Title: Effects of ethylene-related plant growth regulators on blueberry ripening and postharvest storage

Progress Report

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Objectives: To evaluate the RATE of plant growth regulators, ACC, 1-MCP and AVG on ethylene and carbon dioxide evolution, ripening, and postharvest fruit quality in blueberries.

Justification and Description: Blueberry is an important fruit crop with numerous health benefits. With an increase in production and yield in blueberries over the past few years, it is important to develop tools that help to manipulate and/or synchronize ripening and in maintaining postharvest fruit quality during storage. Our own preliminary data indicate that blueberries can store well for 3-5 weeks depending on the cultivar, however supermarket fruit are often bruised and of poor quality. If we can better understand factors important for regulating blueberry ripening and postharvest storage, we can manipulate the progression of ripening, and improve or maintain fruit quality during postharvest storage.

During ripening, individual blueberry fruit on the branch mature at different rates resulting in a non-uniform ripening period extending over 2-3 weeks (Suzuki et al., 1997). However, factors that regulate ripening in blueberries is poorly understood. In terms of ripening, fruits can be classified as climacteric or non-climacteric. In climacteric fruits such as tomato, banana, and apple, ripening is accompanied by a peak in respiration (measured by an increase in CO_2 or O_2 consumption) and a burst in ethylene production. Ethylene production in climacteric fruits is required for normal ripening. However in non-climacteric fruits such as strawberry and grape, the role of ethylene and/or others signals are not very clearly understood (Klee and Giovannoni, 2011). <u>The mechanism of climacteric or non-climacteric respiration and ethylene production in the progression of fruit ripening is not clear in blueberry.</u> Some previous studies observed an increase in respiration and ethylene during ripening in blueberry suggesting a potential climacteric nature to the ripening process (Windus et al., 1976; El-Agamy et al., 1982; Suzuki et al., 1997). Further, external application of the ethylene-releasing compound, Ethephon, can accelerate progression of ripening (Eck et al., 1970; Dekazos, 1976; Ban et al., 2007). However, several other studies have classified blueberry as a non-climacteric fruit that does not show a substantial climacteric rise in respiration or ethylene evolution (Frenkel et al., 1972).

Some of our own preliminary data show that within 4-7 d after Ethephon application, compared to the control, the proportion of ripe fruit increased from 42% to 61% in ‘Premier’ and 46% to 83% in ‘Powderblue’, two rabbiteye blueberry genotypes. This indicated that the application of Ethephon can hasten the progression of ripening and reduce the time to harvest of blueberry fruit. In addition, some of our work from summer 2017, indicated that both rabbiteye and southern highbush blueberries display a peak in respiration and ethylene during the initiation of the ripening period of fruit development (Fig. 1).

After harvest of fruit, physiological changes such as softening, increased sugar accumulation and susceptibility to pathogens can rapidly deteriorate fruit quality. In many other fruit crops use of ethylene inhibitors affects ethylene production and/or perception, delays ripening, and maintains fruit quality during postharvest storage. Once ethylene is produced it binds to receptors and coordinates downstream signaling to regulate ripening and changes in fruit quality. Blocking ethylene biosynthesis (using aminoethoxyvinylglycine; AVG) or blocking ethylene perception (using 1-methylcyclopropene; 1-MCP) can down-regulate ethylene responses. Previous studies have indicated the use of 1-MCP to delay ripening and improve postharvest fruit quality in a number of crops such as apple, apricots, banana, avocado, plums, and tomato (Watkins, 2008). In blueberries, treatment with 1-MCP has been attempted before but has yielded mixed results on fruit quality after storage. In highbush blueberries, treatment with 1-MCP did not alter postharvest shelf life (DeLong et al., 2003). On the other hand, 1-MCP treatment in rabbiteye blueberries enhanced ethylene production and accelerated loss of fruit firmness (Maclean and Nesmith, 2011). In the above studies 1-MCP treatment was applied after harvest as a postharvest treatment well after the progression of the ripening stage of fruit development. The production of ethylene in blueberries is high during the initiation and early stages of ripening (and lowers considerably after harvest; Fig. 1), therefore application of 1-MCP after harvest may not be effective in retarding ripening and improving postharvest storage as these events are already under progression. Hence, preharvest treatments of 1-MCP during ripening (when ethylene production is high) may be important and need to be evaluated to determine the effects of 1-MCP on ripening and fruit quality. A single study on preharvest application of 1-MCP in blueberries indicated that the timing of application and differences in response among cultivars may induce variable effects on shelf-life (Blaker and Olmstead, 2014). It is possible that the variation in response to 1-MCP among cultivars is due to differences in ethylene production during ripening among cultivars, but this has not yet been explored.

The role of the ethylene biosynthesis inhibitor, AVG in delaying ripening, increasing firmness and preventing postharvest fruit drop has been studies in apples, pears, peaches and plum (Altuntas and Ozturk, 2013). In blueberries, AVG applications have been evaluated for bloom delay (Dekazos, 1979) and for abscission mitigation (Malladi et al., 2012). However, the effect of preharvest applications of AVG on ripening and its effect on fruit quality after harvest have not been investigated so far.

Experimental Methods and Results

Objective 1: To determine the evolution of ethylene and CO_2 in southern highbush and rabbiteye blueberry varieties during ripening and postharvest storage

Fruit were harvested from five southern highbush cultivars, ‘Emerald’, ‘Miss Alice Mae’, ‘Miss Lilly’, ‘Miss Jackie’, and ‘Rebel’ and six rabbiteye cultivars ‘Alapaha’, ‘Brightwell’, ‘Krewer’, ‘Powderblue’, ‘Premier’, and ‘Titan’, from various farms: Emerald, Miss Alice Mae and Rebel from Cornelius Farm, Manor, GA; Miss Jackie, Miss Lilly, Alapaha, Brightwell, Krewer, and Titan from UGA Blueberry Research Farm, Alapaha, GA, and Powderblue and Premier from Durham Horticulture Farm, Watkinsville, GA. Fruit were harvested at various developmental stages, which included: early growth stages, S1, S2 and S3; immature green; and ripening stages green, pink and ripe. Ripe fruit were brought back to be placed in the cooler at 4 °C and ~90% relative humidity for postharvest stages between 8 to 26 days. Early growth stages of S1, S2 and S3 were collected for only Premier and S3 for Powderblue. Respiration rates were determined in all southern highbush and rabbiteye blueberry cultivars. Ethylene production was quantified from four southern highbush (Emerald, Miss Jackie, Miss Lilly, Rebel) and four rabbiteye (Brightwell, Powderblue, Premier, Titan) blueberry genotypes.

Determination of respiratory rate: Fruit respiration was determined by measurement of CO_2 release at various stages of fruit development (as described above) using a closed system. Ten grams of fruit were placed in a 495 ml-jar for 1 hour. Headspace samples (50 mL) were analyzed by a CO_2 analyzer (Quantek, MA, United States, Model 902P) and results were expressed as $\mu L \cdot g^{-1} \cdot hr^{-1}$.

Determination of ethylene production: Ethylene production were measured using a closed system with fruit from various developmental stages. Around 25 grams of fruit were placed in a 135 mL jar for 4 hours. Headspace samples (1 ml) were analyzed by GC-17A gas chromatography (GC, Shimadzu, Japan) equipped with a Hayesep-N micropacked column and a flame ionization detector. The temperature of the injection port and the detector of GC were set at 200 °C. The column temperature was 60 °C for 4 min increased by 20 °C/min to 150 °C, and held at 150 °C for 1 min. Helium was used as the carrier gas. The peak area from the resulting chromatograph and a standard curve generated using standard ethylene gas was used to determine the concentration of ethylene and expressed as ($nL \cdot g^{-1} \cdot hr^{-1}$).

Preliminary results

Increase in respiratory rate during blueberry ripening.

Overall, the patterns of respiration were similar across the blueberry cultivars studied (Fig. 1). In general, a respiratory peak was observed at the pink stage (Fig. 1A). Respiratory rates in southern highbush blueberry fruit were generally higher than that in rabbiteye blueberry (Fig. 1A and B). However, differences of respiration among cultivars were marginal, except for Rebel which displayed substantially higher respiratory rate than some of the other southern highbush blueberries.

Increase in ethylene during blueberry ripening

Ethylene production increased in both southern highbush and rabbiteye blueberries during ripening (Fig. 2). However, the pattern of ethylene evolution was different between southern highbush and rabbiteye blueberries. In southern highbush blueberry cultivars, the peak in ethylene levels during ripening declined during postharvest storage (Fig. 2A) whereas in rabbiteye blueberry fruit, ethylene did not further decline after ripening but remained constant during postharvest storage (Fig. 2B). In addition, ethylene concentration differed among cultivars. In southern highbush blueberries, Miss Lilly and Rebel had higher while Suziblue and Rebel had lower ethylene production (Fig. 2 A). In rabbiteye blueberries, 'Titan' and 'Premier' had higher while 'Powderblue' had lower ethylene production (Fig. 2 B).

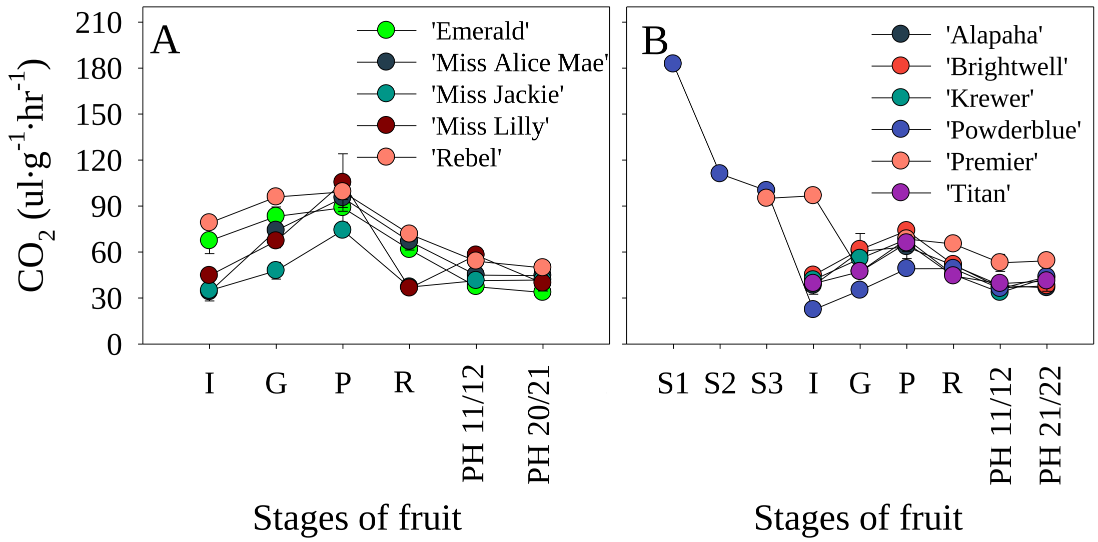

Fig. 1. The changes in respiration rates of southern highbush (A) and rabbiteye (B) blueberries during ripening and postharvest stages in 2018. I: Immature green, G: green, P: pink, R: ripe, PH: postharvest (days), S1-3: Stage 1-3. Data shown are means ± standard errors.

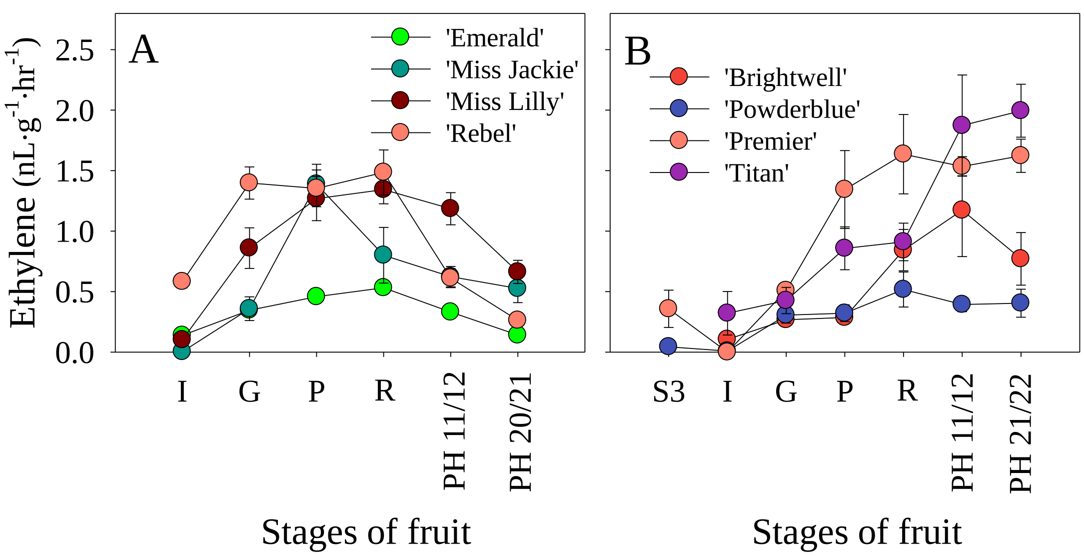

Fig. 2. The changes in ethylene production of southern highbush (A) and rabbiteye blueberries (B) during ripening and postharvest stages in 2018. I: Immature green, G: green, P: pink, R: ripe, PH: postharvest (days), S3: stage 3. Data shown are means ± standard errors.

Objectives 2 and 3: To study the effect of preharvest application of 1-MCP and AVG, on the production of ethylene, CO_2 and ripening; and to evaluate the effect of 1-MCP and AVG on postharvest fruit quality during storage.

The effect of the ethylene precursor, ACC (1-aminocyclopropane-1-carboxylic acid), the ethylene biosynthesis inhibitor, AVG and ethylene perception inhibitor 1-MCP (1-methylcyclopropene) was determined. Powderblue plants at the Durham Horticulture Farm in Watkinsville, GA were treated with control (water), 250 $mg \cdot L^{-1}$ ACC (0.1% Latron B-1956 as surfactant), 300 $mg \cdot L^{-1}$ AVG (0.05% silwet L77 as surfactant), or 6600 $mg \cdot L^{-1}$ 1-MCP in summer 2018. Each PGR treatment had its own control due to separate surfactant requirements of these PGRs. The experimental design was randomized complete block design with four replicates (blocks) for ACC and AVG treatments. The 1-MCP treatment with its control used a completely randomized design with four replicates. All other methods to determine fruit ripening and postharvest fruit quality attributes were followed as outlined above.

Preliminary results indicate that similar to ethephon, ACC promoted blueberry fruit ripening. ACC treated fruit had lower proportion of green fruit and had higher proportion of pink and ripe fruit than in control (by 2.3 and 3-fold respectively) at 4 days after treatment and this trend continued until 10 days after treatment (Fig. 3 A-C). ACC treated fruit displayed lower total soluble solids at 0 and 28 days of cold storage (Table 2). In addition, it resulted in lower juice pH at 28 days cold storage (Table 2).

The ethylene inhibitors, AVG and 1-MCP did not delay fruit ripening. AVG treated fruit had higher proportion of ripe fruit than in control (by 1.5-fold) at 2 days after treatment (Fig. 3 D-F). In addition, 1-MCP treated fruit had higher proportion of pink fruit than in control (by 2-fold) at 4 days after treatment and had lower proportion of green fruit at 7 days after treatment (Fig. 3 G-I). As Powderblue has low endogenous ethylene production (described above), the effect of ethylene inhibitors on this cultivar may be limited. 1-MCP treated fruit had higher firmness (puncture) at harvest (Table 1). AVG treated fruit had lower titratable acidity and higher juice pH at harvest (0 day of cold storage, Table 2).

Conclusions:

Southern highbush and rabiiteye blueberry cultivars displayed an increase in the rate of respiration and ethylene evolution at the onset of ripening. There were some blueberry type differences in these traits. Further, there were also some genotype-based differences in the above traits. Overall, the PGR data indicate that ACC promotes fruit ripening in blueberry, while the other PGRs, AVG and 1-MCP, did not display an effect on Powderblue fruit ripening. The effects of PGRs are often cultivar dependent. As Powderblue appears to display low endogenous ethylene production, it may not be as responsive to treatments that lower ethylene biosynthesis or perception. Hence, it is important to evaluate the effects of these PGRs on multiple genotypes and different types of blueberry. This will be the focus of further research. Additionally, the effects of these PGR applications on ethylene emission and respiration will also be evaluated.

Potential Impact:

Data from this study demonstrate differences in respiration and ethylene evolution characteristics across blueberry types and genotypes. This information is potentially useful in better determining harvest and postharvest fruit quality characteristics of these fruit. Further, this information may be used in better defining postharvest storage conditions for these fruit types. The data presented here also shows that PGRs such as ACC may have potential in concentrating ripening in blueberry fruit. This can potentially reduce the number of harvests and reduce production costs. Further evaluation of these PGRs will determine the broad applicability of these PGRs on southern highbush and rabbiteye blueberry genotypes. This can potentially reduce the number of harvests and reduce production costs. Further evaluation of these PGRs will determine the broad applicability of these PGRs on southern highbush and rabbiteye blueberry genotypes.

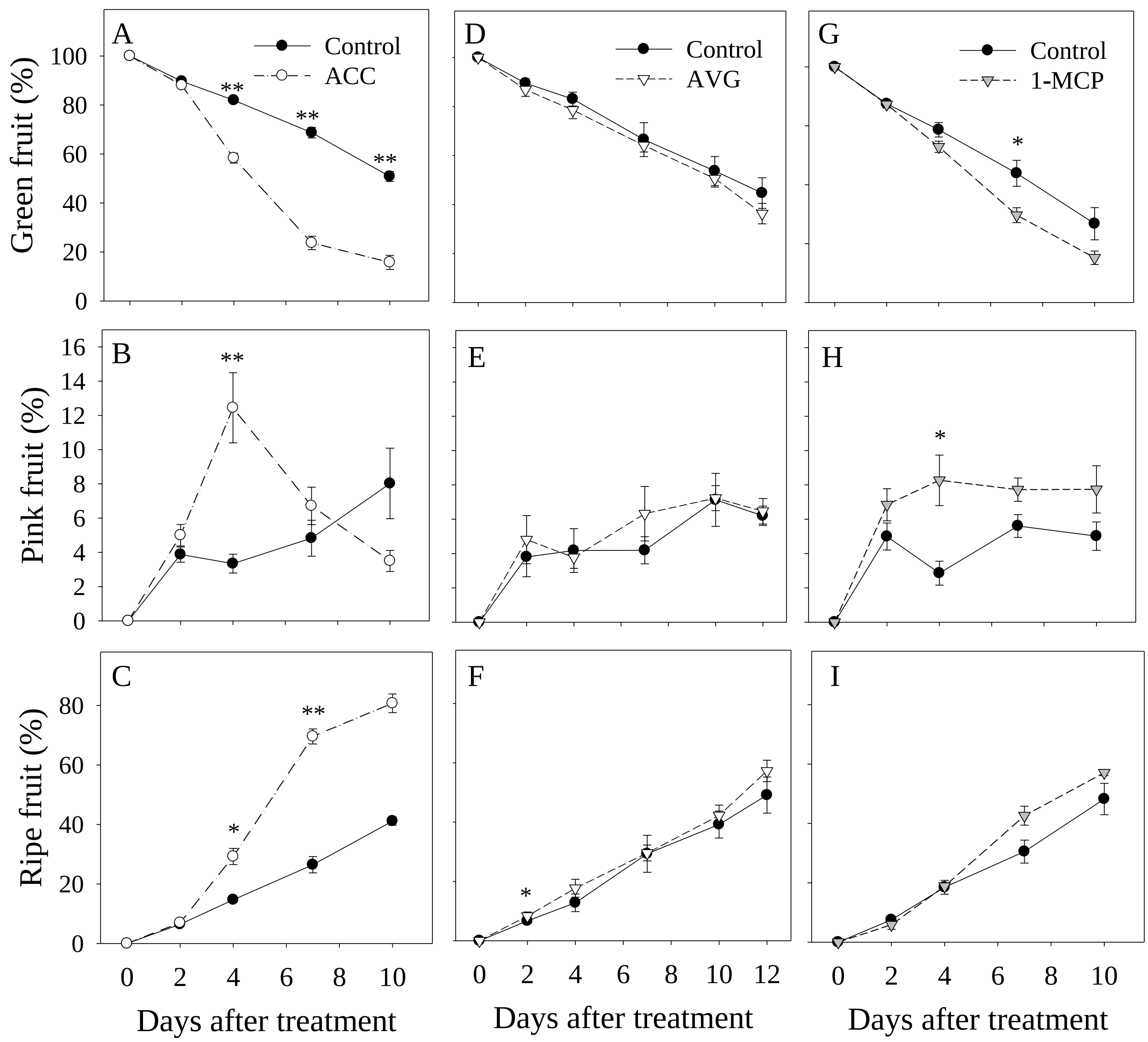

Fig. 3. Effect of preharvest treatments with water (control), ACC, AVG, and 1-MCP on ripening in Powderblue blueberry.

Table 1. Effect of preharvest treatment with ACC, AVG, and 1-MCP on fruit texture and weight sampled at 0, 14, and 28 days cold storage at 4 ^{o}C in Powderblue blueberry in 2018.

	Berry Texture								
	Compression (kgF)			Puncture (kgF)			Berry Weight (g)		
Treatment	**0 d**	**14 d**	**28 d**	**0 d**	**14 d**	**28 d**	**0 d**	**14 d**	**28 d**
Control	0.25	0.22	0.21	0.16	0.15	0.16	1.07	0.98	0.83
ACC	0.25	0.24	0.24	0.16	0.15	0.16	0.94	0.97	0.86
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Control	0.24	0.21	0.20	0.17	0.14	0.15	1.03	0.97	0.88
AVG	0.24	0.21	0.19	0.17	0.15	0.16	1.01	0.95	0.98
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Control	0.23	0.20	0.20	0.15	0.13	0.15	1.11	1.12	0.98
1-MCP	0.26	0.26	0.23	0.17	0.16	0.16	1.08	1.03	1.03
Significance	*NS*	*NS*	*NS*	*0.0388*	*NS*	*NS*	*NS*	*NS*	*NS*

Table 2. Effect of preharvest treatment with ACC, AVG, and 1-MCP on fruit quality sampled at 0, 14, and 28 days cold storage at 4 ^{o}C in Powderblue blueberry in 2018.

	Total Soluble Solids (^{o}Brix)			Titratable Acidity (%)			Juice pH		
Treatment	**0 d**	**14 d**	**28 d**	**0 d**	**14 d**	**28 d**	**0 d**	**14 d**	**28 d**
Control	14.73	14.63	14.63	0.54	0.46	0.49	3.22	3.32	3.36
ACC	12.75	13.40	13.10	0.63	0.54	0.60	3.19	3.27	3.26
Significance	*0.0362*	*NS*	*0.0388*	*NS*	*NS*	*NS*	*NS*	*NS*	*0.0258*
Control	14.63	15.75	15.30	0.63	0.45	0.58	3.17	3.27	3.66
AVG	15.25	15.35	15.63	0.47	0.53	0.51	3.24	3.27	3.33
Significance	*NS*	*NS*	*NS*	*0.0307*	*NS*	*NS*	*0.0392*	*NS*	*NS*
Control	14.98	15.20	15.40	0.50	0.47	0.44	3.26	3.33	3.36
1-MCP	15.45	14.95	15.28	0.55	0.52	0.43	3.25	3.28	3.37
Significance	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*

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