.6 million in 2020 (Monterey County, 2020; Santa Barbara County, 2020; Santa Cruz County, 2020).

Countries that produce a notable quantity of blackberries for either processing or the fresh market included Serbia, Hungary, Mexico. Chile, U.S., and China (Strik et al., 2007; AgMRC, 2021). Off-season imports are supplied largely by Chile and Mexico. Chile leads in frozen imports, and the frozen blackberry market was valued at $24.3 million in total imports in 2021 (AgMRC, 2021). Two Mexican states, Michoacán and Jalisco, contribute most of the off-season fresh blackberry imports to the U.S. Total fresh market imports to the U.S. in 2021 were valued at $318 million (AgMRC, 2021). According to a 2020 report generated by Freshlook/IRI (IRi Worldwide, Chicago, IL), blackberry sales contributed 9.2% of retail dollars among the top five berry categories (California Strawberry Commission, 2022). Other berry categories accounted for in this data included strawberry, blueberry, raspberry, and miscellaneous berries (California Strawberry Commission, 2022).

The movement of certain foods being identified as "superfoods" and the market value associated with these foods may account for the increasing demand and production of blackberries. Storage and processing influence the shelf-life degradation of these antioxidants, and studies have been done to examine quantity and quality in juice extracts from different cultivars (Wang and Lin, 2000). 'Chester Thornless', 'Hull Thornless', and 'Triple Crown' were analyzed for antioxidant content at three stages of fruit development. This research also compared antioxidant content of various cultivars of red raspberries, black raspberries, and strawberries (Wang and Lin, 2000). Blackberry cultivars differ in their total anthocyanin content as well as the composition of individual anthocyanins (Wang and Lin, 2000). Overall, the study concluded that blackberries and black raspberries contained higher overall levels of anthocyanins and total phenolics than strawberries and red raspberries.

## Blackberry Breeding Programs

The University of Arkansas System Division of Agriculture (UA) fruit breeding program was started in 1964 by Dr. Jim Moore and has been directed by Dr. John Clark since 1997. The UA blackberry breeding program is unique in that the primary goal is to develop blackberry cultivars for the fresh market, whereas the other major public breeding program at USDA Horticultural Crops Research Unit in Corvallis, OR, primarily targets cultivar-release for the processing market in the. The UA breeding program is credited with releasing several prickle-free, erect, floricane-fruiting cultivars including ‘Navaho’, ‘Osage’, ‘Ouachita’, ‘Natchez’, and ‘Sweet-Ark™ Caddo’ (Clark, 2013; Clark and Moore, 2005, 2008; Moore and Clark, 1989; Clark et al., 2019). Other public fresh-market blackberry breeding programs also exist at Cornell, University of Florida, and North Carolina State University. There are also private companies competing to release fresh-market blackberry cultivars. Overall, fresh-market blackberry breeding has contributed improvements in erect growth habit, postharvest potential, prickle-free canes, reduced stature, season extension, berry size, fruit flavor, and primocane-fruiting habit.

## Prickles in Blackberry

Prickle-free canes are an important trait that has successfully been introduced into fresh-market blackberries. Multiple heritable sources of prickle-free canes exist in blackberry. The source of prickle-free canes used in the UA breeding program is ‘Merton Thornless’-derived and recessively inherited. In tetraploid blackberry seedlings, small trichomes that are visible along the margins of cotyledons act as a phenotypic marker for the presence of prickles (Castro et al., 2013; Coyner, 2005; Scott et al., 1957). The presence of trichomes on cotyledon margins indicates presence of prickles, and breeding programs can discard prickly seedlings at this stage based on this phenotypic marker. The glands are also sometimes nearly indistinguishable, so a genetic marker could improve reliability of seedling screening, eliminate the need to examine each seedling individually under magnification, and provide genetic information for breeders to use in making crossing plans based on known allele dosages for genotyped material. Implementation of a genetic marker for prickles would be most valuable paired with screening for other important traits (e.g. primocane fruiting, dwarf growth habit, fruit firmness, and flavor) simultaneously.

## Novel Plant Architecture in Blackberry

The UA breeding program is currently exploring applications of 'novel' plant stature. Novel in this context refers to brachytic dwarfs, which display reduced internode length with typical leaf and fruit size, and this type of dwarfism has been observed in peach, another member of the Rosaceae family (Hollender et al., 2016). Overall, there have been five novel cultivars released either entirely derived from UA germplasm or under breeding agreements with private collaborators: 'Sharon's Delight', 'Black Cascade™', 'Purple Opal™', 'Baby Cakes®', and 'Superlicious™'. 'Baby Cakes' was released under a license agreement with Star® Roses in 2016 and was the first dwarf cultivar patented (Clark and Boches, 2016). All currently released novel cultivars are marketed towards home garden plantings, although novel plants with improved storage potential likely have a place in commercial production. Plants with reduced stature and variation in columnar architecture are also of interest because of their potential applications in substrate, long cane, or controlled environment production.

## Gibberellic Acid: Dwarfism and PGRs

Gibberellic acid influences several different metabolic processes in plants, including cell elongation, seed dormancy, floral production, sex expression, and embryo development (Camara et al., 2018; Gupta and Chakrabarty, 2013; Hedden and Thomas, 2012). Variation in plant height has frequently been associated with the GA signaling and/or biosynthesis pathway. The metabolic pathway of gibberellic acid (GA) biosynthesis is associated with dwarfism in many plant genera. Mutations in GA synthesis-related genes may be associated with brachytic dwarfism in tetraploid blackberry germplasm.

Plant growth regulators (PGRs) are commonly used to alter physiological processes in plants. Many PGRs target the GA biosynthesis pathway by altering levels of bioactive GA in plants (Rademacher, 2000). Prohexadione calcium (P-Ca) is an example of a PGR that reduces levels of active GA by targeting the GA biosynthesis pathway (Rademacher, 2000). Chemicals such as P-Ca have an important role in managing vigor in horticultural and agronomic crops as a means to minimize labor inputs and increase product output (de Oliveira et al., 2021; Kashirskaya et al., 2021; Rademacher, 2000). Alternatively, PGRs that increase active GA levels and growth could be any form of bioactive $GA_{20}$-derivatives ($GA_3$, $GA_4$, or $GA_7$) in plants. Applications of various GAs are commonly used horticultural crop and plant production. In wine and table grape (*Vitis labrusca* L., *Vitis vinifera* L., and *Vitis* hybrids) production, GA applications have an important role due to physiological responses including size increase of seedless table grape and rachis elongation which helps control for fungal diseases (Dimovska et al., 2014; Pahi et al., 2020). In cut flower production, the effect of GAs on stem elongation, floral bud development, floral size, and other attributes have made exogenous applications common in production (Coelho et al., 2018; Cornea-Cipcigan et al., 2020; Edrisi et al., 2017; Shakarami et al., 2013). In blackberry, PGRs are not used in commercial production. Currently, P-Ca is being explored for potential management of cane vigor, and $GA_3$ applications have shown promise in promoting budbreak in low-chill regions (Kon et al., 2020; Lin and Agehara, 2020).

## Molecular Breeding Overview

Molecular breeding refers to the application of knowledge of molecular biology to a breeding program with the goal of increasing genetic gain per breeding cycle. Genotypic and phenotypic data is paired to explore functional regions of the genome. This information can then be used to design molecular markers to assist in the selection process (Peace, 2017). Marker-assisted seedling screening has the potential to reduce labor, space requirements, resource consumption (water, fertilizer, etc.), and increase the speed at which improved cultivars are released when paired competently with classical breeding. Screening seedlings for plant height and the absence of prickles is valuable, particularly when paired with screening for other traits such as primocane-fruiting, erectness, fruit quality/yield, and postharvest characteristics. It is a general objective in many blackberry breeding programs to develop markers for highly heritable traits.

RosBREED is a collaborative research effort funded by the USDA-SCRI (Specialty Crop Research Initiative), which emphasizes DNA testing and breeding methods based on genotypic data specific to Rosaceous crops. RosBREED focuses on seven fruit crops, all of which belong to the Rosaceae family: apple, blackberry, peach, pear (*Pyrus* spp. L.), strawberry, sweet cherry (*Prunus avium* L.) and tart cherry (*Prunus cerasus* L.), and the ornamental rose plant (*Rosa spp.*). This program has expanded the possibilities for molecular breeding in fruit crops, and targets to inform breeders about how to successfully integrate DNA-informed breeding tools into breeding programs.

## Molecular Research in *Rubus*

Fresh-market erect and semi-erect blackberry breeding germplasm is composed of autotetraploids, which exhibit multisomic inheritance patterns (2n=4x=28) (Clark et al., 2007;

Finn and Clark, 2017). Autopolyploidy impacts segregation ratios, breeding techniques, and fertility among different ploidy levels (Meng and Finn, 2002). Autotetraploids can segregate into five different genotypic dosage classes for a single polymorphism with two alleles: nulliplex (aaaa), simplex (Aaaa), duplex (AAaa), triplex (AAAa), and quadriplex (AAAA). Autopolyploidy has made the process of combining multiple recessive traits (e.g. prickle-free canes and primocane fruiting) a long process, slowed the momentum of understanding the genetics of blackberry, and created challenges in development of molecular tools for this crop.

Few molecular tools exist for tetraploid blackberry. Types of markers that have been useful in the past include simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD). These types of markers did not allow for high-throughput genotyping and often lacked sufficient marker density to create high-quality maps (Bourke et al., 2018; Graham et al., 2004; Sargent et al., 2007; Stafne et al., 2005). Next-generation sequencing (NGS) has become a more cost-effective endeavor in recent years and yields better coverage and depth of sequencing data. Single nucleotide polymorphisms (SNPs) are currently the most common type of polymorphisms used in mapping and quantitative trait loci (QTL) discovery and have the power to efficiently and accurately genotype large populations with high informational output (Bourke et al., 2018; Thomson, 2014). Expressed sequence tags based on SSRs (EST-SSR), RAD-seq (restriction site associated DNA sequencing), and GBS (genotyping by sequencing) have been used for linkage map construction in tetraploid blackberry, but can be computationally difficult to manage in analyses (Armour, 2021; Castro et al., 2013; Weber, 2014).

TetraploidMap software was used to develop the first linkage map of tetraploid blackberry, detecting the primocane-fruiting and prickle-free cane loci (Castro et al., 2013; Hackett et al., 2007). A biparental population derived from a cross of two UA cultivars, ‘Prime-

Jim®' and 'Arapaho', was used to develop the linkage map, which was constructed with 119 markers assigned to seven linkage groups (LG). The prickle-free locus mapped to LG 4, and the primocane-fruiting locus mapped to LG 7 (Castro et al., 2013). Exact locations of the genes controlling these two traits are still unknown, and no diagnostic markers have been developed for either trait due to lack of sufficient marker coverage (Castro et al., 2013). However, this study did yield flanking markers for the prickle-free locus in blackberry. The flanking markers are RH_MEa0005aH07 at the distance of 4 cM and RH_MEa0011dG03b-265 at a distance of 21 cM (Castro et al., 2013). A second linkage map of tetraploid blackberry was developed using RAD-seq (Weber, 2014). The RAD-seq linkage map was constructed from a mapping population derived from 'Chester Thornless' × 'Prime Jim®'. Weber (2014) reported twenty times the marker density of the previous SSR-based map (Castro et al., 2013) and had over 3,800 markers. Both studies positioned the prickle-free locus on LG4 (Castro et al., 2013; Weber, 2014) based physical locations determined by a red raspberry reference assembly. QTL have been mapped for fruit ripening/softening, bud break to fruit ripening duration, pubescence, *Phytophthora* root rot resistance, flavor volatiles, and dwarfism in raspberry (Graham et al., 2004, 2006, 2009; Paterson et al., 2013; Sargent et al., 2007; Simpson et al., 2017). As of 2019, 91 QTL had been identified in *Rubus*, though some traits had multiple QTL (Molina-Bravo et al., 2019).

Most genetic analysis software available to date has been developed for application to diploid species. Polyploids are generally highly heterozygous, and exist in multiple heterozygous states (Bourke et al., 2018; Castro et al., 2013). There are three possible heterozygous states at a single biallelic locus; simplex, duplex, and triplex. Allele frequency can affect quantitative variation in some traits, and this is referred to as allele dosage. There have been fewer advances in molecular applications for polyploid species due to complexity and obstacles of analysis. Until recently, the software for genetic analysis in polyploids was limited because programs designed for diploids failed to account for multiple heterozygous states and autopolyploid segregation ratios (Bourke et al., 2018; Hackett et al., 2017). Availability of efficient genotyping methods, mapping software, association analysis software, and quantitative trait loci analysis specifically optimized for polyploid species are improving, but gaps still exist in reliability of these tools and broad applications are a challenge (Bourke et al., 2018).

Genome-wide association studies (GWAS) are widely used to identify marker-trait associations in diverse populations. GWAS produces higher resolution genetic information than linkage mapping, particularly when using high-density SNPs. Recently, an RNA-seq analysis and GWAS were performed to identify candidate genes for the prickle-free trait in red raspberry (Khadgi and Weber, 2020, 2021). The prickle-free QTL was mapped to chromosome 4 at 33.5-35.1 Mb physical position based on an unpublished *R. idaeus* reference genome (Khadgi and Weber, 2021). The most promising candidates identified by Khadgi and Weber (2021) were a MYB16-like protein related to epidermal cell differentiation, agamous-like MADS-box protein AGL30 related to prickle development in *Solanum viarum*, and protein trichome birefringence-like 2 related to trichome development are proposed based on BLASTp results from *Rosa chinensis* (Khadgi and Weber, 2021).

A high-quality reference genome for blackberry has not been available for previous research. The chromosome-scale genome assembly of (*R. argutus* Link. and *R. ulmifolius* Schott), two diploid blackberries, were pertinent in facilitating reliable genetic studies of tetraploid blackberry and will rapidly allow for advances in understanding the blackberry genome (Worthington et al., 2020). The development of new software that is compatible with polyploid species has been pivotal in genetic mapping and marker associations of these important but largely under-researched crops. Availability of software that incorporates allele dosage information in polyploids and were crucial to this project. Recently, an R package called GWASpoly was developed which can account for kinship while simultaneously identifying marker-trait associations in autotetraploids (Rosyara et al., 2016). GWASpoly has been successfully applied in analyses of marker-trait associations for multiple traits in autotetraploid southern highbush blueberry (*Vaccinium* sp.) (Ferrão et al., 2018), and the pipeline used in this study followed blueberry GWAS methodology.

The goals of this research were to perform a GWAS and find tightly linked markers or intragenic markers associated with prickle-free canes and plant height in tetraploid blackberry. Previously identified significant regions associated with prickles and plant height in *Rubus* were targeted in sequencing. Identification of reliable markers for 'Merton Thornless'-derived prickle-free breeding selections and plant height in blackberry are valuable, applicable marker-assisted seedling screening in tetraploid blackberry. Both prickle-free canes and dwarfism are traits of interest which have functional applications in cultivar development. Eventually, the markers or genes proposed will be validated and applied in complement with markers for fruit quality attributes and primocane fruiting habit, which will result in faster releases of superior blackberry cultivars from fresh-market blackberry breeding programs.

Applications of two plant growth regulators which cause inverse physiological responses (GA and P-Ca) was conducted to complement the molecular aspect of the plant height research. Three cultivars of erect blackberry with unique architectural features were used to examine whether GA deficiency or insensitivity contribute to plant height variation in tetraploid blackberry.

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# CHAPTER I

# GENETIC CONTROL OF PRICKLES AND PLANT HEIGHT IN BLACKBERRY

## Abstract

Blackberry (*Rubus* L. subgenus *Rubus* Watson) has gained traction in the fresh-fruit market over the past twenty years. This market growth can be attributed to increased consumer awareness and demand, expanded production systems and regions, and availability of elite cultivars which yield, ship, and store well. Prickle-free plants have contributed to this expansion because prickles pose a food safety concern in fresh fruit, negatively affect fruit quality, and cause injury during harvest and plant management processes. Blackberries with reduced stature have gained interest in the ornamental home garden market, and may also be adaptable to controlled-environment, high-density systems. Breeding blackberries is a time- and labor-intensive process which would benefit from implementation of marker assisted selection. The objective of this study was to identify the genetic region responsible for prickle-free canes and quantitative trait loci (QTL) for plant height in autotetraploid blackberry using a genome-wide association study (GWAS). Three hundred and seventy-four blackberry cultivars and breeding selections were discretely surveyed for prickle presence, and 267 genotypes were evaluated for plant height variation. The prickle locus was located on chromosome four at 30.48 to 34.31 Mb. The peak marker for prickles, located at 33.64 Mb on Ra04, correctly predicted the phenotype of 97.3% of genotypes in the GWAS panel. A total of five potential candidate genes and transcription factors related to prickles were identified in this region including a squamosa promoter-binding protein-like domain 6 (*SPL6*), a MYB domain protein 16 (*MYB16*), an agamous-like MADS-box protein 30 (*AGL30*), a homeobox-leucine zipper protein IV (*HOX3*), and a trichome birefringence-like 27 protein (*TBL27*). Two significant QTL located on

chromosomes four and six at 25.95 to 26.53 Mb and 13.94 to 22.40 Mb, respectively, were associated with plant height. A total of five potential candidate genes for plant height were identified near peak markers for each QTL, including two GA20-oxidase genes (*GA20ox1*), one GA2-oxidase gene (*GA2ox6*), and two DELLA receptor-of-GA-like 1 (*RGL1*) genes.

## Introduction

Blackberry (*Rubus* L. subgenus *Rubus* Watson) is a specialty crop with an increasing share of the fresh berry market. The growth in demand and rising production costs have resulted in a need for cultivars that are adaptable to many environments and cultural management approaches. Since the mid-twentieth century, multiple traits such as prickle-free canes, overall plant health, erect cane habit, ripening time, reduced plant stature, primocane-fruiting habit, and fruit quality attributes have been enhanced through conventional blackberry breeding efforts. Conventional methods of blackberry breeding are costly and require a great deal of time. Development and implementation of molecular tools in blackberry breeding programs are needed to shorten breeding cycles and continue growing the industry for this high-demand specialty crop.

Erect and semi-erect fresh-market blackberries are autotetraploid, which complicates genetic research. Autopolyploids are difficult to study because of their high degree of heterozygosity, multisomic inheritance, and challenges with accurately calling allele dosage (Bourke et al., 2018). Resources for analyzing blackberry genetics have also been limited by the lack of a well-developed reference genome specific to the crop (Foster et al., 2019; Worthington et al., 2020). In the past five years, multiple open-source software programs which are capable of scrutinizing autopolyploid genetic data have been developed, along with a chromosome-scale reference genome of diploid blackberry (Brůna et al., 2022; Rosyara et al., 2016). Advances in sequencing technology have improved the accuracy and affordability of genotyping in polyploid crops. Next generation sequencing (NGS) platforms, such as Capture-Seq (RAPiD Genomics, Gainesville, FL), offer the ability to target specific regions for exome sequencing. The advantages of Capture-Seq over genotyping by sequencing (GBS) are capability to target known, functional regions of the genome with custom-designed probes for genes related to traits of interest and greater read depth that enables accurate allele dosage calling in polyploids.

Prickles are a burden in blackberry production systems because they are a food safety concern and can cause mechanical damage to fruit and shorten shelf life (Weber, 2014). A common goal of fresh-market blackberry breeding is to release cultivars which lack “thorns” or more correctly, prickles. Often these terms are used interchangeably in literature. Prickles are borne of cortical and epidermal cells, which differentiates them from thorns and spines botanically (Kellogg et al., 2011; Trinklein, 2013). Prickle-free blackberries can be attained from multiple sources. Non-heritable sources of the prickle-free trait are borne of a chimera in which the L1 layer of tissue, or epidermis, is responsible for the trait expression. In order to maintain trueness to type, cells must be propagated only from the mutated apical meristem, rendering this source of the prickle-free trait not useful in breeding. There is a dominant, heritable source of prickle-free blackberries found in ‘Austin Thornless’. This source is known for deleterious effects, strong association with trailing growth habit, and only being prickle-free over 30 cm in height (Clark et al., 2007). A recessive, heritable source of prickle-free blackberry canes, which was derived from a backcross of ‘John Innes’ and released as ‘Merton Thornless’, also exists (Scott et al., 1957). University of Arkansas System Division of Agriculture (UA) blackberry breeders have used this recessive source for prickle-free canes in breeding. The ‘Merton Thornless’ source of prickle-free canes has benefits over other sources in that the trait is stable and provides a reliable phenotypic marker at a young seedling stage (Clark et al., 2007), but the process of developing prickle-free cultivars took many years because introgression of recessive traits is a lengthy process, particularly for autopolyploids (Coyner et al., 2005). ‘Merton Thornless’ is also a trailing cultivar, which conflicted with the goal of erect plant architecture in the UA fruit breeding program, adding time to remove the trailing tendency in prickle-free progeny.

The genetic control of prickle-free blackberry canes has been explored in previous studies. Two previous linkage mapping studies revealed that the locus for prickles in tetraploid blackberry is on the distal portion of LG4, although the regions identified were quite large due to low marker density (Castro et al., 2013; Weber, 2014). Most recently, a GWAS on red raspberry (*R. idaeus* L.) placed the prickle locus at 33.5 to 35.1 Mb on chromosome 4 of (Khadgi and Weber, 2021). Promising candidate genes proposed in that region included a trichome birefringence-like 2, a MYB16-like transcription factor, and *AGL30* (a MADS-box protein) (Khadgi and Weber, 2021). All the possible candidate genes identified in the red raspberry prickle locus have a role in epidermal or trichome regulation, development, and/or differentiation (Khadgi and Weber, 2020, 2021).

A trichome is an outgrowth that forms from the epidermis of a plant. Trichomes can be categorized into two broad classifications: glandular and non-glandular (Huchelmann et al., 2017). The difference between these two groups is that glandular trichomes form a mass, or “head” that is rich in secondary metabolites (Huchelmann et al., 2017; Kellogg et al., 2011). Kellogg et al. (2010) examined prickle formation on canes of blackberry cultivars ‘Arapaho’ and ‘Prime-Jim®’, red raspberry cultivars ‘Heritage’ and ‘Canby’, and the rose hybrid cultivar ‘Radtko’ (*Rosa hybrida* L.). Kellogg et al. (2011) conducted morphological studies of developing prickles and found that blackberry seedlings can have glandular and non-glandular trichomes. Only glandular trichomes developed into prickles, and non-glandular trichomes were present on the developing canes and leaves of prickle-free plants. Only the plants that had both glandular and non-glandular trichomes present developed prickles. Lignification began at the top of the trichome, working down towards the attachment point on the epidermis (distal-proximal) (Kellogg et al., 2011). This led the researchers to conclude that the secondary metabolites present in the glandular trichome heads may signal prickle development.

A notable amount of variation for plant height exists in the UA blackberry germplasm. Novel blackberry plants were first discovered in the UA breeding program in 2002. Novel in this context refers to brachytic dwarf plants that exhibit shortened internodes without reduction of the size of other structures (e.g. leaves, flowers, and fruits). The term novel is used rather than dwarf because many of these plants often have other unique architectural qualities like compact, columnar growth. The first novel plant, 'APF-44', was selected in 2002 from a population of primocane-fruiting blackberry seedlings and had an average mature height of less than 1 m (Worthington and Clark, 2020). Since then, the novel breeding initiative in the UA breeding program has grown in importance, accounting for 10-20% of crosses made in recent years.

Novel blackberries have generated interest in the global home garden market. 'Sharon's Delight', 'Baby Cakes®', and 'Superlicious™' are examples of novel UA breeding selections that have been commercialized for home gardeners (Clark and Boches, 2016; Worthington and Clark, 2020). There are also two partially-UA-derived novel releases, 'Black Cascade™' and 'Purple Opal™' that are commercially available in the home-garden space and were developed collaboratively with Hargreaves Plants. Plant height and internode length are quantitatively inherited in blackberry, and many UA breeding selections have slightly reduced internode length and plant size relative to older cultivars. A recently released floricane-fruiting cultivar, 'Sweet-Ark® Ponca', is classified as 'semi-dwarf' because the plant exhibits columnar growth habit, reduced internode length, and delayed primocane development, but ultimately reaches a final height typical of standard architecture blackberry plants. Blackberry plants with unique architecture may be ideal for emerging blackberry production systems such as substrate culture, tabletop production, long cane production, and vertical farming. In order for novel blackberry plants to be recognized as a viable option for commercial fruit production, the UA breeding program has been working to improve yield, precocity, and fruit quality of these unique plants.

Dwarfism is an important trait in many crops. Dwarf cereal crops have shown improved yield and reduced lodging. Many genes responsible for dwarf phenotypes in horticultural and agronomic crops are related to gibberellic acid (GA) biosynthesis and metabolism (Hedden and Thomas, 2012; Peng and Harberd, 1997; Peng et al., 1997, 1999; Thomas and Sun, 2004). A few examples of dwarfing genes related to the GA transduction pathway include GA-insensitive (*GAI*) and GA-insensitive dwarf (*GID*) genes. The following have been described previously: *GID1* in peach (*Prunus persica* L.) (Hollender et al., 2016)*, GID1a, GID1b and GID1c* in *Arabidopsis thaliana* (Thomas and Sun, 2004)*, VvGAI1* in wine grapes (*Vitis vinifera* L.) (Boss and Thomas, 2002)*,* and *Rht-B1* in wheat (*Triticum aestivum* L.) (Peng et al., 1999). These genes all encode DELLA proteins in the GRAS family of transcriptional regulators. DELLA proteins are responsible for negatively regulating GA uptake (Eckardt, 2007; Peng et al., 1997; Thomas and Sun, 2004). Biologically active forms of GA degrade the DELLA protein, allowing cell elongation to resume. GA-oxidases are crucial enzymes which regulate levels of GAs in plants and are related to the biosynthesis pathway (Hedden and Phillips, 2000). Plants expressing GA biosynthesis-related mutations are often dwarf and may have other physiological abnormalities

(Dijkstra et al., 2008; Hu et al., 2017; Huang et al., 2010; Ford et al., 2018; Lo et al., 2008; Schomburg et al., 2003; Yan et al., 2017). It is currently unknown what causes brachytic dwarfism in the UA novel selections, however, mutations in GA signaling or synthesis-related pathways are probable targets commonly associated with dwarf phenotypes in many plant species.

The objective of this research was to perform a genome-wide association study to identify loci associated with plant height and prickles in erect and semi-erect blackberry germplasm. The results of this project will be used to develop markers for plant height and prickles to screen seedlings in fresh-market blackberry breeding programs. Marker assisted selection for prickle-free and reduced height plants will increase the pace of progress in blackberry breeding while reducing time and more efficiently utilizing labor.

## Materials and Methods

### *Plant Material and Phenotypic Data Collection*

This study was conducted at the UA Fruit Research Station (FRS) in Clarksville, AR (lat. 35°31’5”, long. 35°31’5”). This site is in USDA hardiness zone 7b, and the soil type is predominantly Linker fine sandy loam (Typic Hapludults). Genotypes evaluated in this study included tetraploid erect and semi-erect blackberry cultivars and UA breeding selections. All genotypes were grown in single, un-replicated ten-plant plots, which were not trellised and received overhead irrigation as needed. Plots received herbicide and insecticide applications as needed throughout the year as well as a minimal spray program for common blackberry pathogens including anthracnose [*Elsinoe veneta* (Burkh.) Jenkins] and cane and leaf rust [*Kuehneola uredines* (Link) Arthur]. Plots were tipped at 107 cm each June following the onset of the log vegetative growth phase of primocanes in order to manage height and induce lateral fruiting branches.

Plots were evaluated discretely for presence or absence of prickles on plants. Eight cultivars (Black Satin, Brazos, Chester Thornless, Darrow, Eldorado, Loch Ness, Merton Thornless, and Raven) used as parents in many fresh-market blackberry breeding programs that were not grown at FRS were also included in the GWAS analysis for prickles. Leaf tissue for these plants was provided by the USDA National Clonal Germplasm Repository and phenotypes were taken from Clark et al. (2007). Two diploid blackberry germplasm accessions [‘Hillquist’ (*R. argutus* Link.) and ‘Burbank Thornless’ (*R. ulmifolius inermis* Schott)] were grown in plots at FRS and included in the prickle GWAS. Prickle ratings for 374 genotypes were compiled for final analysis.

Plant height (cm) measurements were collected from five first-year canes (primocanes) chosen at random from each plot. Height data were collected prior to tipping on a single date each year; 23 June and 24 June for 2020 and 2021, respectively. Plots must be planted for approximately one year prior in order to produce representative primocanes, so the newest selections in the UA breeding program plus tissue accessions acquired from the USDA National Germplasm Repository in Corvallis, OR were not included in height evaluation. Height data for 267 individuals were used for final analysis.

*Heritability Calculations and BLUPs*

Phenotypic data for plant height were transformed into a single best linear unbiased predictor (BLUP) for each genotype using PROC MIXED (SAS Institute, Inc., Cary, NC). Harmonic means for years and replicates were calculated in the R package *dplyr*. Broad-sense heritability was calculated using the equation $\mathrm{H} = \sigma_G^2/(\sigma_G^2 + \sigma_{GY}^2/y + \sigma^2/yr)$, where $\sigma_G^2$

represents genotypic variation, $\sigma_{GY}^2$ represents genotype by year variation, $\sigma^2$ represents residual effects, and $r$ and $y$ represent the harmonic means for number of replicates and years, respectively.

*Genotyping and SNP Filtering*

DNA was extracted from young leaves of each genotype following a modified cetyltrimethylammonium bromide (CTAB) method (Porebski et al., 1997). The samples were quantitated with an Invitrogen Qubit 1 Fluorimeter dsDNA-BR assay kit (Thermo Fisher Scientific, Waltham, MA). Standardized DNA concentrations of 40 µg/mL for each genotype in the panel were sent to RAPiD Genomics (Gainesville, FL) for genotyping.

The Capture-Seq platform was used for genotyping. A total of 35,054 biotinylated 120-mer Capture-Seq probes were designed using the 'Hillquist' reference genome (Brůna et al., 2022). Probes were chosen to target known regions and pathways of interest associated with multiple traits (17,100 probes), including genes associated with prickles and the GA pathway, and to ensure good coverage of the genome and account for linkage disequilibrium (LD) (17,954 probes). Illumina HiSeq2000 was used to perform paired-end sequencing. MOSAIK (Lee et al., 2014) was used to align cleaned and trimmed sequencing data to the 'Hillquist' genome. Freebayes (Garrison and Marth, 2012) was used to perform variant calling. A dataset of biallelic SNP markers with an average read depth of at least 150 per sample and a minor allele frequency of > 0.01 was generated using VCFtools (Danecek et al., 2011). This dataset was then converted to probabilistic tetraploid allele dosage calls and filtered for quality in UpDog (Gerard et al., 2018). Markers estimated to have greater than 0.05 posterior proportion of individuals mis-genotyped were excluded from association analysis.

*Association Analysis*

GWASpoly (Rosyara et al., 2016) was used for association analysis. The binary prickle data and primocane height BLUPs were used for the phenotypic input. Numeric allele dosage scores for each genotype which corresponded to the SNPs generated from Capture-Seq and the subsequent filtering steps functioned as the genotypic input. Data were analyzed using a mixed model controlling for population structure with a random effect kinship matrix calculated using the leave-one-chromosome-out (LOCO) method (Listgarten et al., 2012; Yang et al., 2014) and a fixed Q matrix generated by STRUCTURE (Pritchard et al., 2000) with the number of informative groups determined by the delta K statistic (Evanno et al., 2005). All available gene models (additive, 1-dom, 2-dom, diplo-general, diplo-additive, and general) were tested. The LOD thresholds for significant SNP detection were determined with a Bonferroni correction at $\alpha = 0.05$. The fit.QTL function in GWASpoly was used to calculate p-values and percent variance explained by significant QTL.

*Possible Candidate Gene Identification*

Patterns of LD decay around significant loci were analyzed using the mldest function in the R package ldsep (Gerard, 2021). This function iteratively computes pairwise comparisons of SNPs to predict LD on specific chromosomes and in specific regions. A 2.5 Mb region on both sides of the peak marker in each significant region were used to calculate LD and select appropriate regions for exploring potential candidate genes. The ‘Hillquist’ V1 genome assembly (Brůna et al., 2022) contained 38,503 genes that were functionally annotated through interrogation of the Swiss-Prot, Araport11, NCBI nr, Refseq and TrEmbl protein databases with BLAST+ blastp-fast algorithm. Genes within the regions determined by LD calculations flanking peak SNPs with functional annotations related prickles or plant height were considered potential candidate genes.

## Results

### *Phenotypic Results*

A total of 374 genotypes in the GWAS panel were evaluated for presence or absence of prickles. Thirty-eight genotypes (10.2%) had prickles and the remaining 336 genotypes (89.8%) were prickle-free (Supplementary Table 1). Overall, 267 genotypes were evaluated for plant height (Supplementary Table 1), with 244 and 227 genotypes evaluated in June 2020 and 2021, respectively. Normalized genotypic BLUPs of plant heights for both years ranged from 41.2 – 184.5 cm, and the mean plant height in all cultivars and selections surveyed was 113.8 cm across both years (Fig. 1). The broad sense heritability of plant height was 0.81, suggesting this trait is highly heritable.

### *Association Analysis*

Phenotypic and genotypic data were analyzed in GWASpoly to detect significant SNPs non-randomly associated with prickles and plant height. A total of 81,064 SNPs were generated by CaptureSeq and passed quality filtering steps. The maximum genotype frequency was set to 0.95, and minor allele frequency (MAF) was adjusted appropriately by trait in GWASpoly resulting in unique final SNP datasets for prickle and plant height association analysis. The Q matrix used to control for population structure was composed of K=6 column vectors, as determined by the delta K statistic. All available gene action models in GWASpoly were tested initially. The most biologically relevant models for tetraploid blackberry were also those that uncovered the most informative SNPs, so results reported are based on the additive, simplex-dominant-alternate, and simplex-dominant-reference models. The Bonferroni correction at $\alpha =$

0.05 was used to determine a minimum likelihood of the odds (LOD) value for significant SNPs. Under the additive model the minimum significant LOD was 6.1, and under the simplex-dominant models the LOD significance thresholds were 5.3 and 6.1 for the alternate and reference models, respectively.

*Prickles.* The MAF threshold for the analysis of prickles was set to 0.02, resulting in 76,045 SNPs used in the association study. QQ-plots did not show evidence of systemic bias for the models evaluated in this study (Fig. 2a). A single locus associated with the prickle-free trait was detected containing 384 significant SNPs from 30484542 to 34309626 bp chromosome Ra04 (Fig. 3a). The peak SNP in the prickle-free locus was at 33636565 bp and had an LOD value of 75.28 under the simplex dominant gene model. The peak marker correctly predicted the phenotype (prickly vs prickle-free) of 364 of 374 genotypes (97.3%) in the panel.

*Plant height.* The association analysis for plant height included 76,804 SNPs with MAF greater than 0.05. No systemic bias for the tested models was evident in the QQ-plots (Fig. 2b). Two QTL for plant height were detected on chromosomes Ra04 and Ra06 (Fig. 3b). The QTL on chromosome Ra04 contained 39 significant SNPs located from 25959770 to 26529505 bp. The peak SNP in this QTL was located at 26408960 bp and had an LOD value 8.09 under the simplex dominant model. The QTL on Ra06 contained 35 significant markers located from 13940761 to 22402316 bp. The peak SNP on Ra06 was located at 16203569 bp and had an LOD value 8.19 under the additive gene model. The most highly significant SNPs in the QTL on chromosomes Ra04 and Ra06 explained 15% and 13% of observed phenotypic variance for plant height in the GWAS panel according to the fit.QTL function in GWASpoly.

*Possible Candidate Gene Identification*

*Prickles.* Linkage disequilibrium was calculated 2.5 Mb upstream and downstream of the peak marker for prickles on chromosome Ra04 and the peak markers for plant height in the QTL on chromosome Ra04 and Ra06 using the mldest function of the ldsep R package (Gerard, 2021). Linkage disequilibrium in the region of the prickle-free locus was extensive, with markers 2.2 Mb apart correlated at $r^2 = 0.1$. Therefore, a 2 Mb region on each side of the peak marker was considered appropriate to explore for possible candidate genes and transcription factors (Fig. 4a). Possible candidate genes and transcription factors were identified based on described relationships with epidermal, trichome, and/or prickle development. Five possible candidates with roles in prickle formation were identified within or near the prickle-free locus (Table 1). A squamosa promoter-binding protein-like domain 6 (*SPL6*), Ra_g19233, was located at 32260895 to 32263859 bp within the prickle-free locus and 1.3 Mb from the peak marker. A MYB domain protein 16 (*MYB16*), Ra_g19351, was located at 32802206 to 32803665 bp within the prickle-free locus and 832 kb from the peak marker. An agamous-like MADS-box protein 30 (*AGL30*), Ra_g19365, was located at 32877483 to 32880709 bp within the prickle-free locus and 755 kb from the peak marker. A homeobox-leucine zipper protein (*HOX3*), Ra_g19498, was located at 33599420 to 33601244 bp within the prickle-free locus and 35 kb from the peak marker. Lastly, a trichome birefringence-like 27 protein (*TBL27*), Ra_g19762, was found at 34904416 to 34905885 bp, outside of the prickle-free locus and 1.26 Mb from the peak marker associated with prickles.

*Plant Height.* Linkage disequilibrium in the region of the plant height QTL on chromosomes Ra04 and Ra06 decayed more rapidly than around the prickle-free locus (Fig. 2b-c). Therefore, possible candidate genes were explored in a 1 Mb region on each side of the peak

SNPs for each QTL. Five possible candidate genes with described relationships with the GA-pathway were identified within or near the Ra04 and Ra06 QTLs (Table 1). On Ra04, all three possible candidates were outside the significant QTL region (25959770 to 26529505 bp), but within 1 Mb of the peak marker at 26408960 bp. Two neighboring DELLA protein RGL1-like (*RGL1*) genes, Ra_g18239 and Ra_g18240, were located on Ra04 at 26962868 to 26963329 bp and 26963434 to 26963916 bp, respectively. These *RGL1* genes were 553 kb and 554 kb from the peak marker in the plant height QTL on chromosome Ra04. A gibberellin 2-dioxygenase 6 (*GA2ox6*), Ra_g18309, was located at 27361510 to 27364509 bp on Ra04, 952 kb from the peak marker in the QTL. The remaining two possible candidates were gibberellin 20-oxidase 1 (*GA20ox1*) genes located on Ra06 within the significant QTL region from 13940761 to 22402316 bp (Table 1). One of the *GA20ox1* genes, Ra_g27457, was located at 16987118 to 16988405 bp, 783 kb from the peak marker, and the second *GA20ox1* gene, Ra_g27461, was located at 17054993 to 17056276 bp, 851 kb from the peak marker in the QTL region.

## Discussion

### *Prickles*

A major locus associated with the 'Merton Thornless' source of the prickle-free trait in tetraploid blackberry was identified on chromosome 4 at 30.48 to 34.31 Mb in this study. Previous biparental mapping studies by Castro et al. (2013) and Weber (2014) both placed the 'Merton Thornless' prickle-free locus in large regions on the distal portion of LG4. The prickle-free locus mapped by Castro et al. (2013) was flanked by SSR markers at 4 and 25 cM. Weber (2014) mapped the prickle-free locus to a position between 0 and 1.17 Mb from the end of chromosome 4 on an unpublished draft genome of red raspberry and found that the flanking markers from Castro et al. (2013) aligned to physical positions 1.07 and 8.43 Mb from the end of chromosome 4. The prickle-free locus in diploid red raspberry was also placed in a 1.6 Mb (33.54–35.15 Mb) region on chromosome 4 of the same unreleased red raspberry genome using GWAS analysis performed in a biparental population (Khadgi and Weber, 2021). It is difficult to compare the exact physical position of the prickle-free locus identified in this study with previously mapped loci because the red raspberry genome used by Weber (2014) and Khadgi and Weber (2021) is not publicly available. However, orthologs of three of the possible candidate genes identified in this study, *MYB16*, *AGL30*, and *TBL27*, were recently proposed in red raspberry as possible candidates related to prickles based on their proximity to significant markers (Khadgi and Weber, 2021). Overall, the placement of the prickle-free locus on chromosome Ra04 at 30.48-34.31 Mb corroborates previous mapping studies and suggests that the genetic control of prickle-free traits in red raspberry and 'Merton Thornless' derived tetraploid blackberries is similar.

The peak SNP associated with prickles at 33636565 bp on chromosome Ra04 correlated to phenotypic data well, with only 10 out of 374 genotypes incorrectly predicted. Of the 10 genotypes inaccurately predicted by the SNP, only 'Burbank Thornless' had a prickle-free phenotype but was predicted to have three prickle-free alleles and one prickly allele. 'Burbank Thornless' (*R. ulmifolius inermis*, PI 554060), is a semi-erect diploid prickle-free blackberry accession. A prickle-free diploid *R. ulmifolius* plant was used as the original source of the prickle-free trait in 'Merton Thornless', but it is unclear if 'Burbank Thornless' is a clone of the *R. ulmifolius* plant used in the development of 'Merton Thornless'. The other nine genotypes inaccurately predicted by the peak marker were prickly breeding selections that had four copies of the prickle-free allele. Interestingly, all nine of these prickly breeding selections were recently derived from a prickly Lebanese blackberry plant that was first used in crosses at UA in 2009. It is unclear if the genetic mechanism controlling the prickle-free trait is different from the rest of the GWAS panel in ‘Burbank Thornless’ and the Lebanese-derived germplasm or if recombination between the candidate gene controlling prickle development and the peak SNP at 33636565 bp limit the predictive ability of the SNP in these genotypes.

Prickles have been hypothesized to be modified trichomes (Kellogg et al., 2011; Khadgi and Weber, 2020), although this is still unconfirmed and alternative hypotheses have been proposed (Zhou et al., 2021). A relationship between stomata, trichome, and prickle development has been proposed previously (Chalvin et al., 2020; Torii, 2021). Khadgi and Weber (2021) proposed three possible candidate genes with functions related to trichome development near significant SNPs in the prickle-free locus in red raspberry; a *MYB16*-like transcription factor, the agamous-like MADS-box gene *AGL30*, and trichome birefringence-like 2 (*TBL2*). Two of the three candidates proposed in red raspberry (*MYB16* and *AGL30*) were located within the prickle-free locus in tetraploid blackberry. A homolog of *TBL2*, trichome birefringence 27 (*TBL27*) was found just outside the prickle-free locus and 1.27 Mb from the peak SNP associated with prickles in tetraploid blackberry. Two additional genes located on Ra04 between 30.48-34.31 Mb with functions related to trichome development, the squamosa promoter-binding-like protein 6 (*SPL6*) and the homeobox domain leucine zipper HOX3 (*HOX3*), are also proposed as possible candidate genes for the prickle-free trait.

The myleoblastosis (*MYB*) protein-coding genes belong to the *R2R3-MYB* subfamily of transcription factors (Chalvin et al., 2020). This family of proteins is involved in many functions related to stomatal regulation, trichome formation, and cuticle and wax formation (Baumann et al., 2007; Chalvin et al., 2020; Oshima and Mitsuda, 2013; Yang et al., 2022). Two *R2R3-MYB* genes, *MYB16* and *MYB88*, were found within the QTL for blackberry prickles. *MYB16* regulates cuticle formation in reproductive organs and trichomes (Oshima et al., 2013; Oshima and Mitsuda, 2013). Downregulation of *MYB16* is associated with reduced production of glandular trichomes, which could inhibit prickle development if prickles are indeed modified trichomes or share a related metabolic pathway as hypothesized (Baumann et al., 2007). Khadgi and Weber (2020) found that *MYB16* was significantly down-regulated in the epidermis of prickle-free plants relative to prickly raspberry plants in a transcriptome analysis, making this an especially interesting candidate for the prickle-free trait. A gene encoding a MYB88 transcription factor protein (*MYB88*) (Ra_g19414) was also located at 33143949 to 33147906 bp within the QTL and 488 kb from the peak marker. *MYB88* has been described to function in stomata formation, and while not a likely candidate for prickles, this gene is interesting because of the proposed associations that exist in the origins of stomata, trichomes, and prickles (Torii, 2021).

The possible candidate gene located nearest the peak SNP was a homeobox domain leucine zipper HOX3 (*HOX3*). *HOX3* is classified as an HD-ZIP IV, which have known functions in glandular trichome initiation and could be involved in R2R3-MYB/HD-ZIP IV complexes that differentiate trichome function (Chalvin et al., 2020). An established relationship between *HOX3* and trichome development exists. In *Gossypium* sp. (cotton), a *HOX3* transcription factor has been identified that is associated with trichome elongation (Shan et al., 2014).

*AGL30* is a transcription factor in the MADS-box family. Khadgi and Weber (2020) found that five MADS-box transcription factors were differentially expressed between prickle-free and prickly plants, and *AGL30* specifically was downregulated in prickle-free epidermis of *Solanum viarum* Dunal (Pandey et al., 2018). Another little-studied but promising candidate gene for the prickle-free trait in blackberry was *TBL27*, a member of the trichome birefringence (*TBR*) gene family, which was located 1.27 Mb from the peak prickle-free marker. *TBR* functions in formation and deposition of cellulose on the secondary cell wall (Bischoff et al., 2010; Potikha and Delmer, 1995). Mutant *tbr Arabidopsis* plants were unable generate secondary wall cellulose in trichomes found on leaves and stems (Potihka and Delmer, 1995).

The last possible candidate gene for the prickle-free trait, *SPL6,* has been expressed at various degrees in transgenic *Arabidopsis* and tobacco (*Nicotiana tabacum* L.) plants. Mutants overexpressing the *SPL6* transcription factor had high trichome concentrations of on leaf margins in contrast to the wild types, which had smooth rosette leaf margins (Ma et al., 2019). This is interesting, given that prickly tetraploid blackberry plants have trichomes on cotyledon leaf margins, while prickle-free blackberries have smooth cotyledon leaf margins. In maize (*Zea mays* L.), triple-knockout mutants of without functioning *ZmSPL10*, *ZmSPL14* and *ZmSPL26* genes completely lacked trichomes but had higher densities of stomata, further confirming the existence of a common pathways for formation of stomata and non-stomata epidermal tissues (Kong et al., 2021).

All possible candidates for the ‘Merton Thornless’ prickle-free trait in tetraploid blackberry have designated roles in stomata formation and trichome development and differentiation. Transcriptome analysis specific to tetraploid blackberry could be utilized to determine if these possible candidate genes are expressed at different levels in prickly and prickle-free genotypes. Resequencing of prickly and prickle-free genotypes may also help to uncover nonsynonymous mutations within these potential candidate genes.

### *Plant Height*

Very little research has been conducted on the genetic control of plant height in *Rubus*. One study investigated dwarfism in a red raspberry mapping population. The researchers proposed that dwarfism in red raspberry was monogenic and recessively inherited based on segregation ratios observed in the study, and the dwarf locus was mapped to LG6 at 36.5 cM (Sargent et al., 2007). In contrast, plant height was quantitatively inherited in this blackberry GWAS panel and ranged from 41.2–184.5 cm. Two large-effect QTL explaining 15% and 13% of observed phenotypic variance for plant height in the GWAS panel were discovered on chromosomes Ra04 and Ra06. Five possible candidate genes and transcription factors for plant height in tetraploid blackberry were identified in and around the two significant QTL. These potential candidates included one GA 2-oxidase gene (*GA2ox6*), two GA 20-oxidase genes (*GA20ox1*), and two DELLA *RGL1* genes.

GA-oxidases have important roles in the GA biosynthesis pathway. There are three types of GA-oxidase enzymes that belong to the superfamily 2OG-Fe(II) oxygenase (2-oxoglutarate-dependent dioxygenase and Fe (II)-dependent oxygenase) (He et al., 2019). The possible candidate gene identified on Ra04 in this study was a *GA2ox6*. GA2ox enzymes convert bioactive GAs and/or their precursors to inactive forms (Hedden and Phillips, 2000). Mutations in various *GA2ox* genes have been shown to induce dwarf or semi-dwarf phenotypes in rice (*Oryza sativa* L.), wheat, *Arabidopsis*, tobacco, and multiple *Solanum* species (Dijkstra et al., 2008; Ford et al., 2018; Huang et al., 2010; Lo et al., 2008; Schomburg et al., 2003). Overexpression of *GA2ox6* in rice resulted in a semi-dwarf phenotype with that was reversible with exogenous GA applications (Huang et al., 2010). Endogenous levels of GA post-application were found to be similar to levels observed in the wild-type, indicating the *GA2ox6* dwarf was GA-deficient rather than GA-insensitive (Huang et al., 2010).

The two *GA20ox1* genes were identified as possible candidate genes within the plant height locus on chromosome Ra06. *Ga20ox* mutants have been associated with dwarf phenotypes in *Arabidopsis* and several crop species. The "green revolution genes" used to develop high-yielding dwarf cultivars of rice and barley (*Hordeum vulgare* L.) are orthologs of *AtGA20ox1* (Barboza et al., 2013). In coconut (*Cocos lucifera* L.), *GA20ox1* has been identified, and tall phenotypes were found to have three times the expression level of dwarfs, leading researchers to conclude *CnGA20ox1* is causal for height variation in coconut (Boonkaew et al., 2018). Dwarf phenotypes are important in watermelon (*Citrullus lantanus* L.) breeding and production, and *ClaGA20ox*, has been proposed as a possible candidate for dwarfism in watermelon (Dong et al., 2018). Both GA20ox- and GA2ox-induced dwarfism are reversible with exogenous applications of bioactive GA (Bulley et al., 2005; Lo et al., 2008; Schomburg et al., 2003), indicating mutations in these biosynthesis pathways lead to GA-deficiency.

Aside from the two distinct types of GA-oxidase genes identified, two additional genes related to GA regulation (*RGL1*) were identified near the QTL on Ra04. The GRAS (GAI, RGA, and SCARECROW) family of proteins contains a subfamily called DELLA proteins (Muangprom et al., 2005). Within DELLA are repressor-of-GA-like (*RGL)* genes. *RGL1* has been characterized in *Arabidopsis* and negatively regulates an array of GA responses (Thomas and Sun, 2004). The DELLA subfamily also includes some genes directly associated with dwarfism, including a "green revolution" *GAI* gene with orthologs identified in multiple cereal crops (Boss and Thomas, 2002; Muangprom et al., 2005; Peng et al., 1999). Despite having a known role in the GA signaling pathway, expression studies involving *RGL1* have demonstrated that this gene primarily affects floral development in *Arabidopsis* (Wen and Chang, 2002; Yu et al., 2004), indicating it is a less probable candidate for dwarfism.

Possible candidate genes identified in this study all have regulatory roles in the GA pathways. *RGL1* functions as a suppressor of GA-signaling, *GA2ox6* has a role in down-regulating bioactive GAs, and *GA20ox1* has a role in facilitating GA production. The GA-oxidase genes found in this study are supported by previous research as having a role in dwarfism, however *RGL1* is not a strong candidate due to being more influential in floral development. Development of reliable markers to screen for plant height in blackberry will be useful for planning crosses as well as screening seedlings. Currently, populations segregating for height include plants with normal stature that are planted near the reduced-stature seedlings. The ability to eliminate plants with tall stature early in the breeding process would increase the ability to see potential plant performance in dwarfs because they would not be competing for vital resources such as light, nutrients, and water. Validation of markers and possible candidate genes associated with plant height in tetraploid blackberry will follow this research.

## Conclusion

A single locus controlling prickles and two QTL associated with plant height were identified in tetraploid blackberry in this research. The locus associated with prickles, containing five possible candidate genes and transcription factors, was located on Ra04 at 30484542 to 34309626 bp and fit the simplex dominant gene model. The location of the prickle-free locus is corroborated by other studies conducted in biparental raspberry and blackberry mapping populations (Castro et al., 2013; Khadgi and Weber 2021; Weber, 2014). One QTL, near three possible candidate genes associated with plant height, was located on Ra04 at 25959770 to 26529505 bp and fit the simplex dominant gene model. The second QTL contained two possible candidate genes associated with plant height and was located on Ra06 at 13940761 to 22402316 bp and fit the additive gene model. Three possible candidate genes identified, one *GA2ox6* and two *GA20ox1* genes, are involved in the GA-biosynthesis pathway. The remaining two candidates were *RGL1* genes that have a role in the GA-signaling pathway but are more highly expressed in reproductive versus vegetative organs. Validation of the possible candidate genes and transcription factors discovered in this project will continue. Implementation of marker assisted selection for prickle-free and dwarf seedlings will enhance output from fresh-market blackberry breeding programs.

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## Tables and Figures

Table 1. Possible candidate genes for prickles and plant height in blackberry.

| Trait | Gene ID | Chromosome | Start bp | Stop bp | Functional annotation | Database |
|---|---|---|---|---|---|---|
| Prickles | Ra_g19233.t1 | Ra04 | 32260895 | 32263859 | SPL6 | SwissProt |
| Prickles | Ra_g19351.t1 | Ra04 | 32802206 | 32803665 | MYB16 | SwissProt |
| Prickles | Ra_g19365.t1 | Ra04 | 32877483 | 32880709 | MADS-BOX AGAMOUS 30 | SwissProt |
| Prickles | Ra_g19498.t1 | Ra04 | 33599420 | 33601244 | HOX3 (HD-ZIP IV) | RefSeq |
| Prickles | Ra_g19762.t1 | Ra04 | 34904416 | 34905885 | TRICHOME BIREFRINGENCE 27 | Araport11 |
| Plant Height | Ra_g18309.t1 | Ra04 | 27361510 | 27364509 | GA2ox6 | Araport11 |
| Plant Height | Ra_g18239.t1 | Ra04 | 26962868 | 26963329 | DELLA protein RGL1-like | NCBI nr |
| Plant Height | Ra_g18240.t1 | Ra04 | 26963434 | 26963916 | DELLA protein RGL1-like | NCBI nr |
| Plant Height | Ra_g27457.t1 | Ra06 | 16987118 | 16988405 | GA20ox1 | TrEmbl |
| Plant Height | Ra_g27461.t1 | Ra06 | 17054993 | 17056276 | GA20ox1 | TrEmbl |

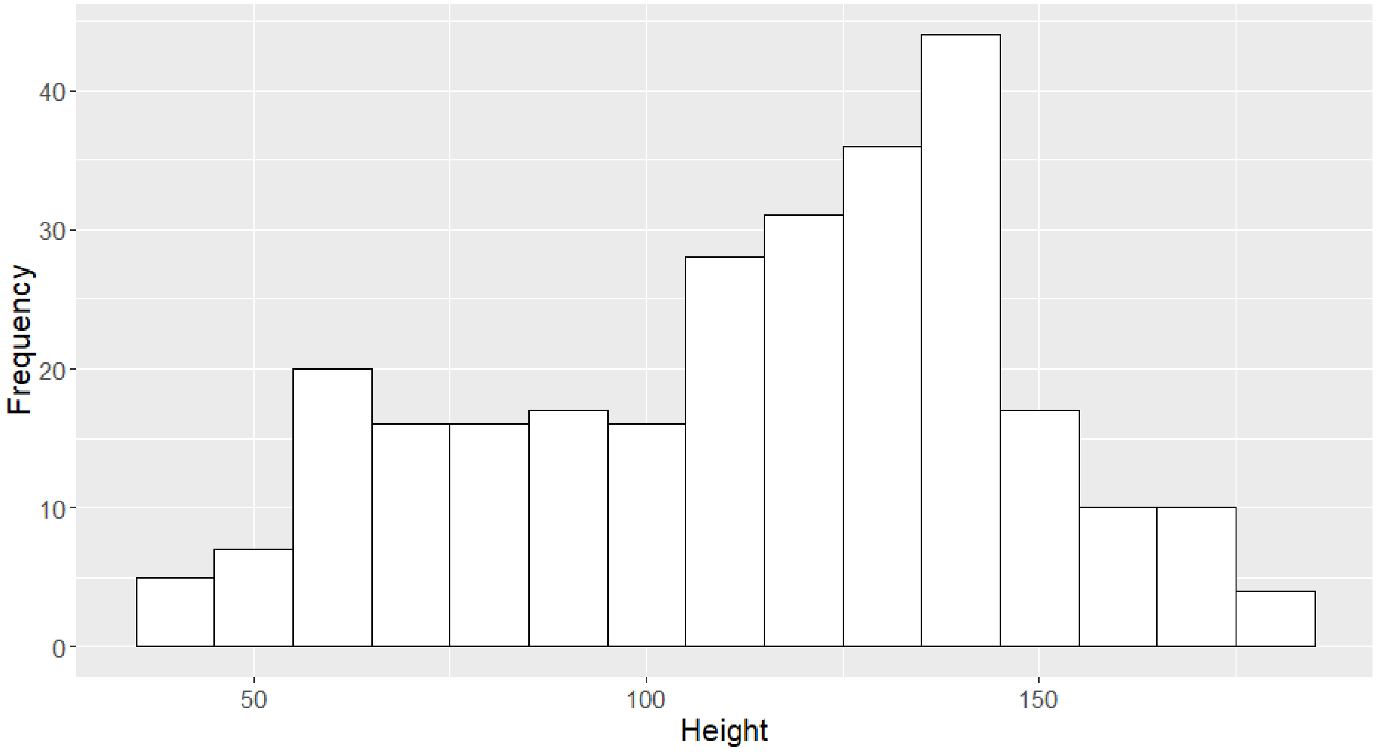

Fig. 1. Histogram constructed from normalized BLUPs of plant height (cm) collected over 2 years in a panel 267 genotypes.

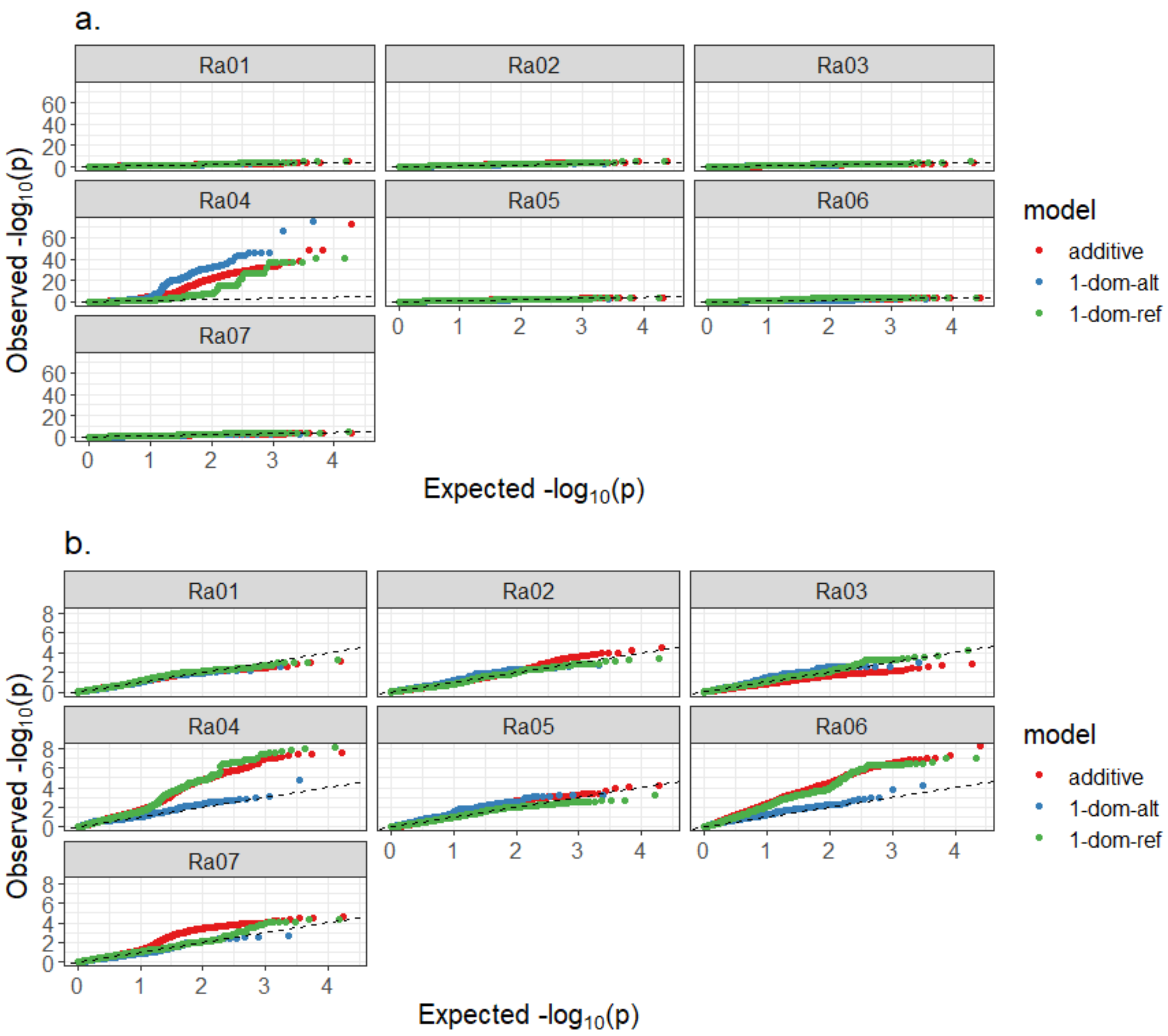

Fig. 2. QQ-plots for (a) prickles and (b) plant height showing the observed versus expected distribution of p-values for markers on each *R. argutus* chromosome. Deviation from the fitted dashed line represents rejection of the null hypothesis that markers are not associated with traits.

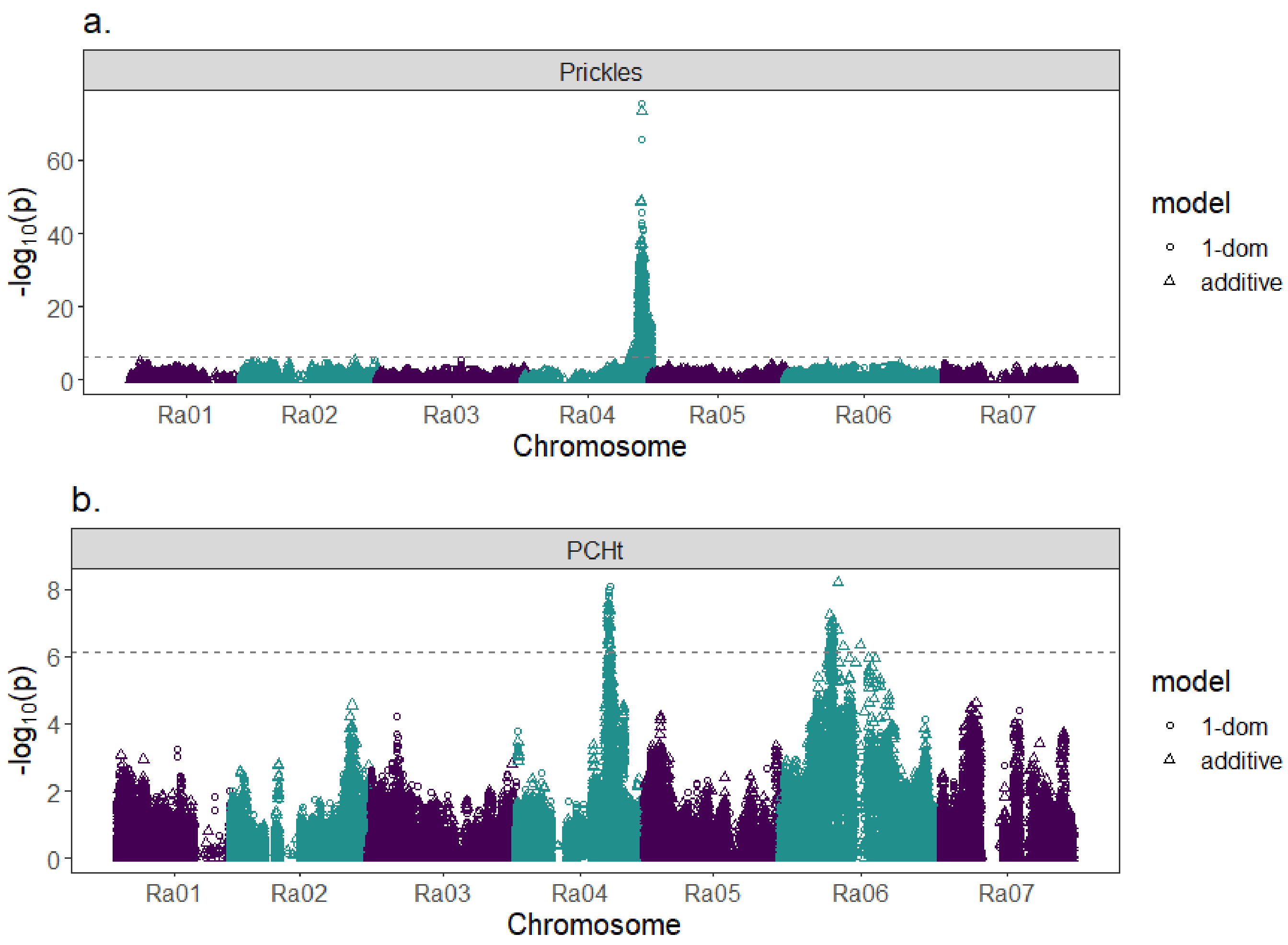

Fig. 3. Manhattan plots for blackberry (a) prickles and (b) plant height. The dashed lines represent the Bonferroni threshold of α = 0.05.

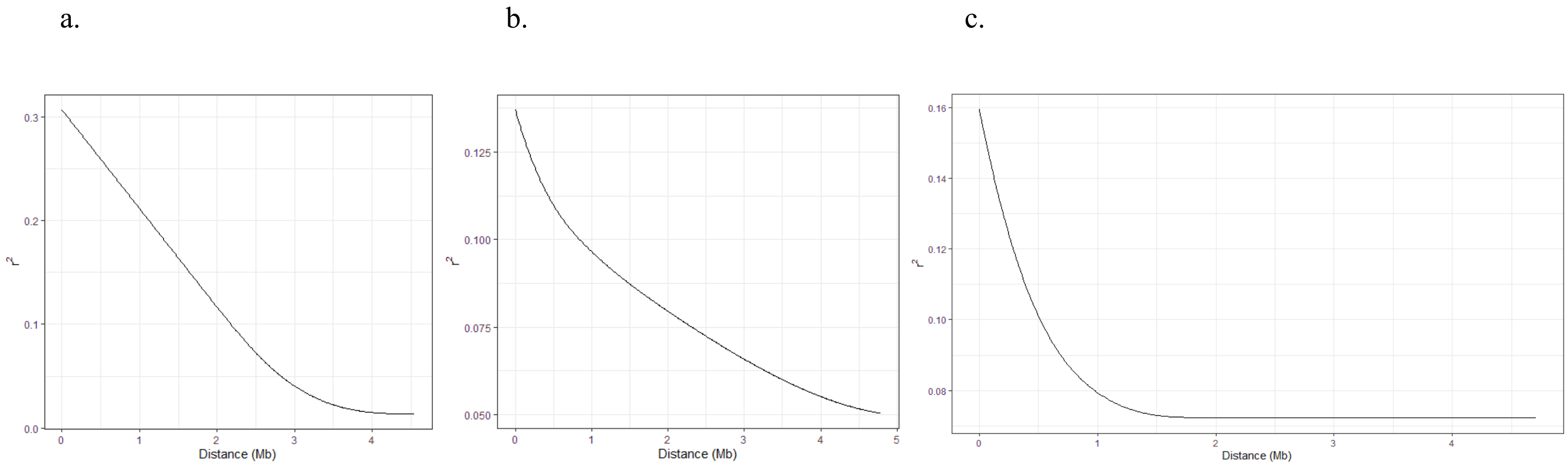

Fig. 4. Plots showing estimates of LD decay in the 5 Mb region around peak SNPs associated with (a) prickles on chromosome Ra04 at 33,636,565 bp, (b) plant height on chromosome Ra04 at 26,408,960 bp, and (c) plant height on chromosome Ra06 at 16,203,569 bp.

# CHAPTER II

# INFLUENCE OF PLANT GROWTH REGULATORS ON BLACKBERRY PLANTS WITH UNIQUE ARCHITECTURE

## Abstract

The use of plant growth regulators (PGRs) and genetics to control plant height in blackberry production systems could potentially reduce labor costs associated with summer pruning/tipping and prevent infections of cane blight (*Leptosphaeria coniothyrium*) on primocanes. Plant growth regulators are used to manipulate growth patterns in many horticultural crops, but they are not used in blackberry production. Blackberry plants with brachytic dwarfism that have been developed for the ornamental home-garden market may have an important role in tabletop and controlled-environment production systems of blackberry in the future. This study was conducted to gain a baseline understanding of the effect that two PGRs, prohexadione calcium (P-Ca) and gibberellic acid ($GA_3$), have on the plant architecture of three blackberry cultivars with varying growth habit. The cultivars used in this experiment were 'Baby Cakes®', a dwarf ornamental cultivar, 'Sweet-Ark® Ponca', a fresh-market cultivar with reduced internode length and standard height, and 'Natchez', a standard architecture cultivar. Each PGR was tested at three treatment rates in two locations. The most consistent effect of $GA_3$ observed in this study was an increase in the number and size of lateral branches. In Arkansas, all cultivars responded similarly to $GA_3$ treatments with increased number, length, and biomass of laterals as well as increased total stem biomass and total biomass of all tissue types. In North Carolina, the lateral biomass of all cultivars was increased by $GA_3$ treatments, but only 'Natchez' had increased lateral number and only 'Natchez' and 'Ponca' had longer laterals on $GA_3$ treated plants. P-Ca applications reduced internode length and reduced primocane height at both locations and on all cultivars. However, in instances where cultivar by P-Ca rate interactions were significant, the effect of P-Ca was strongest in ‘Natchez’ and weakest in ‘Baby Cakes’. Exogenous $GA_3$ and P-Ca may have applications on managing plant height in dwarf and semi-dwarf blackberries, but these results suggest that their responses to PGRs will be subtler than in standard-height cultivars.

## Introduction

Blackberry (*Rubus* L. subgenus *Rubus* Watson) production has increased steadily over the past two decades, but production costs associated with labor and other inputs are rising (Worthington et al., 2020). Tipping is a cultural management technique used for managing plant height and inducing lateral fruiting branches by removing the top 3-5 cm of actively growing primocanes (Strik et al., 2012). The timing of tip removal is important, and growers generally tip when primocanes are approximately 1 – 1.5 m tall and still in the exponential (log) growth stage (Strik et al., 2012; Thompson and Strik, 2009). The drawbacks of tipping are the high cost of labor, redistribution of harvest laborers, and increased susceptibility to disease associated with wounds inflicted by tip removal (Brannen and Krewer, 2012; Strik et al., 2012). Disease occurrence from wounds caused by cane blight (*Leptosphaeria coniothyrium* (Fuckel) Sacc.) is problematic, particularly in regions where fresh wounds may be subject to rain (Brannen and Krewer, 2012).

Methods of controlling vegetative vigor also need to be optimized for new high-density and controlled-environment blackberry production systems. Long-cane production is an example of a high-density system in which potted plants produce primocanes in soilless substrate at a high-density, are given a period of chill via cold storage, and are then moved to conditions that favor flowering and fruit development (Oliveira et al., 2002; Sønsteby et al., 2013). Often long-cane plants fruit in a greenhouse or high tunnel to produce off-season fruit and maximize profitability (Carew et al., 2000; Heiberg et al., 2008; Sønsteby et al., 2013). Long-cane systems offer flexibility in ripening time when paired with high tunnel production, and this pairing may boost yields, increase fruit size and quantity of marketable fruit, and require fewer pest/pathogen management strategies (Heiberg et al., 2008; Sønsteby et al., 2013). The long-cane production system has successfully been implemented in raspberry (*Rubus idaeus* L.) and is being researched for blackberry. Vertical farming is another example of a high-density system and a relatively new concept which integrates horticulture, engineering, and computer science into food production (Al-Kodmany, 2020; Benke and Tomkins, 2017; Kozai et al., 2016). Plants are given optimum amounts of water, nutrients, and artificial light in production, and the result can be high yields, lower transport distance, and high-quality produce (Al-Kodmany, 2020; Benke and Tomkins, 2017). The vertical farm model has been proposed as a long-term solution to loss of arable land, water limitations in prominent production regions, and food security for the growing human population (Al-Kodmany, 2020; Benke and Tomkins, 2017).

There is substantial interest in adapting these systems for caneberry production (Lovett, 2022; Miller, 2021; Growing Produce, 2021), but management of vegetative vigor and plant size will be a major challenge. Strawberry (*Fragaria x ananassa* Duch.) production in controlled-environment production systems, including tabletop production in greenhouses and vertical farming, is increasingly important, and caneberry production may be headed in the same direction based on recent interest (Samtani et al., 2019). Alternative management techniques are needed to decrease labor costs, optimize plant architecture, and reduce disease occurrence in both field-based and controlled-environment blackberry production systems. One possible strategy to achieve these goals is the use of plant growth regulators (PGRs).

Chemical PGRs are used to control reproductive attributes, manipulate vegetative growth patterns, and induce shoot and root development in production systems with minimal labor inputs (Rademacher, 2000). Plant growth regulators have been applied to manage vigor of field grown row and tree crops, as well as greenhouse-produced ornamentals (Rademacher, 2000). Gibberellic acid (GA) and prohexadione calcium (P-Ca) are two PGRs commonly used to control plant height and vegetative vigor in horticultural crops.

Gibberellins are a group of endogenous hormones that contribute to the regulation of terminal shoot growth (Camara et al., 2018; Hedden and Thomas, 2012). Approximately 140 types of GA have been identified, which can be broken down into two basic categories: biologically active forms and precursors (Talon et al., 1990). The differentiation between the biologically active forms of GA is their structure and carbon content. Biologically active GA is composed of 19 carbon atoms, whereas precursors contain 20 carbon atoms (Talon et al., 1990, Hedden and Thomas, 2000). The most common forms of GA used in horticultural crop production are $GA_3$ and $GA_{4+7}$, and, in general, $GA_3$ is used to affect vegetative plant growth and fruit size, and $GA_{4+7}$ is most commonly used to influence fruit size and quality. Some ornamental plants, particularly cut flowers, receive GA treatment to elongate flower stems, increase flower size, and shorten the time period for flowers to develop (Coelho et al., 2018; Cornea-Cipcigan et al., 2020; Edrisi et al., 2017; Shakarami et al., 2013). In fruit crops, GA is used to manage cluster density and increase berry size in wine and table grapes (*Vitis* spp.) (Dimovska et al., 2014; Pahi et al., 2020), crop load on apple (*Malus* x *domestica* Borkh.) (Bertelsen and Tustin, 2002), peach (*Prunus persica* L.) (Coneva and Cline, 2006), and citrus (*Citrus* spp.) (Garmendia et al., 2019) trees, and postharvest attributes of cherry (*Prunus avium* L.) and apricot (*Prunus americana* Marsh.) (Canli et al., 2014; Canli and Orhan, 2009; Lenahan et al., 2006). Research on how blackberry responds to exogenous GA has been limited. One study found that $GA_3$ applications increased lateral branch length and arrested primocane elongation in blackberries, while not affecting biomass or cane number (Malik and Archbold, 1992). More recently, $GA_3$ has been found to increase blackberry budbreak and improve yield on a cultivar-specific basis in low chill regions (Lin and Agehara, 2020). However, this study did not examine vegetative attributes and noted floral abortion to be problematic in association with $GA_3$.

Prohexadione calcium is classified as an acylcyclohexanedione and functions as a plant growth retardant. The metabolic pathway of GA is disrupted by P-Ca, rendering bioactive forms of GA inactive and unavailable for plant processes (Rademacher, 2000). Low concentration drench applications of P-Ca to mother plants were found to improve the quantity and quality of 'Maehyang' strawberry runners, a desirable effect in strawberry propagation (Kim et al., 2019). Fruiting strawberry plants showed decreased runner formation and increased crown branching (a desirable effect in fruit production), increased floral buds, and a positive effect on yield in response to P-Ca applications (Hytönen et al., 2008). In apple, P-Ca applications have been shown to effectively reduce shoot growth, increase yield, and protect against some common pathogenic apple diseases (Kashirskya et al., 2021; Wallis and Cox, 2020).

While not currently registered for use on blackberry, research is under way to determine the effect P-Ca on caneberries. In raspberry, P-Ca applications to 'Willamette' were found to increase yield and the number of fruiting laterals and nodes per meter, while reducing cane height and fruit acidity without a negative effect on other chemical fruit attributes (Poledica et al., 2012). Similar to results in raspberry, Milivojević et al. (2017) found that P-Ca applications positively affected fruit weight, soluble solids content, and yield on two blackberry cultivars, 'Loch Ness' and 'Triple Crown'. In the same study, plant height was reduced and number of nodes per meter increased as a result of P-Ca application. P-Ca reduced the height and internode length of primocanes on two blackberry cultivars, ‘Osage’ and ‘Von’, in a recent study conducted in North Carolina (Kon et al., 2020a). There was no consistent impact of P-Ca on yield in the first year of the study. However, P-Ca treatments reduced yield of both cultivars during second season of the study (Kon et al., 2020a, 2020b).

Another possible solution to managing blackberry vigor is use of plants with brachytic dwarfism or reduced internode length. Height variation in blackberry is quantitative trait, and expression exists on a continuous spectrum. Brachytic dwarfism is a form of reduced stature in which internode length is shortened, but other plant organs, such as leaves and fruit, remain a normal size (Hollender et al., 2016). Blackberry plants with reduced stature were first discovered in a seedling population at the University of Arkansas Division of Agriculture (UA) Fruit Research Station (FRS) in 2002 (Worthington and Clark, 2020). Dwarf blackberry cultivars have been released for the ornamental home-garden market and have recently become of interest for high-density and controlled-environment production systems (Lovett, 2022; Worthington and Clark, 2020). Plants have also been discovered in UA blackberry germplasm that exhibit reduced internode length but ultimately still reach a final height comparable to prevalent cultivars used in commercial production. In this study, these reduced internode plants that are still an average height are referred to as “semi-dwarf”, and the term “standard height” is used to describe blackberry plants with typical height and internode length.

The genetic cause of dwarfism in blackberry is unknown, however, mutations in genes related to GA biosynthesis and metabolism are associated dwarfism in many plant genera (Hedden and Phillips, 2000). Gibberellic acid ($GA_3$) and P-Ca typically have opposite physiological responses in plants but have not previously been studied on dwarf and semi-dwarf blackberry. Given that dwarfs and semi-dwarfs may have altered GA-biosynthesis pathways compared to standard height cultivars, it is unclear how applications of these PGRs will affect the architecture of these unique blackberry plants. The objective of this study was to determine the effects of $GA_3$ and P-Ca applied in separate experiments on the vegetative vigor of container-grown standard-height, semi-dwarf, and dwarf blackberry cultivars.

## Materials and Methods

### *Plant Material*

Three blackberry cultivars with varying plant architecture were used in this study: 'Baby Cakes®' ('Baby Cakes'), 'Sweet-Ark® Ponca' ('Ponca'), and 'Natchez'. 'Baby Cakes' is a home-garden, brachytic dwarf cultivar which typically reaches a maximum height of approximately 1.5 m without manipulation (Clark and Boches, 2016). 'Ponca' is considered to be a reduced-internode length plant because the plants reach a height typical of standard plants but have shortened internodes and vigorous primocane growth occurs later in the season than typically observed in standard-height plants (Clark, 2020). For this study, 'Ponca' was informally referred to as a "semi-dwarf" plant. 'Natchez' is a standard-height plant with architecture that is typical of cultivated blackberries and shows typical, expected patterns for primocane emergence and height development (Clark and Moore, 2008).

'Baby Cakes' plugs were obtained from Star® Roses (West Grove, PA), and 'Ponca' and 'Natchez' plugs were obtained from Agristarts (Apopka, FL). Plugs that were about 5 cm in height and actively growing were transplanted to 7.5 L nursery pots filled with Pro-Mix BX General Purpose growing media (Premier Tech Horticulture, Quakertown, PA) with Osmocote Classic 14N–4.2P–11.6K (The Scotts Company, Marysville, OH) incorporated at 36 g/container. Plants were kept in a greenhouse until late April at each site, then moved to a trellised and dripirrigated area for the duration of the experiment. Each plant was allowed to grow only one primocane to allow uniform effects to be observed. Additional canes that emerged from the crown of the plants were removed prior to initial PGR applications and throughout the experiment. Plants were irrigated as needed and trained to grow upright on a trellis with three tiers. Wires were spaced at 60 cm intervals (Fig. 1).

### *Experimental Design*

The $GA_3$ and P-Ca experiments were conducted separately in parallel on two separate trellises for application purposes. Each experiment was a factorial with three cultivars and three PGR rates organized as a completely randomized design with six replicates per treatment combination. This study was conducted at two locations during the summer of 2021: The Mountain Horticultural Crops Research and Extension Center in Mills River, NC, and the UA Fruit Research Station (FRS) in Clarksville, AR.

### *Plant Growth Regulator Applications*

Applications of $GA_3$ and P-Ca began when 'Natchez' plants averaged 30 cm in height, which was 21 June, 2021 and 8 July, 2021 at Arkansas and North Carolina, respectively. Weekly $GA_3$ (Purified $GA_3$ Solution; Phytotech Labs, Lenexa, KS) treatments were applied for 5 weeks at the North Carolina location and 6 weeks at the Arkansas location. A root zone drench method was used for $GA_3$ applications. Three rates were tested for $GA_3$: 0 (control), 250, and 500 $mg{\cdot}L^{-1}$. Each treatment, including the control, included Silwet™ L-77 (Momentive Performance Materials, Waterford, NY) surfactant at a rate of 0.125% (v/v).

Three P-Ca (Kudos 27.5 WDG; Fine Americas, Inc., Walnut Creek, CA) applications were made biweekly at both locations at three rates: 0 (control), 125, and 250 $mg{\cdot}L^{-1}$. Each P-Ca treatment 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.

*Data Collection and Analysis*

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 at 21 d intervals 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 destructive biomass of leaf and cane segments. Leaf surface area and root biomass were only measured at the North Carolina location. Biomass samples were dried at 35 °C for 7 d. Area under height progress curve (AUHPC), change in plant height over the course of the experiment, average internode length, and total leaf biomass were calculated at the conclusion of the experiment. Modeled after the area under disease progress curve (Shaner and Finney, 1977), AUHPC was used to analyze height progress over the course of the experiment. Overall, 16 response variables were measured or calculated in this experiment (Table 1).

Data for the $GA_3$ and P-Ca experiments were analyzed separately as completely randomized factorial 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. Pooled analysis revealed 10 response variables with significant location interactions for $GA_3$ and 11 response variables with location interactions for P-Ca. Therefore, results for the North Carolina and Arkansas sites are presented separately. Mean separation was performed using Tukey's Honest Significant Difference (HSD) at $\alpha = 0.05$. Regression analysis was performed for variables with significant rate and/or genotype by rate interactions effects to expose significant linear and quadratic relationships. Graphics were generated in RStudio version 4.0.4 (R Core Team, Vienna, Austria) using the package ggplot2.

## Results

### *Gibberellic Acid*

Significant differences among genotypes were detected for most response variables, as was expected given that the three cultivars were specifically chosen for their varying plant architecture. Leaf area, which was only measured at North Carolina, was the only response variable that did not have a significant cultivar main effect in a least one location in the $GA_3$ experiment (Table 2). Significant differences in lateral stem biomass among cultivars were found in North Carolina but not Arkansas. Significant differences among cultivars without interaction effects were observed for 11 response variables in both locations: AUHPC, height change, final diameter, number of nodes, internode length, cane biomass, cane leaf biomass, lateral stem biomass, lateral leaf biomass, total leaf biomass, total stem biomass, total biomass (Tables 2-3). As expected, 'Baby Cakes' was shorter than 'Natchez' at both locations as measured by height change and AUHPC (Table 3; Figs. 1-2). 'Baby Cakes' also had significantly shorter internodes than 'Ponca' and 'Natchez' in both locations. In Arkansas, no significant differences in AUHPC or height change were detected between 'Ponca' and 'Natchez', while in North Carolina 'Ponca' was more similar to 'Baby Cakes'. 'Ponca' had the thickest stem diameter in both locations. In Arkansas, biomass segments were generally lowest for 'Baby Cakes' and highest for 'Ponca'. Biomass results in North Carolina were complex, but 'Natchez' had significantly higher biomass than 'Baby Cakes' for all segments except lateral leaf biomass. 'Natchez' had higher cane and root biomass than 'Ponca' in North Carolina, but no significant differences were detected between these two cultivars for total biomass or lateral stem, total stem biomass, and total leaf segments (Table 3).

Rate of $GA_3$ significantly affected multiple response variables (Tables 2, 4, 5). No significant cultivar by $GA_3$ rate interactions were detected in Arkansas, and only lateral number and length were found to have significant cultivar by $GA_3$ rate interaction at North Carolina (Table 2). In Arkansas, number of laterals, lateral length, lateral stem biomass, lateral leaf biomass, total stem biomass, and total biomass of all cultivars increased with higher treatment rates. $GA_3$ rate had a quadratic effect on lateral length, total stem biomass, and total biomass in Arkansas (Tables 4-5). There was linear effect of $GA_3$ on the number of laterals, lateral stem biomass, and lateral leaf biomass in Arkansas.

In North Carolina, AUHPC, cane leaf biomass, and root biomass were reduced by exogenous $GA_3$ treatments, while lateral stem biomass and lateral leaf biomass increased with $GA_3$ application across all cultivars (Tables 4-6). Leaf area was significantly reduced compared to the control with 250 $mg \cdot L^{-1}$ treatment but not the 500 $mg \cdot L^{-1}$ treatment (Table 6). Significant cultivar by rate interaction effects were found for lateral number and length in North Carolina (Tables 2, 5). Application of $GA_3$ increased the number of laterals on 'Natchez' plants, with an average of 1.17, 2.50, and 4.50 laterals for the 0, 250, and 500 $mg \cdot L^{-1}$ rates, respectively (Table 5). In contrast, there was no significant effect of $GA_3$ on the number of laterals in 'Baby Cakes' or 'Ponca' plants in North Carolina. Applications of $GA_3$ increased lateral length for both 'Ponca' and 'Natchez', but had no effect on the lateral lengths of 'Baby Cakes'. Although the effect of $GA_3$ rate on cane leaf biomass in North Carolina was significant (Table 2), no linear or quadratic effect of treatment rate on cane leaf biomass was found in regression analysis (Table 4). The quadratic model fit best for all other response variables with significant $GA_3$ rate effects (Tables 4-6). In general, $GA_3$ applications increased the number, length, and biomass of laterals and either did not affect or reduced overall cane height (Figs. 1-2; Table 4).

*Prohexadione Calcium*

As in the $GA_3$ experiment, many significant differences among cultivars were identified in the parallel P-Ca experiment. All 16 response variables measured had significant cultivar main effects in at least one location, though no significant differences among cultivars were detected for number of laterals in Arkansas or total biomass in North Carolina (Tables 7-8). 'Ponca' plants had the thickest cane diameter in both locations, and 'Ponca' also had significantly fewer nodes than other cultivars in North Carolina. 'Baby Cakes' had the longest laterals in both locations, though laterals were notably longer on all cultivars in North Carolina compared to Arkansas. 'Ponca' had the highest total biomass and the highest biomass for canes, total stems, leaves on canes, and total leaf components in Arkansas, and there was no significant difference between 'Ponca' and 'Baby Cakes' for the lateral stem and leaf biomass components. While total biomass did not differ among cultivars in North Carolina, 'Natchez' had the highest cane biomass and 'Baby Cakes' had the highest lateral stem and leaf biomass components (Table 8).

Although cultivar was the most common main effect detected in the P-Ca trial, P-Ca rate and cultivar by P-Ca rate interaction effects were also significant for many variables (Tables 7, 9, 10). There was a significant quadratic effect of P-Ca rate on internode length at both locations across all cultivars, with shortened internodes resulting from both 125 $mg \cdot L^{-1}$ and 250 $mg \cdot L^{-1}$ treatment rates (Table 9). In North Carolina increasing P-Ca rates had a negative linear effect on the number of nodes across all cultivars. In Arkansas a linear reduction in nodes with increasing

P-Ca rate was only found for ‘Natchez’, and there was no significant effect of P-Ca rate on number of nodes in ‘Baby Cakes’ and ‘Ponca’ (Table 10). Increasing rates of P-Ca resulted in linear reduction of AUHPC in Arkansas with no significant cultivar by rate interaction effect (Table 10, Figs. 1b, 3a). A significant cultivar by rate interaction effect was detected for AUHPC in North Carolina. P-Ca applications reduced AUHPC in all cultivars, and the effect was greatest for ‘Natchez’ and least for ‘Baby Cakes’ (Fig. 3b). The linear regression model fit best for the effect of P-Ca rate on AUHPC in ‘Baby Cakes’ and ‘Natchez’, while the quadratic model fit best for ‘Ponca’. Significant cultivar by P-Ca rate interaction effects were detected for height change in both locations (Table 10). All cultivars had reduced growth of main canes when treated with P-Ca, but the effect was strongest in ‘Natchez’, intermediate in ‘Ponca’, and weakest in ‘Baby Cakes’ (Fig. 3). The linear regression model fit best for the effect of P-Ca on height change in ‘Baby Cakes’ and ‘Natchez’ grown in Arkansas and ‘Baby Cakes’ and ‘Ponca’ grown in North Carolina, while the quadratic regression model fit best for ‘Ponca’ in Arkansas and ‘Natchez’ in North Carolina.

There were no significant effects of P-Ca rate or cultivar by rate interaction on total biomass or any biomass components in Arkansas (Table 7). In North Carolina P-Ca applications reduced total biomass, cane biomass, lateral stem biomass, and total stem biomass of all cultivars with no significant cultivar by rate interaction (Table 9). The linear regression model fit best for cane biomass and the quadratic model fit best for the other biomass components. Root biomass increased linearly with P-Ca application treatment rate on ‘Natchez’ only (Table 6). The effect of P-Ca on leaf area and biomass was complicated. Leaf area and biomass of ‘Baby Cakes’ had a quadratic relationship to P-Ca rate, with lower leaf area and biomass at the 125 $mg \cdot L^{-1}$ rate compared to the 250 $mg \cdot L^{-1}$ treatment and untreated plants (Table 6, 10). Leaf area of ‘Natchez’ decreased linearly with increasing treatment rate of P-Ca, and leaf area of ‘Ponca’ was not affected by P-Ca. There was no significant effect of P-Ca rate on leaf biomass of ‘Ponca’ or ‘Natchez’ in North Carolina (Table 10).

## Discussion

PGRs are not currently used in blackberry production systems, and little research has been conducted to evaluate the potential value of these growth-altering substances. Innovative strategies for managing plant height and vigor are needed to mitigate labor costs in current production systems and adapt existing blackberry cultivars to emerging high-density production systems. Neither $GA_3$ nor P-Ca have previously been tested on dwarf or semi-dwarf blackberry. The response of these unique phenotypes to each PGR, which influence the GA pathway differently, may assist in understanding underlying genetic causes for height variation, while simultaneously establishing if there is a benefit to applying PGRs to dwarf and semi-dwarf plants in commercial production.

### *Gibberellic Acid*

The most consistent effect of $GA_3$ observed in this study was an increase in the number and size of lateral branches. In Arkansas there was no significant cultivar by $GA_3$ rate effect on any response variable, and the number of laterals, lateral length, lateral stem biomass, lateral leaf biomass, total stem biomass, and total biomass of all cultivars increased with higher treatment rates. In North Carolina lateral stem and leaf biomass increased in response to $GA_3$ application across all cultivars, but significant cultivar by rate interaction effects were found for lateral number and length. For response variables with significant cultivar by rate interactions, the effect of $GA_3$ was generally greatest in ‘Natchez’, intermediate in ‘Ponca’, and lowest in ‘Baby Cakes’. $GA_3$ applications increased the number of laterals in ‘Natchez’ and the length of laterals on

‘Natchez’ and ‘Ponca’, but had no effect on either response variable in ‘Baby Cakes’ in North Carolina. Exogenous $GA_3$ applications also reduced AUHPC, cane leaf biomass, and root biomass across all cultivars in North Carolina. In North Carolina the quadratic regression model fit best for almost all response variables with significant rate effects, while there was a mix of linear and quadratic responses to increasing $GA_3$ rates for the variables measured in Arkansas.

Results of this study were consistent with previous findings. Standard height ‘Chester Thornless’ blackberry plants showed elongated laterals and reduced primocane height upon exposure to $GA_3$ (Malik and Archbold, 1992). In the same study, no significant response to $GA_3$ was identified for cane number, leaflet number, or cane, leaf, and root biomass response variables. It is unclear if lateral-specific tissues were included in leaf and stem biomass estimates by Malik and Archbold (1992). Lateral biomass (stems and leaves) increased with $GA_3$-treated plants for both leaves and stem matter at both locations in the present study. Total stem biomass and total biomass of all tissues increased with $GA_3$ applications in Arkansas, but this finding could be related to pooling biomass of different tissue types and the calculation method used for these variables.

The observed effect of $GA_3$ on lateral number is of interest because blackberry yield is correlated to lateral number (Strik et al., 2012). Therefore, exogenous $GA_3$ applications could potentially increase yields and trellis-fill in existing management systems by stimulating lateral growth. However, GA is used to decrease bloom density or induce floral abortion in apple (Bertelsen and Tustin, 2002), citrus (Garmendia et al., 2019), and peach (Coneva and Cline, 2006) production systems, so these effects may counteract any potential yield benefit from increased lateral development. Past research has shown increased budbreak of some floricane-fruiting blackberry cultivars in a low-chill environment (Lin and Agehara, 2020). Vegetative characteristics were not evaluated by Lin and Agehara (2020), and it is unclear if yield benefits would be observed in environments with sufficient chilling. Furthermore, Lin and Agehara (2020) also used a cytokinin to arrest the effects of $GA_3$ on floral abortion, so this should be considered in future evaluations of $GA_3$ for lateral development and its effect on yield. Yield was not evaluated in this study or by Malik and Archbold (1992). Further research is needed to determine the effects of exogenous $GA_3$ on yield in dwarf, semi-dwarf, and standard height blackberries. Additional research is also needed to determine the impact of $GA_3$ application method. The present study used a root zone drench; however, a spray application study would be useful in determining mobility and distribution patterns of this PGR in blackberry.

### *Prohexadione Calcium*

The most important effects of P-Ca treatments observed in this study were reduced primocane elongation and internode length. Significant cultivar by P-Ca rate interactions were discovered for many response variables, including height change in both sites, number of nodes in Arkansas, and AUHPC in North Carolina. In general, the effects of P-Ca applications were strongest in the standard height cultivar (‘Natchez’), intermediate in the semi-dwarf cultivar (‘Ponca’), and weakest in the dwarf cultivar (‘Baby Cakes’). Lateral lengths of all cultivars were shortened in response to P-Ca applications in North Carolina, but no effect was noted in Arkansas. The effects of P-Ca on total biomass and specific tissue biomass varied by test site and were inconclusive. Arkansas results indicated no significant effect of P-Ca on tissue biomass, while cane, lateral stem, and total stem biomass components and total biomass of all organs were reduced at both the 125 $mg \cdot L^{-1}$ and 250 $mg \cdot L^{-1}$ treatment rates in North Carolina. There was no clear pattern to which regression model fit best for the response variables measured in either site. In general, the effect on response variables to increasing P-Ca rate from 0 to 125 $mg \cdot L^{-1}$ was much more pronounced than effect of the increase from 125 mg·L$^{-1}$ to 250 mg·L$^{-1}$ application rates.

The response of standard height blackberries to P-Ca treatments were mostly similar to what had been observed in previous research. The reduction in internode length, primocane height, and AUHPC observed for all cultivars in this study are supported by results found in previous research (Kon et al., 2020a, 2020b; Milivojević et al., 2017; Poledica et al., 2012). The number of laterals were not affected by P-Ca in either location in this study, and previous studies have reported conflicting effects of P-Ca on lateral number in caneberry. Milivojević et al. (2017) and Poledica et al. (2012) found that the number of laterals increased in response to P-Ca, while Kon et al. (2020b) found reduced lateral number in response to P-Ca. No effect of P-Ca on cane diameter was observed in the present study, in contrast to previous research which found that cane diameters were reduced in response to P-Ca (Milivojević et al., 2017; Poledica et al., 2012). The number of nodes on 'Natchez' were reduced by P-Ca applications in North Carolina, which also contradicts previous reports (Kon et al., 2020a; Milivojević et al., 2017; Poledica et al., 2012). Previous research has shown that P-Ca applications have increased yield (Milivojević et al., 2017; Poledica et al., 2012), whereas Kon et al. (2020b) observed no effect on yield in the first year of their study followed by a reduction in yield in the second fruiting season. This is understandable as the applications would not impact floricanes that fruited the year of the applications, but rather the primocanes that were treated and fruited as floricanes the second year. Research on yield effects on standard height plants, as well as dwarf and semi-dwarf plants, is crucial to determining if P-Ca treatments are economically beneficial. Minor reductions in yield associated with P-Ca application may be acceptable if labor costs are reduced synchronously or planting densities can be increased.

### *Impacts of PGRs on Dwarf Blackberry*

Generally, dwarf and semi-dwarf blackberry plants had fewer significant responses to PGR applications than the standard height blackberry, ‘Natchez’. PGR applications impacted all cultivars similarly for several important response variables, including lateral stem biomass in the $GA_3$ study and internode length in the P-Ca study. However, in instances where significant cultivar by PGR rate interactions were observed in one or more study location, the effect of $GA_3$ or P-Ca was strongest in ‘Natchez’, intermediate in ‘Ponca’, and weakest in ‘Baby Cakes’. Mutations in genes related to GA biosynthesis and metabolism are associated dwarfism in many plant genera (Hedden and Phillips, 2000). The genetic cause of dwarfism in blackberry is unknown, and response to exogenous GA applications are useful for understanding what mechanism in the GA pathways is being affected in the plant. Exogenous $GA_3$ applications have restored typical height in dwarf plants with mutations in genes related to GA-deficiency (El-Sharkawy et al., 2012; Huang et al., 2010; Li et al., 2019). In contrast, the reduced effects of PGR applications on ‘Baby Cakes’ relative to ‘Natchez’ observed in this study suggest that dwarf blackberries may be at least partially GA-insensitive rather than GA-deficient, a response similar to brachytic dwarfism observed in peach (Hollender et al., 2016). Exogenous $GA_3$ and P-Ca may have applications in managing plant height in dwarf and semi-dwarf blackberries, but the results of this study suggest that the effects of these PGRs will be subtler than in standard height cultivars. It is unlikely that the difference in response could be compensated for by increasing PGR rate considering that a quadratic regression model was the best fit for many response variables measured in this study.

## Conclusion

The use of PGRs and plants exhibiting natural, genetic variation in height and architecture each have system-specific benefits to consider. This study concluded that $GA_3$ and P-Ca applications each influenced vegetative growth of blackberry differently. In general, $GA_3$ increased lateral number, length and biomass, while P-Ca arrested cane height development and reduced internode length and number. ‘Natchez’ plants were most responsive to PGR applications, while ‘Ponca’ and ‘Baby Cakes’ had less dramatic responses to P-Ca and $GA_3$ application in many instances. P-Ca applications are effective for reducing plant height and could be a useful alternative to tipping. Early research on the response of standard blackberry to P-Ca indicated a need to mitigate the undesirable effects this chemical has on lateral development, and our study shows that $GA_3$ may be a useful solution to the negative yield impact observed by Kon et al. (2020b). Delayed height development in the presence of P-Ca could be useful for vigor management early in the growing season, while applications of $GA_3$ may be beneficial later in the season to recover unintended effects of P-Ca on lateral production, indicating these PGRs may have complementary roles in blackberry production. Use of $GA_3$ and P-Ca are most promising for effective vigor management in field and long-cane blackberry production systems, although they have not been tested simultaneously on the same plant. Genetically dwarf plants likely fit best in vertical farming and tabletop production models of blackberry, whereas semi-dwarf plants would be ideal for field and long-cane production given their high number of nodes. Utilizing dwarf and semi-dwarf genetics is a way around incurring the cost associated with purchasing PGRs, so this should be considered by growers when establishing new plantings and choosing a production approach. A cost-benefit analysis is needed to determine whether use of PGRs is indeed a more economical approach to managing vigor and controlling cane blight than management practices currently in place. Long-term evaluation of the effects of $GA_3$ and P-Ca on fruit quality and yield are needed to determine if they are viable alternatives to current management techniques or fit in emerging production systems. Since neither evaluation of plants with varying architecture nor use of PGRs in blackberry production have been well-defined, this research provided a baseline understanding of the potential that varying plant architecture and PGRs may have in existing and emerging cultural management systems.

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## Tables and Figures

Table 1. Description of response variables measured and calculations used in the final analyses of evaluating physiological responses of blackberry cultivars to exogenous applications of gibberellic acid and prohexadione calcium.

| Response variables | Description of measurement or calculation |
|---|---|
| Area Under Height Progress Curve (AUHPC)$^{z}$ | $AUHPC = \sum_{i=1}^{n} \left[\frac{H_{i+1} + H_i}{2}\right] [X_{i+1} - X_i]$ |
| Height change (cm) | The difference in initial height and final cane height |
| Diameter (mm) | The final diameter of the base of the cane |
| Nodes (no.) | The final count of nodes on the main cane |
| Internode length (cm) | Final plant height divided by the number of nodes |
| Laterals (no.) | The number of laterals present at experiment termination |
| Lateral length (cm) | The mean sum of lateral lengths taken on a per plant basis |
| Cane biomass (g) | The dry weight of the main cane tissue |
| Cane leaf biomass (g) | The dry weight of the leaves taken from the main cane |
| Lateral stem biomass (g) | The dry weight of lateral stems |
| Lateral leaf biomass (g) | The dry weight of the leaves taken from the laterals |
| Total leaf biomass (g) | The sum of dry weights of main cane leaves and lateral leaves |
| Total stem biomass (g) | The sum of dry weights of the main cane and lateral stems |
| Total biomass (g) | The sum of dry weights for all cane, stem, and leaves |
| Root biomass (g)$^{x}$ | The dry weight of the plant's root system |
| Leaf area (cm$^2$) | The sum of total leaf area |

$^{z}H_i$ is the height (cm) measured at the $i^{th}$ observation, $X_i$ is the time period (days) at the $i^{th}$ observation, and $n$ is the overall number of observations.

$^{x}$Root biomass and leaf area were only measured in North Carolina.

Table 2. P-values of main effects and interactions of cultivar and gibberellic acid (GA$_3$) treatment rates on blackberry plants at two locations in 2021 generated by analysis of variance.

| Response variable | Cultivar | GA$_3$ rate | Cultivar by GA$_3$ rate | Cultivar | GA$_3$ rate | Cultivar by GA$_3$ rate |
|---|---|---|---|---|---|---|
| | *Arkansas* | | | *North Carolina* | | |
| AUHPC$^z$ | <0.01$^y$ | 0.85 | 0.93 | <0.01 | 0.05 | 0.51 |
| Height change (cm) | <0.01 | 0.71 | 0.94 | <0.01 | 0.09 | 0.86 |
| Diameter (mm) | <0.01 | 0.27 | 0.74 | 0.01 | 0.99 | 0.76 |
| Nodes (no.) | <0.01 | 0.83 | 0.30 | <0.01 | 0.10 | 0.94 |
| Internode length (cm) | <0.01 | 0.92 | 0.22 | <0.01 | 0.56 | 0.42 |
| Laterals (no.) | <0.01 | <0.01 | 0.41 | <0.01 | <0.01 | <0.01 |
| Lateral length (cm) | 0.03 | <0.01 | 0.33 | <0.01 | <0.01 | <0.01 |
| Cane biomass (g) | <0.01 | 0.21 | 0.74 | <0.01 | 0.24 | 0.65 |
| Cane leaf biomass (g) | <0.01 | 0.96 | 0.80 | <0.01 | 0.03 | 0.64 |
| Lateral stem biomass (g) | 0.37 | 0.04 | 0.76 | 0.04 | <0.01 | 0.11 |
| Lateral leaf biomass (g) | 0.04 | <0.01 | 0.53 | <0.01 | <0.01 | 0.43 |
| Total leaf biomass (g) | <0.01 | 0.31 | 0.67 | <0.01 | 0.10 | 0.99 |
| Total stem biomass (g) | <0.01 | <0.01 | 0.46 | <0.01 | 0.30 | 0.70 |
| Total biomass (g) | <0.01 | 0.05 | 0.62 | <0.01 | 0.16 | 0.97 |
| Root biomass (g) | nm$^x$ | nm | nm | <0.01 | 0.03 | 0.65 |
| Leaf area (cm$^2$) | nm | nm | nm | 0.33 | 0.04 | 0.97 |

$^z$AUHPC = area under height progress curve.

$^y$Results are significant at $p \leq 0.05$.

$^x$nm = not measured.

Table 3. Mean separation results among three blackberry cultivars for variables with significant cultivar effects in at least one location in the $GA_3$ experiment conducted in Arkansas and North Carolina in 2021.

| | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| Variable | BC[z] | Ponca | Natchez | BC | Ponca | Natchez |
| | *Arkansas* | | | *North Carolina* | | |
| AUHPC[y] | 1438 b[x] | 3687 a | 4026 a | 1476 C | 2452 B | 3771 A |
| Height change (cm) | 40.29 b | 125.49 a | 118.47 a | 36.52 B | 51.21 B | 91.68 A |
| Diameter (mm) | 4.02 c | 7.00 a | 4.85 b | 8.42 B | 9.62 A | 8.29 B |
| Nodes (no.) | 25.30 b | 37.08 a | 38.33 a | 28.33 A | 21.89 B | 29.28 A |
| Internode length (cm) | 2.31 b | 3.99 a | 4.22 a | 2.75 B | 4.17 A | 5.01 A |
| Laterals (no.)[w] | 2.24 b | 5.67 a | 5.20 a | 1.78 | 4.22 | 2.72 |
| Lateral length (cm) | 61.59 b | 119.19 ab | 127.80 a | 55.03 | 126.07 | 123.26 |
| Cane biomass (g) | 2.93 c | 26.19 a | 10.85 b | 4.66 C | 9.72 B | 12.63 A |
| Cane leaf biomass (g) | 7.92 c | 31.77 a | 17.12 b | 12.45 B | 11.93 B | 18.23 A |
| Lateral stem biomass (g) | 7.34 a | 8.83 a | 5.96 a | 2.55 B | 4.45 A | 3.89 AB |
| Lateral leaf biomass (g) | 2.29 b | 5.23 a | 3.43 ab | 5.69 AB | 8.16 A | 4.46 B |
| Total leaf biomass (g) | 10.21 c | 37.00 a | 20.55 b | 18.14 B | 20.08 AB | 22.69 A |
| Total stem biomass (g) | 10.28 b | 35.02 a | 16.81 b | 7.21 B | 14.17 A | 16.52 A |
| Total biomass (g) | 20.49 c | 72.02 a | 37.36 b | 25.34 B | 34.25 A | 39.21 A |
| Root biomass (g) | nm[v] | nm | nm | 5.72 C | 11.58 B | 14.61 A |

[z] BC = Baby Cakes®; Ponca = Sweet-Ark® Ponca.

[y] AUHPC = Area under height progress curve.

[x] Means with the same letter within location in each row are not significantly different by Tukey's honest significant difference, $p \leq 0.05$. Lower-case letters indicate mean separation at Arkansas and upper-case letters indicate mean separation at North Carolina.

[w] Mean separation was not performed for main effect of cultivar on number of laterals or lateral length in North Carolina as cultivar by $GA_3$ rate interaction effects were significant.

[v] nm=not measured.

Table 4. Results of regression analysis for response variables with a significant rate effect in at least one location in the GA$_3$ experiment conducted on blackberry plants in Arkansas and North Carolina in 2021.

| Rate | AUHPC[z] | Cane leaf biomass (g) | Lateral stem biomass (g) | Lateral leaf biomass (g) | Total stem biomass (g) | Total biomass (g) |
|---|---|---|---|---|---|---|
| | | | *Arkansas* | | | |
| 0 mg·L$^{-1}$ | 3143[y] | 19.08 | 4.32 | 1.50 | 15.69 | 36.27 |
| 250 mg·L$^{-1}$ | 2961 | 19.14 | 8.73 | 4.20 | 22.15 | 45.49 |
| 500 mg·L$^{-1}$ | 3048 | 18.59 | 9.09 | 5.25 | 24.26 | 48.11 |
| Regression model | NS[x] | NS | L | L | Q | Q |
| P-value[w] | - | - | <0.01 | <0.01 | 0.01 | 0.02 |
| | | | *North Carolina* | | | |
| 0 mg·L$^{-1}$ | 2824 | 16.28 | 2.84 | 5.16 | 12.82 | 34.25 |
| 250 mg·L$^{-1}$ | 2500 | 13.66 | 2.95 | 5.01 | 11.77 | 30.44 |
| 500 mg·L$^{-1}$ | 2375 | 12.67 | 5.10 | 8.13 | 13.31 | 34.11 |
| Regression model | Q | NS[v] | Q | Q | NS | NS |
| P-value | <0.01 | - | <0.01 | <0.01 | - | - |

[z] AUHPC = Area under height progress curve.

[y] Results represent overall least square means of the three blackberry cultivars (Baby Cakes®, Sweet-Ark® Ponca, and Natchez) at the specified treatment rate.

[x] L = linear model; Q = quadratic model; NS = not significant.

[w] P-value is for the specified regression model.

[v] ANOVA results indicated a significant effect of rate on cane leaf biomass (p = 0.03). However, regression analysis did not detect a significant linear or quadratic relationship.

Table 5. Results of regression analysis for response variables with a significant cultivar by rate interaction effect at the North Carolina site in the $GA_3$ experiment conducted on blackberry plants in Arkansas and North Carolina in 2021. No significant cultivar by rate interactions were found in Arkansas.

| | Laterals (no.) | | | Lateral length (cm) | | |
|---|---|---|---|---|---|---|
| Rate | BC[z] | Ponca | Natchez | BC | Ponca | Natchez |
| | | | *Arkansas*[y] | | | |
| 0 mg·L$^{-1}$ | | 1.98 | | | 38.8 | |
| 250 mg·L$^{-1}$ | | 5.67 | | | 122.1 | |
| 500 mg·L$^{-1}$ | | 5.47 | | | 147.8 | |
| Regression model | | L[x] | | | Q | |
| P-value | | <0.01[w] | | | 0.04 | |
| | | | *North Carolina* | | | |
| 0 mg·L$^{-1}$ | 1.50 | 4.83 | 1.17 | 47.1 | 136.4 | 54.9 |
| 250 mg·L$^{-1}$ | 2.00 | 2.83 | 2.50 | 56.5 | 90.5 | 92.1 |
| 500 mg·L$^{-1}$ | 1.83 | 5.00 | 4.50 | 61.5 | 151.3 | 237.7 |
| Regression model | NS | NS | Q | NS | Q | Q |
| P-value | - | - | 0.01 | - | <0.01 | <0.01 |

[z] BC = Baby Cakes®; Ponca = Sweet-Ark® Ponca.

[y] Overall least square means of all three blackberry cultivars at each specified treatment rate are presented for Arkansas because no significant cultivar by $GA_3$ rate interaction effect was detected.

[x] L = linear model; Q = quadratic model; NS = not significant.

[w] P-value is for the specified regression model.

Table 6. Results of regression analysis for the effect of PGR rate on root biomass and leaf area response variables, which were measured exclusively at the North Carolina site.

| | BC[z] | Ponca | Natchez | BC | Ponca | Natchez |
|---|---|---|---|---|---|---|
| Rate | | Root biomass (g) | | | Leaf area ($cm^2$) | |
| | | | *$GA_3$* [y] | | | |
| 0 mg·$L^{-1}$ | | 12.31 | | | 2710 | |
| 250 mg·$L^{-1}$ | | 10.29 | | | 2349 | |
| 500 mg·$L^{-1}$ | | 9.32 | | | 2777 | |
| Regression model | | Q[x] | | | Q | |
| P-value | | <0.01[w] | | | <0.01 | |
| | | | *P-Ca* | | | |
| 0 mg·$L^{-1}$ | 8.17 | 9.83 | 12.13 | 2589 | 1968 | 2321 |
| 125 mg·$L^{-1}$ | 6.98 | 15.23 | 22.03 | 2122 | 1808 | 2278 |
| 250 mg·$L^{-1}$ | 6.78 | 14.07 | 27.47 | 2940 | 1785 | 1924 |
| Regression model | NS | NS | L | Q | NS | L |
| P-value | - | - | <0.01 | 0.03 | - | 0.04 |

[z] BC = Baby Cakes®; Ponca = Sweet-Ark® Ponca.

[y] Overall least square means of all three blackberry cultivars at each specified treatment rate are presented for $GA_3$ because no significant cultivar by $GA_3$ rate interaction effect was detected.

[x] L = linear model; Q = quadratic model; NS = not significant.

[w] P-value is for the specified regression model.

Table 7. P-values of main effects and interactions of cultivar and prohexadione calcium (P-Ca) treatment rates on blackberry plants at two locations in 2021 generated by analysis of variance.

| Variable | Cultivar | P-Ca rate | Cultivar by P-Ca rate | Cultivar | P-Ca rate | Cultivar by P-Ca rate |
|---|---|---|---|---|---|---|
| | | *Arkansas* | | | *North Carolina* | |
| AUHPC[z] | <0.01[y] | <0.01 | 0.20 | <0.01 | <0.01 | 0.01 |
| Height change (cm) | <0.01 | <0.01 | 0.01 | <0.01 | <0.01 | <0.01 |
| Diameter (mm) | <0.01 | 0.23 | 0.59 | <0.01 | 0.49 | 0.35 |
| Nodes (no.) | <0.01 | 0.03 | 0.04 | <0.01 | <0.01 | 0.15 |
| Internode length (cm) | <0.01 | <0.01 | 0.72 | <0.01 | <0.01 | 0.14 |
| Laterals (no.) | 0.06 | 0.19 | 0.59 | <0.01 | 0.51 | 0.19 |
| Lateral length (cm) | <0.01 | 0.16 | 0.55 | <0.01 | <0.01 | 0.98 |
| Cane biomass (g) | <0.01 | 0.07 | 0.64 | <0.01 | <0.01 | 0.62 |
| Cane leaf biomass (g) | <0.01 | 0.53 | 0.23 | <0.01 | 0.07 | 0.09 |
| Lateral stem biomass (g) | <0.01 | 0.21 | 0.28 | <0.01 | <0.01 | 0.70 |
| Lateral leaf biomass (g) | 0.04 | 0.42 | 0.30 | <0.01 | 0.11 | 0.43 |
| Total leaf biomass (g) | <0.01 | 0.67 | 0.16 | 0.02 | 0.53 | 0.03 |
| Total stem biomass (g) | <0.01 | 0.29 | 0.62 | 0.02 | <0.01 | 0.67 |
| Total biomass (g) | 0.01 | 0.33 | 0.61 | 0.22 | <0.01 | 0.15 |
| Root biomass (g) | nm[x] | nm | nm | <0.01 | <0.01 | <0.01 |
| Leaf area (cm$^2$) | nm | nm | nm | <0.01 | 0.25 | 0.02 |

[z] AUHPC = Area under height progress curve.

[y] Results are significant at $p \leq 0.05$.

[x] nm = not measured.

Table 8. Mean separation results among three blackberry cultivars for variables with significant cultivar effects in at least one location in the P-Ca experiment conducted in Arkansas and North Carolina in 2021.

| | Cultivar | | | | | |
|---|---|---|---|---|---|---|
| Variable | BC[z] | Ponca | Natchez | BC | Ponca | Natchez |
| | | *Arkansas* | | | *North Carolina* | |
| AUHPC[y] | 1057 c[x] | 1892 b | 2567 a | 1382[w] | 1879 | 2976 |
| Diameter (mm) | 3.94 b | 6.44 a | 4.05 b | 8.16 B | 10.68 A | 7.71 B |
| Nodes (no.) | 21.28 | 30.72 | 31.17 | 29.22 A | 20.44 B | 28.90 A |
| Internode length (cm) | 2.31 b | 3.99 a | 4.22 a | 1.79 C | 3.15 B | 3.71 A |
| Laterals (no.) | 2.08 a | 1.72 a | 0.83 a | 2.94 A | 3.78 A | 0.94 B |
| Lateral length (cm) | 38.43 a | 17.11 b | 12.53 b | 74.18 A | 38.66 B | 32.71 B |
| Cane biomass (g) | 1.73 b | 9.70 a | 4.46 b | 4.65 C | 8.00 B | 10.03 A |
| Cane leaf biomass (g) | 6.08 c | 22.63 a | 12.33 b | 13.21 B | 12.12 B | 18.24 A |
| Lateral stem biomass (g) | 6.24 a | 3.55 ab | 1.22 b | 3.84 A | 1.74 B | 1.11 B |
| Lateral leaf biomass (g) | 1.39 a | 0.87 ab | 0.42 b | 8.51 A | 6.16 A | 2.04 B |
| Total leaf biomass (g) | 7.47 c | 23.50 a | 12.75 b | 21.72 | 18.28 | 20.28 |
| Total stem biomass (g) | 7.97 b | 14.16 a | 5.68 b | 8.49 B | 9.74 AB | 11.14 A |
| Total biomass (g) | 15.43 b | 51.76 a | 18.43 b | 30.20 A | 28.02 A | 31.43 A |

[z] BC = Baby Cakes®; Ponca = Sweet-Ark® Ponca.

[y] AUHPC = Area under height progress curve.

[x] Means with the same letter within location in each row are not significantly different by Tukey's honest significant difference, $p \leq 0.05$. Lower-case letters indicate mean separation at Arkansas and upper-case letters indicate mean separation at North Carolina.

[w] Mean separation was not performed for main effect of cultivar on AUHPC and total leaf biomass in North Carolina or number of nodes in Arkansas as cultivar by P-Ca rate interaction effects were significant.

Table 9. Results of regression analysis for response variables with a significant rate effect in at least one location in the P-Ca experiment conducted on blackberry plants in Arkansas and North Carolina in 2021.[z]

| Rate | Internode length (cm) | Lateral length (cm) | Cane stem biomass (g) | Lateral stem biomass (g) | Total stem biomass (g) | Total biomass (g) |
|---|---|---|---|---|---|---|
| | | | | *Arkansas* | | |
| 0 mg·L$^{-1}$ | 2.93 | 30.60 | 6.86 | 3.19 | 10.97 | 40.11 |
| 125 mg·L$^{-1}$ | 1.89 | 15.28 | 4.90 | 2.88 | 7.78 | 22.73 |
| 250 mg·L$^{-1}$ | 1.73 | 22.19 | 4.12 | 4.94 | 9.06 | 22.78 |
| Regression model | Q[y] | NS | NS | NS | NS | NS |
| P-value | <0.01[x] | - | - | - | - | - |
| | | | | *North Carolina* | | |
| 0 mg·L$^{-1}$ | 3.42 | 100.22 | 9.31 | 4.28 | 13.59 | 33.98 |
| 125 mg·L$^{-1}$ | 2.61 | 23.67 | 6.82 | 1.15 | 7.98 | 27.30 |
| 250 mg·L$^{-1}$ | 2.62 | 21.66 | 6.55 | 1.25 | 7.80 | 28.37 |
| Regression model | Q | Q | L | Q | Q | Q |
| P-value | 0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.03 |

[z] Results represent overall least square means of the three blackberry cultivars (Baby Cakes®, Sweet-Ark® Ponca, and Natchez) at the specified treatment rate.

[y] L = linear model; Q = quadratic model; NS = not significant.

[x] P-value is for the specified regression model.

Table 10. Results of regression analysis for response variables that had a significant cultivar by P-Ca rate in at least one location in the P-Ca experiment conducted on blackberry plants in Arkansas and North Carolina in 2021.[z]

| | BC[y] | Ponca | Natchez | BC | Ponca | Natchez | BC | Ponca | Natchez | BC | Ponca | Natchez |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rate | | AUHPC[x] | | | Height change (cm) | | | Nodes (no.) | | | Total leaf biomass (g) | |
| | | | | | | | *Arkansas* | | | | | |
| 0 mg·L$^{-1}$ | | 2398 | | 34.75 | 79.00 | 105.17 | 20.50 | 33.00 | 38.00 | | 15.04 | |
| 125 mg·L$^{-1}$ | | 1601 | | 18.00 | 28.67 | 33.33 | 20.33 | 30.50 | 30.00 | | 14.95 | |
| 250 mg·L$^{-1}$ | | 1519 | | 14.33 | 23.67 | 18.83 | 23.00 | 28.67 | 25.50 | | 13.72 | |
| Model | | L[w] | | L | Q | L | NS | NS | L | | NS | |
| P-value | | <0.01[v] | | <0.01 | 0.01 | <0.01 | - | - | <0.01 | | - | |
| | | | | | | | *North Carolina* | | | | | |
| 0 mg·L$^{-1}$ | 1641 | 2345 | 3742 | 42.60 | 46.63 | 108.27 | | 30.33 | | 22.10 | 18.35 | 20.72 |
| 125 mg·L$^{-1}$ | 1258 | 1613 | 2747 | 21.73 | 8.82 | 35.90 | | 24.72 | | 18.25 | 18.09 | 21.62 |
| 250 mg·L$^{-1}$ | 1246 | 1678 | 2439 | 23.42 | 10.07 | 16.72 | | 23.50 | | 24.80 | 18.40 | 18.51 |
| Model | L | Q | L | L | L | Q | | L | | Q | NS | NS |
| P-value | 0.01 | 0.02 | 0.01 | 0.03 | 0.01 | <0.01 | | <0.01 | | 0.04 | - | - |

[z] For response variables with no significant cultivar by P-Ca rate interaction (AUHP, results represent the overall least square mean of the three blackberry cultivars (Baby Cakes®, Sweet-Ark® Ponca, and Natchez) at each specified treatment rate.

[y] BC = Baby Cakes®; Ponca = Sweet-Ark® Ponca.

[x] AUHPC = Area under height progress curve.

[w] L = linear model; Q = quadratic model; NS = not significant.

[v] P-value is for the specified regression model.

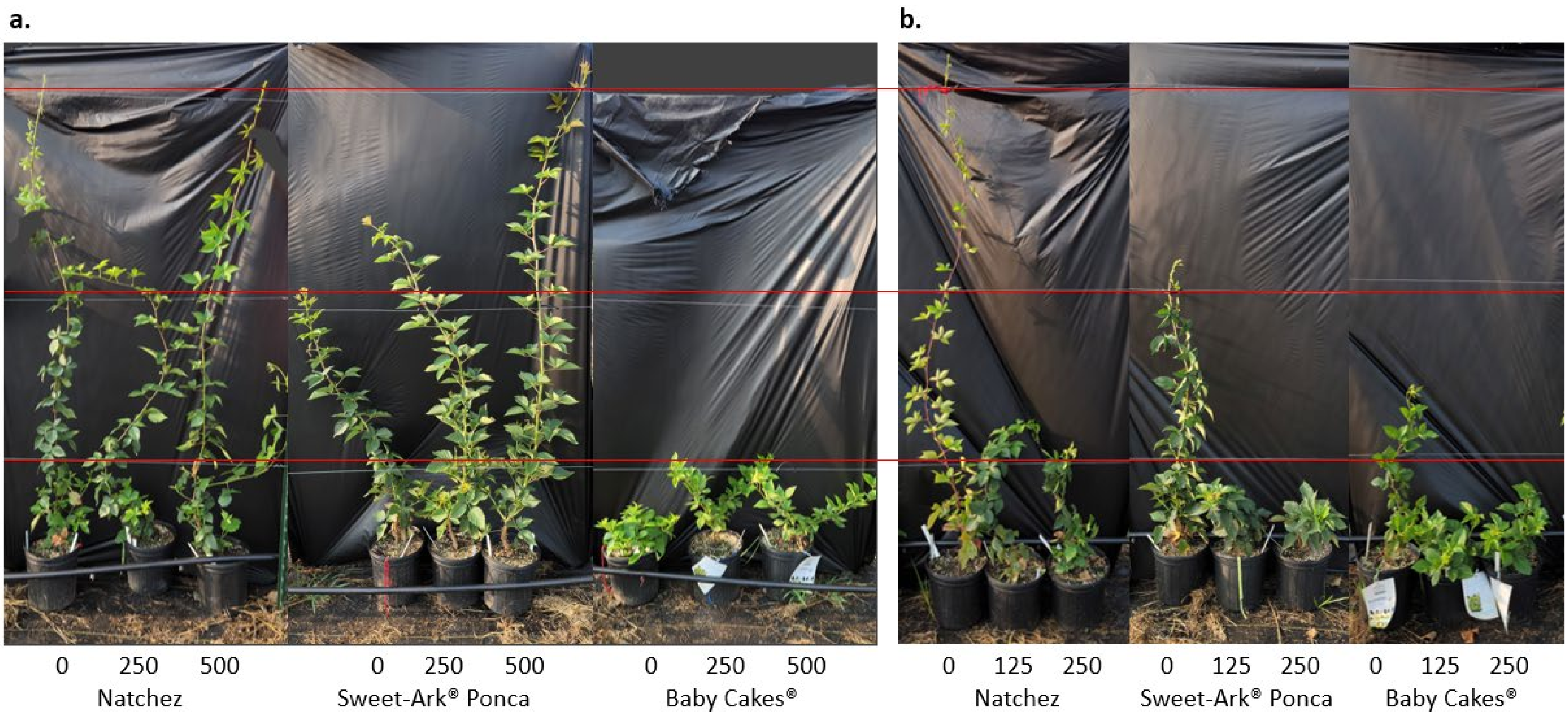

Fig.1. Three cultivars of blackberry plants treated with three rates of (a) gibberellic acid ($GA_3$) and (b) prohexadione calcium at Arkansas. Red lines highlight the trellis wires at approximately 60, 120, and 180 cm.

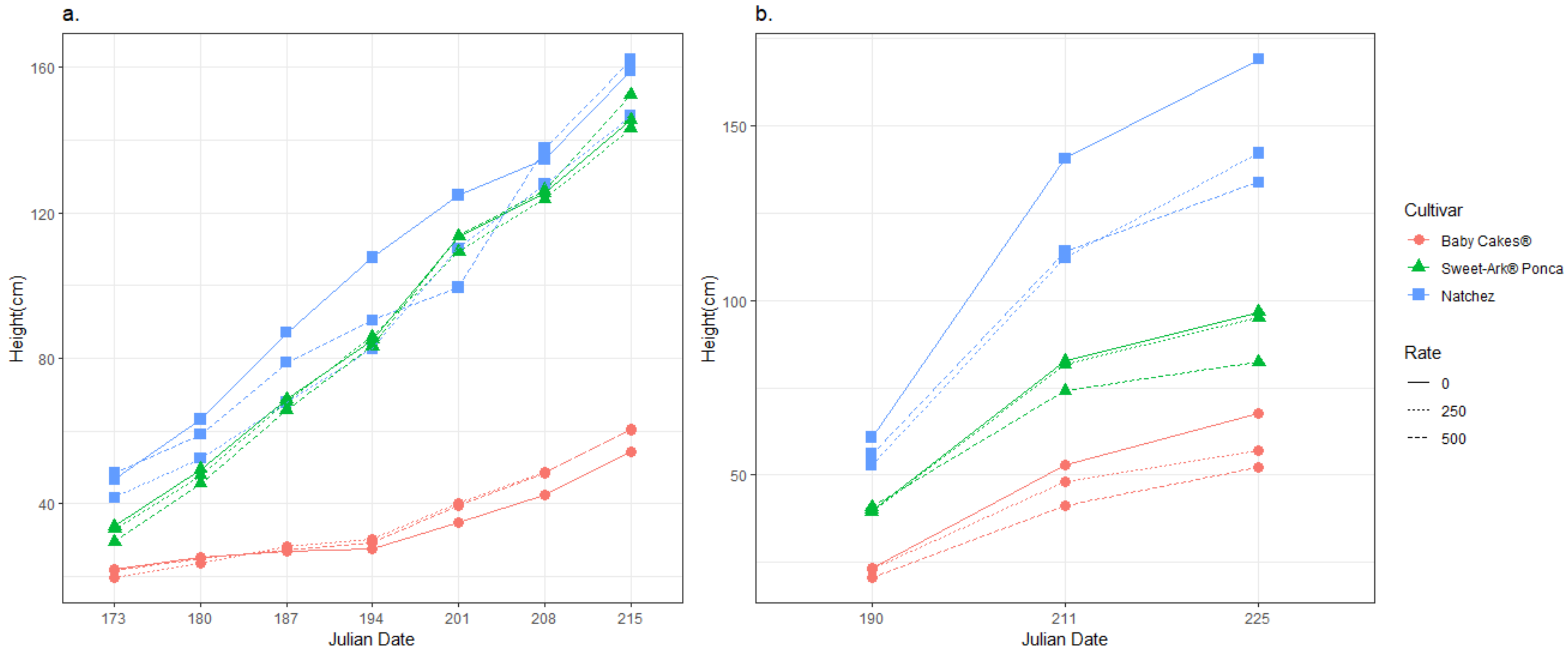

Fig. 2. Effects of $GA_3$ root zone drench applications on primocane height progression of three cultivars of blackberry in two locations; (a) Arkansas and (b) North Carolina.

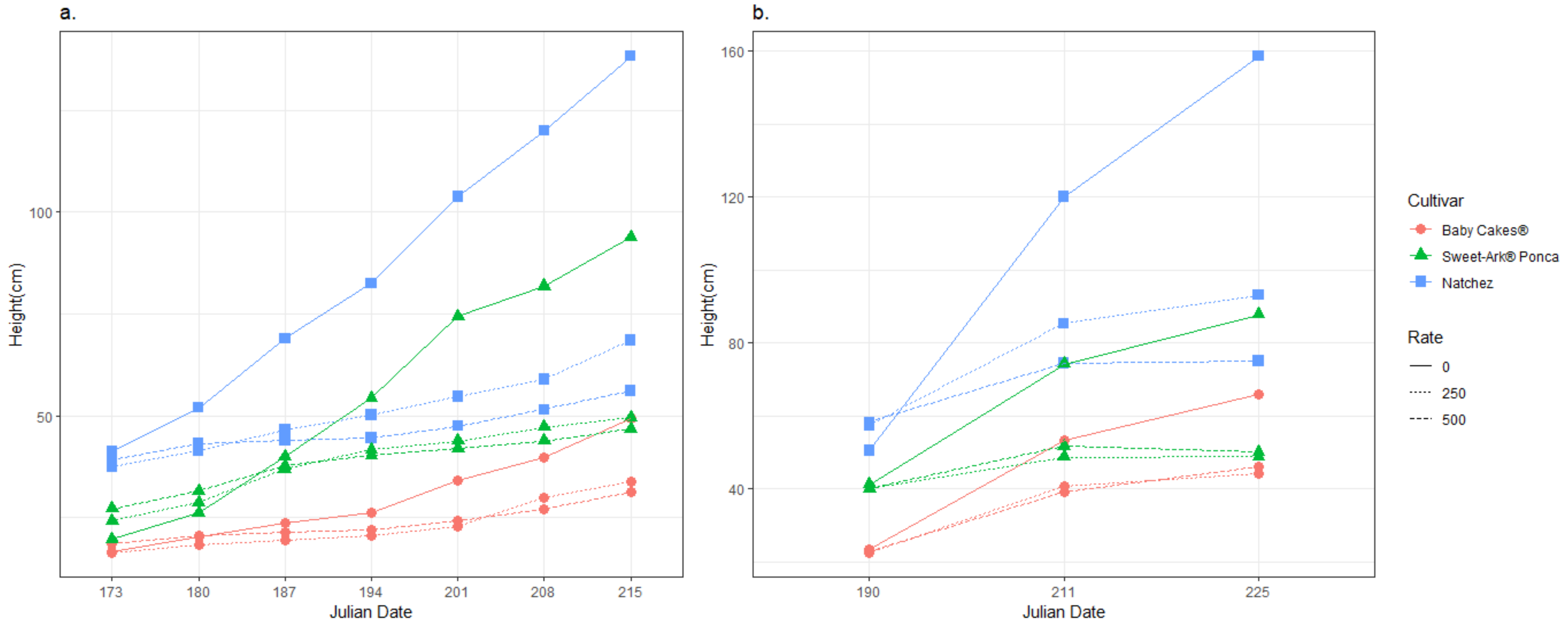

Fig. 3. Effects of P-Ca spray applications on primocane height progression of three cultivars of blackberry in two locations; (a) Arkansas and (b) North Carolina.

# OVERALL CONCLUSION

Despite growth in the fresh blackberry industry, development of new tools for breeding and evolution of growing systems have evolved little over the past few decades. Rising labor costs and disease pressure from cane blight, particularly in regions with significant annual rainfall, have presented new challenges in production that demand innovative approaches. The development of alternative methods for managing vigor of existing cultivars, as well as interest in new blackberry cultivars suitable for emerging high-density production systems has become more apparent; however traditional breeding is a time- and labor-intensive process. Genetic resources have been difficult to apply to erect and semi-erect blackberry given its ploidy, lack of sufficient marker coverage, and the lack of a high-quality reference genome. Recently, new tools have become available and enabled molecular breeding techniques for this increasingly popular fruit crop. The aim of this research was to perform a genome-wide association study (GWAS), and initiate the process for marker development for prickles and plant height in tetraploid blackberry. Complementary to the plant height aspect of the GWAS, two plant growth regulators (PGR), gibberellic acid ($GA_3$) and prohexadione calcium (P-Ca), with inverse effects on endogenous GA levels in plants were evaluated for their vegetative effects and potential uses in production systems on standard, semi-dwarf, and dwarf blackberry plants.

The GWAS uncovered a single locus controlling prickles and two QTL associated with plant height in tetraploid blackberry. The locus associated with prickles, containing five possible candidate genes and transcription factors, was located on Ra04 at 30484542 to 34309626 bp and fit the simplex dominant gene model. The location of the prickle-free locus is corroborated by other studies. One QTL, near three possible candidate genes associated with plant height, was located on Ra04 at 25959770 to 26529505 bp and fit the simplex dominant gene model. The second QTL contained two possible candidate genes associated with plant height and was located on Ra06 at 13940761 to 22402316 bp and fit the additive gene model. Three possible candidate genes identified, one *GA2ox6* and two *GA20ox1* genes, are involved in the GA-biosynthesis pathway. The remaining two candidates were *RGL1* genes that have a role in the GA-signaling pathway but are more highly expressed in reproductive organs and less likely to influence vegetative growth. Validation of the possible candidate genes and transcription factors discovered in this project will continue. Implementation of marker-assisted selection for prickle-free and dwarf seedlings will enhance valuable output from fresh-market blackberry breeding programs and help fulfill the need for cultivars that fit well into existing and high-density production systems.

The PGR study concluded that $GA_3$ and P-Ca applications each influenced vegetative growth of blackberry differently. In general, $GA_3$ increased lateral number, length and biomass, while P-Ca arrested cane height development and reduced internode length and number. ‘Natchez’ plants were most responsive to PGR applications, while ‘Ponca’ and ‘Baby Cakes’ had less dramatic responses to P-Ca and $GA_3$ application in many instances. P-Ca applications are effective for reducing plant height and could be a useful alternative to tipping primocanes. Early research on the response of standard blackberry cultivars to P-Ca indicated a need to mitigate the undesirable effects this chemical has on lateral development, and our study shows that $GA_3$ may be a useful solution to the negative yield impact observed. Delayed height development in the presence of P-Ca could be useful for vigor management early in the growing season, while applications of $GA_3$ may be beneficial later in the season to recover unintended effects of P-Ca on lateral production, indicating these PGRs may have complementary roles in blackberry production. Use of $GA_3$ and P-Ca are most promising for effective vigor management in field and long-cane blackberry production systems, although they have not been tested simultaneously on the same plant. Genetically dwarf plants likely fit best in vertical farming and tabletop production models of blackberry, whereas semi-dwarf plants would be ideal for field and long-cane production given their high number of nodes. Utilizing dwarf and semi-dwarf genetics is a way around incurring the cost associated with purchasing PGRs, so this should be considered by growers when establishing new plantings and choosing a production approach. A cost-benefit analysis is needed to determine whether use of PGRs is indeed a more economical approach to managing vigor and controlling cane blight than management practices currently in place. Long-term evaluation of the effects of $GA_3$ and P-Ca on fruit quality and yield are needed to determine if they are viable alternatives to current management techniques or fit in emerging production systems. Since neither evaluation of plants with varying architecture nor use of PGRs in blackberry production have been well-defined, this research provided a baseline understanding of the potential that varying plant architecture and PGRs may have in existing and emerging cultural management systems. While challenges will continuously exist in production, we are one step closer to speeding up the process of releasing superior blackberry cultivars that meet grower needs, and a step closer to understanding a new management technique that may be valuable in commercial fresh-market blackberry production.

# APPENDICES

## Appendix A

Supplementary Table 1. Genotypes alongside years of data collection (2020, 2021, or both years), normalized BLUPs for plant height (cm) and discrete ratings on a yes/no basis for presence of prickles used in final analyses.

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| Comanche | Both | 126.5 | y |
| Cherokee | Both | 144.9 | y |
| Cheyenne | Both | 114.4 | y |
| Shawnee | Both | 97.8 | y |
| Choctaw | Both | 144.8 | y |
| Navaho | Both | 144.5 | n |
| Kiowa | Both | 141.2 | y |
| Arapaho | Both | 96.5 | n |
| Chickasaw | Both | 123.2 | y |
| A-1790 | Both | 110.4 | y |
| Apache | Both | 173.9 | n |
| Ouachita | 2020 | 168.8 | n |
| A-1960T | Both | 113.4 | n |
| Natchez | Both | 141.4 | n |
| A-2252T | Both | 100.7 | n |
| Stella | Both | 143.8 | y |
| A-2316T | Both | 149.8 | n |
| Osage | - | - | n |
| A-2416T | Both | 143.9 | n |
| A-2418T | Both | 133.4 | n |
| A-2421 | Both | 137.2 | y |
| Sweet-Ark® Caddo | Both | 133.7 | n |
| A-2444T | Both | 123.5 | n |
| A-2453T | Both | 117.3 | n |
| A-2454T | Both | 119.0 | n |
| A-2473T | Both | 140.7 | n |
| A-2484T | Both | 140.7 | n |
| A-2491T | Both | 93.9 | n |
| A-2496 | Both | 114.1 | y |
| A-2500TN | Both | 43.0 | n |
| A-2506T | 2020 | 77.3 | n |
| A-2518T | - | - | n |
| A-2520T | Both | 163.2 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| A-2522T | Both | 65.6 | n |
| A-2524T | Both | 166.7 | n |
| A-2525TN | Both | 55.5 | n |
| A-2526T | Both | 128.9 | n |
| A-2527T | Both | 135.7 | n |
| A-2528T | Both | 135.4 | n |
| Sweet-Ark® Ponca | Both | 89.7 | n |
| A-2541T | Both | 142.0 | n |
| A-2545 | - | - | y |
| A-2547T | Both | 90.6 | n |
| A-2551TN | 2020 | 59.7 | n |
| A-2560T | Both | 117.2 | n |
| A-2563TN | 2020 | 69.8 | n |
| A-2568T | - | - | n |
| A-2571T | Both | 118.2 | n |
| A-2572T | Both | 154.3 | n |
| A-2575T | Both | 77.3 | n |
| A-2578T | Both | 102.3 | n |
| A-2580T | Both | 95.3 | n |
| A-2583T | - | - | n |
| A-2587T | Both | 134.1 | n |
| A-2596T | Both | 144.6 | n |
| A-2597T | - | - | n |
| A-2598T | - | - | n |
| A-2601T | - | - | n |
| A-2602T | Both | 129.6 | n |
| A-2604T | Both | 117.0 | n |
| A-2605T | - | - | n |
| A-2606T | Both | 108.7 | n |
| A-2609T | 2020 | 132.8 | n |
| A-2610T | Both | 115.2 | n |
| A-2611T | - | - | n |
| A-2613T | Both | 124.1 | n |
| A-2614T | Both | 140.0 | n |
| A-2615T | Both | 101.7 | n |
| A-2616T | 2020 | 140.8 | n |
| A-2617T | Both | 121.9 | n |
| A-2620T | Both | 161.7 | n |
| A-2624T | Both | 136.3 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| A-2625T | Both | 151.7 | n |
| A-2628T | Both | 129.9 | n |
| A-2636T | Both | 120.7 | n |
| A-2637T | Both | 115.0 | n |
| A-2639T | - | - | n |
| A-2642T | Both | 141.4 | n |
| A-2644T | 2020 | 162.7 | n |
| A-2651T | - | - | n |
| A-2652T | Both | 129.6 | n |
| A-2656T | Both | 133.9 | n |
| A-2657T | - | - | n |
| A-2658T | Both | 121.2 | n |
| A-2660T | Both | 135.8 | n |
| A-2663T | Both | 144.2 | n |
| A-2665T | 2020 | 133.1 | n |
| A-2668T | 2020 | 150.6 | n |
| A-2669T | Both | 82.4 | n |
| A-2672T | Both | 135.2 | n |
| A-2673T | Both | 109.3 | n |
| A-2674T | - | - | n |
| A-2676T | - | - | n |
| A-2677T | Both | 144.2 | n |
| A-2678T | Both | 109.2 | n |
| A-2679T | - | - | n |
| A-2680T | Both | 64.2 | n |
| A-2683T | - | - | n |
| A-2684T | Both | 106.8 | n |
| A-2685T | Both | 142.5 | n |
| A-2686T | Both | 97.3 | n |
| A-2687T | Both | 125.7 | n |
| A-2688T | - | - | n |
| A-2700T | Both | 85.0 | n |
| A-2701T | Both | 87.2 | n |
| A-2708T | Both | 139.3 | n |
| A-2709T | Both | 122.9 | n |
| A-2710T | Both | 132.2 | n |
| A-2711T | 2020 | 66.2 | n |
| A-2713T | Both | 120.5 | n |
| A-2716T | Both | 119.1 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| A-2717T | Both | 149.1 | n |
| A-2718T | Both | 81.0 | n |
| A-2720T | 2020 | 102.2 | n |
| A-2722T | Both | 118.1 | n |
| A-2723T | Both | 136.5 | n |
| A-2724T | Both | 142.3 | n |
| A-2725T | 2020 | 104.8 | n |
| A-2726TN | - | - | n |
| A-2728T | - | - | n |
| A-2730T | 2020 | 151.3 | n |
| A-2731T | 2020 | 133.8 | n |
| A-2733T | Both | 94.5 | n |
| A-2734T | - | - | n |
| A-2735T | - | - | n |
| A-2736T | - | - | n |
| A-2738T | 2020 | 179.8 | n |
| A-2739T | 2020 | 166.3 | n |
| A-2740T | Both | 167.1 | n |
| A-2742T | - | - | n |
| A-2743T | Both | 133.5 | n |
| A-2745T | 2020 | 176.0 | n |
| A-2746T | Both | 153.6 | n |
| A-2747T | 2020 | 127.7 | n |
| A-2749T | Both | 141.2 | n |
| A-2751T | Both | 85.3 | n |
| A-2752T | Both | 70.5 | n |
| A-2753T | 2020 | 152.8 | n |
| A-2755T | 2020 | 125.4 | n |
| A-2756T | Both | 138.2 | n |
| A-2757T | Both | 104.4 | n |
| A-2759T | Both | 147.0 | n |
| A-2760T | 2020 | 117.5 | n |
| A-2761T | 2020 | 148.8 | n |
| A-2762T | 2020 | 131.4 | n |
| A-2763T | 2020 | 79.4 | n |
| A-2764T | - | - | n |
| A-2765T | Both | 162.8 | n |
| A-2766T | 2020 | 130.4 | n |
| A-2767T | Both | 77.1 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| A-2768T | Both | 57.2 | n |
| A-2769T | - | - | n |
| A-2770T | 2020 | 184.5 | n |
| A-2771T | Both | 127.6 | n |
| A-2772T | 2020 | 89.1 | n |
| A-2773T | Both | 60.4 | n |
| A-2774T | Both | 130.2 | n |
| A-2775T | - | - | n |
| A-2777T | 2020 | 142.3 | n |
| A-2778T | Both | 110.8 | n |
| A-2780T | Both | 142.6 | n |
| A-2781T | Both | 139.0 | n |
| A-2782T | Both | 167.5 | n |
| A-2783T | Both | 124.2 | n |
| A-2784T | Both | 119.6 | n |
| A-2785T | Both | 159.1 | n |
| A-2786T | 2020 | 160.9 | n |
| A-2787T | Both | 151.3 | n |
| A-2788T | Both | 148.7 | n |
| A-2789TN | Both | 77.3 | n |
| A-2790TN | Both | 50.9 | n |
| A-2791TN | Both | 75.0 | n |
| A-2792TN | Both | 63.0 | n |
| A-2793TN | Both | 69.2 | n |
| A-2794TN | Both | 48.3 | n |
| A-2795TN | 2021 | 65.9 | n |
| A-2796TN | Both | 57.5 | n |
| A-2797T | Both | 107.7 | n |
| A-2798T | Both | 124.6 | n |
| A-2799T | Both | 138.4 | n |
| A-2800T | - | - | n |
| A-2801T | Both | 157.9 | n |
| A-2802T | Both | 130.2 | n |
| A-2803T | 2021 | 119.4 | n |
| A-2804T | 2021 | 126.7 | n |
| A-2805T | 2021 | 121.8 | n |
| A-2806T | 2021 | 133.4 | n |
| A-2807T | 2021 | 111.3 | n |
| A-2808T | 2021 | 79.1 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| A-2809T | 2021 | 130.7 | n |
| A-2810T | 2021 | 112.0 | n |
| A-2811T | 2021 | 93.3 | n |
| A-2812TN | - | - | n |
| A-2813TN | 2021 | 75.4 | n |
| A-2814TN | 2021 | 79.8 | n |
| A-2815T | 2021 | 128.9 | n |
| A-2816T | 2021 | 120.5 | n |
| A-2817T | 2021 | 134.8 | n |
| A-2818T | 2021 | 138.2 | n |
| A-2819T | 2021 | 156.3 | n |
| A-2820T | 2021 | 131.0 | n |
| A-2821T | - | - | n |
| A-2822T | - | - | n |
| A-2823T | 2021 | 143.7 | n |
| A-2824T | 2021 | 142.8 | n |
| A-2825T | - | - | n |
| A-2826T | - | - | n |
| A-2827T | - | - | n |
| A-2828T | - | - | n |
| A-2829T | - | - | n |
| A-2830T | - | - | n |
| A-2831T | - | - | n |
| A-2832T | - | - | n |
| A-2833T | - | - | n |
| A-2834T | - | - | n |
| A-2836T | - | - | n |
| A-2837T | - | - | n |
| A-2838T | - | - | n |
| A-2839T | - | - | n |
| A-2840T | - | - | n |
| A-2841T | - | - | n |
| A-2842TN | - | - | n |
| A-2843TN | - | - | n |
| A-2844TN | - | - | n |
| A-2845TN | - | - | n |
| A-2846TN | - | - | n |
| A-2847TN | - | - | n |
| Prime-Jim | Both | 110.8 | y |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| APF-122 | Both | 111.2 | y |
| Sharon's Delight | Both | 69.7 | y |
| Prime-Ark® Freedom | Both | 115.4 | n |
| Prime-Ark® Traveler | Both | 147.4 | n |
| Black Gem | Both | 105.6 | n |
| Baby Cakes® | Both | 60.3 | n |
| APF-238T | Both | 151.1 | n |
| APF-259TN | 2020 | 59.9 | n |
| Prime-Ark® Horizon | Both | 143.3 | y |
| APF-276TN | Both | 80.5 | n |
| APF-298TN | Both | 92.4 | n |
| APF-318 | Both | 89.9 | y |
| APF-328 | 2020 | 140.6 | y |
| APF-334T | Both | 138.5 | n |
| APF-335T | Both | 149.6 | n |
| APF-338 | - | - | y |
| APF-341TN | 2020 | 53.2 | n |
| APF-345T | - | - | n |
| APF-355TN | Both | 64.3 | n |
| APF-366T | Both | 99.7 | n |
| APF-370T | Both | 135.6 | n |
| APF-372T | Both | 112.5 | n |
| APF-373T | Both | 108.9 | n |
| APF-377TN | Both | 58.7 | n |
| APF-379TN | Both | 69.1 | n |
| APF-386TN | Both | 61.9 | n |
| APF-388T | - | - | n |
| APF-389TN | Both | 56.1 | n |
| APF-392TN | 2020 | 69.5 | n |
| APF-394T | - | - | n |
| APF-399T | - | - | n |
| APF-400T | Both | 114.8 | n |
| APF-404T | Both | 126.5 | n |
| APF-405T | 2020 | 164.0 | n |
| APF-406TN | Both | 63.1 | n |
| APF-407TN | 2020 | 58.5 | n |
| APF-409T | Both | 113.2 | n |
| APF-410T | 2020 | 119.1 | n |
| APF-414TN | Both | 84.0 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| APF-415T | Both | 168.3 | n |
| APF-423TN | - | - | n |
| APF-424TN | 2020 | 67.6 | n |
| APF-425T | Both | 87.0 | n |
| APF-426T | Both | 110.3 | n |
| APF-427T | 2020 | 102.1 | n |
| APF-428T | Both | 137.7 | n |
| APF-430 | - | - | y |
| APF-432T | Both | 128.0 | n |
| APF-434T | - | - | n |
| APF-435T | Both | 143.1 | n |
| APF-437T | 2020 | 134.4 | n |
| APF-439TN | Both | 65.2 | n |
| APF-44 | Both | 59.5 | y |
| APF-440 | Both | 90.1 | y |
| APF-441 | Both | 91.1 | y |
| APF-443T | 2020 | 126.3 | n |
| APF-444TN | Both | 57.9 | n |
| APF-447T | 2020 | 75.4 | n |
| APF-448T | Both | 106.4 | n |
| APF-449TN | Both | 53.9 | n |
| Prime-Ark® 45 | Both | 145.1 | y |
| APF-450TN | Both | 60.9 | n |
| APF-451T | 2020 | 95.6 | n |
| APF-452T | 2020 | 135.3 | n |
| APF-457TN | Both | 41.8 | n |
| APF-458T | Both | 59.5 | n |
| APF-459T | Both | 45.6 | n |
| APF-460 | Both | 112.2 | y |
| APF-464TN | 2020 | 68.6 | n |
| APF-465TN | 2020 | 89.5 | n |
| APF-466T | Both | 89.5 | n |
| APF-467T | 2020 | 111.1 | n |
| APF-468T | Both | 114.6 | n |
| APF-469T | Both | 112.9 | n |
| APF-470T | Both | 64.6 | n |
| APF-471T | Both | 120.7 | n |
| APF-472T | Both | 124.1 | n |
| APF-474T | 2020 | 122.8 | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| APF-475T | Both | 140.2 | n |
| APF-476T | - | - | n |
| APF-477T | Both | 133.8 | n |
| APF-478TN | Both | 41.2 | n |
| APF-479TN | Both | 65.7 | n |
| APF-480TN | Both | 83.7 | n |
| APF-481TN | Both | 76.0 | n |
| APF-482TN | Both | 41.2 | n |
| APF-483TN | 2020 | 74.7 | n |
| APF-484TN | Both | 45.6 | n |
| APF-485T | Both | 108.2 | n |
| APF-486TN | - | - | n |
| APF-487TN | 2021 | 94.7 | n |
| APF-488T | 2021 | 111.1 | n |
| APF-490TN | - | - | n |
| APF-491TN | 2021 | 95.6 | n |
| APF-492T | 2021 | 134.8 | n |
| APF-493 | 2021 | 135.6 | y |
| APF-494 | 2021 | 116.5 | y |
| APF-495T | 2021 | 128.5 | n |
| APF-496T | 2021 | 124.6 | n |
| APF-497T | 2021 | 167.9 | n |
| APF-498T | - | - | n |
| APF-499T | - | - | n |
| APF-500T | - | - | n |
| APF-501T | - | - | n |
| APF-502T | - | - | n |
| APF-503T | - | - | n |
| APF-504T | - | - | n |
| APF-505T | - | - | n |
| APF-506T | - | - | n |
| APF-507TN | - | - | n |
| APF-508TN | - | - | n |
| APF-509TN | - | - | n |
| APF-510TN | - | - | n |
| APF-511TN | - | - | n |
| APF-512TN | - | - | n |
| APF-513TN | - | - | n |
| APF-514TN | - | - | n |

| Genotype | Year of collection plant height | Plant height (cm) normalized BLUPs | Prickles (y/n) |
|---|---|---|---|
| APF-515TN | - | - | n |
| APF-516TN | - | - | n |
| APF-517TN | - | - | n |
| APF-518TN | - | - | n |
| APF-519TN | - | - | y |
| APF-520TN | - | - | y |
| APF-521TN | - | - | n |
| APF-522TN | - | - | n |
| APF-523TN | - | - | n |
| APF-524TN | - | - | y |
| APF-525TN | - | - | n |
| APF-526T | - | - | n |
| Black Magic | Both | 145.8 | y |
| Prime-Jan | - | - | y |
| APF-PBB1 | 2020 | 120.2 | n |
| Black Satin[z] | - | - | n |
| Brazos | - | - | y |
| Burbank Thornless | - | - | n |
| Chester Thornless | - | - | n |
| Darrow | - | - | y |
| Eclipse | Both | 175.5 | n |
| Eldorado | - | - | y |
| Galaxy | Both | 97.4 | n |
| Hillquist | - | - | y |
| Loch Ness | - | - | n |
| Merton Thornless | - | - | n |
| Raven | - | - | y |
| Tupy | Both | 166.7 | y |
| Von | Both | 168.1 | n |

[z] Black Satin, Brazos, Chester Thornless, Darrow, Eldorado, Loch Ness, Merton Thornless, and Raven were not grown at FRS. Prickle phenotypes were taken from Clark et al. (2007).