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The Effect of Ethephon, Abscisic Acid, and Methyl Jasmonate on Fruit Ripening in Rabbiteye Blueberry (*Vaccinium virgatum*)

Yi-Wen Wang [1], Anish Malladi [1], John W. Doyle [1], Harald Scherm [2] and Savithri U. Nambeesan [1,*]

[1] Department of Horticulture, Miller Plant Sciences, University of Georgia, Athens, GA 30602, USA; yiwen.wang25@uga.edu (Y.-W.W.); malladi@uga.edu (A.M.); doylejw@uga.edu (J.W.D.)
[2] Department of Plant Pathology, Miller Plant Sciences, University of Georgia, Athens, GA 30602, USA; scherm@uga.edu
* Correspondence: sunamb@uga.edu

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Abstract: Ripening in blueberry fruit is irregular and occurs over an extended period requiring multiple harvests, thereby increasing the cost of production. Several phytohormones contribute to the regulation of fruit ripening. Certain plant growth regulators (PGRs) can alter the content, perception, or action of these phytohormones, potentially accelerating fruit ripening and concentrating the ripening period. The effects of three such PGRs—ethephon, abscisic acid, and methyl jasmonate—on fruit ripening were evaluated in the rabbiteye blueberry (*Vaccinium virgatum*) cultivars 'Premier' and 'Powderblue'. Application of ethephon, an ethylene-releasing PGR, at 250 mg L^{-1} when 30–40% of fruit on the plant were ripe, accelerated ripening by increasing the proportion of blue (ripe) fruit by 1.5–1.8-fold within 4 to 7 days after treatment in both cultivars. Ethephon applications did not generally alter fruit quality characteristics at harvest or during postharvest storage, except for a slight decrease in juice pH at 1 day of postharvest storage and an increase in fruit firmness and titratable acidity after 15 days of postharvest storage in Powderblue. In Premier, ethephon applications decreased the proportion of defective fruit at 29 days of postharvest storage. Abscisic acid (600–1000 mg L^{-1}) and methyl jasmonate (0.5–1 mM) applications did not alter the proportion of ripe fruit in either cultivar. These applications also had little effect on fruit quality characteristics at harvest and during postharvest storage. None of the above PGR applications affected the development of naturally occurring postharvest pathogens during storage. Together, data from this study indicated that ethephon has the potential to accelerate ripening in rabbiteye blueberry fruit, allowing for a potential decrease in the number of fruit harvests.

Keywords: plant growth regulators; ethephon; abscisic acid; methyl jasmonate; postharvest fruit quality

1. Introduction

Blueberries (*Vaccinium* spp.) contain bioactive compounds which offer potential health benefits and have witnessed a large increase in production over the last two decades [1,2]. Blueberries are native to North America and some common cultivated species include lowbush (*Vaccinium angustifolium* Ait.), northern highbush (*Vaccinium corymbosum* L.), rabbiteye (*V. virgatum* Ait.), and southern highbush (hybrids of *V. corymbosum*, *V. virgatum*, and *V. darrowii* Camp.) [3–5]. During fruit development, blueberry fruit on a branch mature at different rates, resulting in a non-uniform ripening period extending over 2 to 3 weeks [6]. As a result, blueberries intended for the fresh fruit market are hand

harvested three to five times depending on the variety. This makes harvesting a labor intensive and expensive component of blueberry production, requiring up to 520 h of labor/acre and costing up to $0.70 per pound of harvested fruit [7–9]. Concentrating the period of ripening could help reduce the required number of harvests and reduce costs associated with production. Ripening is regulated by multiple plant hormones such as ethylene, abscisic acid, auxins, and jasmonates [10]. External applications of plant growth regulators (PGRs) that influence the levels or activity of these plant hormones may alter the progression of ripening and thereby help in concentrating the period of fruit ripening for efficient harvesting. Therefore, understanding the progression of ripening and developing tools such as PGR applications can help in improving the efficiency of blueberry harvesting.

Fruit ripening is a coordinated process involving changes in fruit texture, color, flavor, and susceptibility to biotic and abiotic factors [11,12]. Although all fruit display these changes during ripening, fruits can be generally classified into one of two types depending on physiological and biochemical changes accompanying the initiation and progression of ripening: climacteric and non-climacteric. In climacteric fruits such as tomato (*Solanum lycopersicum*), banana (*Musa* spp.), and apple (*Malus* × *domestica*), ripening is accompanied by a peak in respiration and ethylene production [11–14]. In such fruits, once ethylene production is triggered at ripening, it is autocatalytic and is one of the key factors that regulate changes associated with ripening. Non-climacteric fruits, such as strawberry (*Fragaria* × *ananassa*) and grape (*Vitis vinifera*), do not exhibit an increase in respiration and ethylene in association with ripening. In these fruits, the role of ethylene and other signals in regulating ripening are not completely understood [12,14–16]. The roles of climacteric respiration and ethylene in the progression of fruit ripening in blueberry are unclear. Some previous studies observed an increase in respiration and ethylene during blueberry ripening, suggesting a potential climacteric nature to the ripening process [6,17,18]. Also, external application of the ethylene-releasing compound ethephon accelerated the progression of ripening and reduced the harvest time in blueberry [19–21]. However, several other studies have classified blueberry as a non-climacteric fruit that does not display a substantial climacteric rise in respiration or ethylene evolution [22]. Hence, further studies are required to better understand the contribution of ethylene in blueberry ripening, and to determine if manipulation of this plant hormone offers a viable option for controlling ripening.

Abscisic acid (ABA), another plant hormone, plays an important role in many developmental processes such as adaptation to stress and seed dormancy. In addition, recent work has suggested a role for ABA during ripening in climacteric as well as non-climacteric fruit. Abscisic acid concentration increases during fruit ripening in apple [23], orange (*Citrus sinensis*) [24], cherry (*Prunus avium*) [25], strawberry [26,27], and grape [28]. In strawberry, decreased expression of *9-cis-epoxycarotenoid dioxygenase* (*FaNCED1*), a gene coding for an enzyme involved in ABA biosynthesis, lowered ABA levels and prevented fruit from ripening normally [27]. In grape, ABA applications improved red color and helped achieve early harvest, underlining its potential for accelerating ripening [29–31]. Further, in tomato, ABA may function upstream of ethylene and induce the expression of ethylene biosynthesis genes to regulate ripening [32,33]. Similarly, in banana, ABA applications may enhance ethylene sensitivity and coordinate ethylene-regulated ripening [34]. In bilberry (*V. myrtillus* L.), which is closely related to blueberry, ABA has been implicated in the regulation of ripening [35]. In highbush blueberry (*V. corymbosum*), ABA levels increase at the onset of ripening and may be involved in regulating the production of flavonoids [36]. However, external applications of ABA delayed ripening and increased fruit firmness in southern highbush blueberry (*V. corymbosum* interspecific hybrids) [37]. Although these studies suggest a potential role for ABA in regulating blueberry ripening, it requires further investigation, especially to determine if external ABA applications can be used to reliably manipulate the progression of this process across different blueberry species.

Jasmonates are another group of phytohormones with well-characterized roles in defense responses and developmental processes such as senescence [38]. Jasmonates have been implicated recently in the regulation of fruit ripening [14,39]. In tomato and apple, jasmonates promoted ethylene biosynthesis by inducing the expression of genes involved in its biosynthesis [40]. In apple,

methyl jasmonate (MeJA) applications influenced the production of aromatic volatiles, an integral component of fruit flavor, in a cultivar-dependent manner [41]. In peach (*Prunus persica*), jasmonates delayed ripening [42]. Although MeJA had a negative effect on ethylene biosynthesis during ripening in peach, it still promoted anthocyanin biosynthesis [43]. In non-climacteric fruits such as cultivated strawberry and Chilean wild strawberry (*Fragaria chiloensis*), application of MeJA increased ethylene evolution and respiration, and promoted color development thereby accelerating ripening [44,45]. In raspberry (*Rubus idaeus*), MeJA application increased flavonoid content, total soluble solids (TSS) content, and total sugars, and lowered titratable acidity (TA), thus influencing multiple ripening characteristics [46]. Together, these emerging data suggest that the effect of jasmonates on fruit ripening may be species-specific, requiring further evaluation in the species of interest. Further, preharvest and postharvest applications of MeJA may not only improve fruit quality but also offer a protective role by limiting pathogen growth as seen in strawberry and peach [39]. MeJA applications on highbush blueberry resulted in changes in total sugar content, total anthocyanin content, and expression of anthocyanin biosynthesis genes in a cultivar-dependent manner [47]. However, the specific role of MeJA in blueberry ripening and its effect on postharvest fruit quality attributes is not clear and has not been investigated previously.

While the effects of multiple PGRs on fruit ripening have been evaluated in various fruit crops, these have not yet been tested extensively in blueberry. Blueberry production could greatly benefit from the use of PGRs that help manipulate the time of ripening. Hence, the main goal of this research was to evaluate three PGRs, ethephon, ABA, and MeJA, for their ability to alter the progression of ripening in two rabbiteye blueberry cultivars. These three PGRs were selected for further study due to previous research suggesting their potential as described above. Furthermore, as preharvest applications of these PGRs can influence postharvest fruit quality and storage characteristics including disease symptom development, the effects of their application on postharvest fruit quality and disease incidence were also evaluated.

2. Materials and Methods

2.1. Plant Material and PGRs

Two rabbiteye blueberry cultivars, Premier and Powderblue (both at 5 years since planting), grown at the Durham Horticulture Farm in Watkinsville, GA were used for this study. All applications were performed when around 30–40% of fruit on the plant were ripe. Whole plants were sprayed using a hand-held sprayer until run-off. For the early-maturing Premier, the treatments consisted of: control (water), 250 mg L^{-1} ethephon, 600 mg L^{-1} ABA, and 0.5 mM MeJA. All treatments were applied on 20 June 2016 along with an adjuvant (0.15% Latron B-1956; Simplot, Lathrop, CA, USA). The doses were determined based on preliminary studies. Applications on Premier were made in the evening close to sunset to minimize photo-destruction of ABA. For the later-maturing Powderblue, the same treatments were applied on 9 July 2016 except that the concentration of ABA and MeJA were increased to 1000 mg L^{-1} and 1 mM, respectively. Due to potential rainfall in the late afternoon, all applications on Powderblue were made early in the morning. For each treatment, four replicates consisting of four individual plants were used in both cultivars.

2.2. Rate of Ripening

Prior to PGR application, three 50 to 100-cm-long shoots, each consisting of a total of approximately 50–100 fruit, were tagged per replicate. Very small immature as well as ripe fruit were removed from the tagged branches. The number of green, pink, and ripe fruit was counted prior to and after PGR applications at regular intervals (2–4 days) up to 11 days and 13 days for Premier and Powderblue, respectively. Fruit counted as pink ranged from having around 25% pink color (75% green) to around 75% pink (25% blue) on the fruit surface. Fruit was considered ripe when the color of

the entire fruit was blue. The percentage of green, pink, and ripe fruit was calculated from these data for each assessment date.

2.3. Postharvest Fruit Quality and Disease Incidence

Two additional shoots containing around 300 fruit (total) were tagged on each replicate to study the effect of PGR applications on postharvest fruit quality and disease incidence. Very small immature and ripe fruit at the time of application were removed. Ripe fruit were hand-harvested approximately 10 days after application of PGRs and split into three groups for postharvest fruit quality analyses. These groups were randomly assigned to one of the following treatment periods for postharvest evaluation: PH + 1 (postharvest + 1 day); PH + 15 (postharvest + 15 days); and PH + 29 (postharvest + 29 days). For postharvest storage, fruit were placed in a walk-in cooler set to 4 °C and a relative humidity of 90–95%. For each storage period and replicate, around 40 fruit were used for fruit quality evaluation and around 60 fruit were used for disease incidence evaluation. For fruit quality analysis, visual evaluation of quality was conducted and weight, texture, pH, TA, TSS content, and berry color were measured. For visual evaluation of fruit quality, 30 fruit per replicate were scored for symptoms of bruising such as tears, dents, leakiness, and appearance of mold to determine the percent defective fruit. Fruit weight was measured on 20 fruit, using a balance (Quintix® Precision Balance, Sartorius, Bohemia, NY, USA). Fruit texture measurements were made using a fruit texture analyzer (GS-15, Güss Manufacturing, Strand, South Africa). Two tests, compression and skin puncture, were performed on 12 fruit per replicate for determining fruit texture by orienting the fruit on the equatorial plane. For compression analyses, a probe with a 15-mm diameter end plate was used with parameters set at a measure speed of 5 mm s^{-1} and measure distance of 1 mm. To measure skin puncture force, a 1.5-mm probe was used with parameters set at a measure speed of 5 mm s^{-1} and measure distance of 3 mm.

For measuring pH, TA, and TSS, juice from around 30 g of fruit was extracted using a blender followed by centrifugation for 10 min at 3901× *g* on a benchtop centrifuge (Allegra X-22, Beckman Coulter Life Sciences, Indianapolis, IN, USA). The supernatant was filtered through cheesecloth. Around 0.3 mL of supernatant was used to determine TSS using a digital handheld refractometer (Atago USA, Belleveue, WA, USA). To determine pH and TA, the supernatant was titrated using an automatic mini titrator (Hanna Instruments, Woonsocket, RI, USA) and alkaline titrant. The titrator has a pH electrode which provided an initial pH value of the supernatant before titration is initiated. For TA, the data are expressed as percent citric acid (CA) equivalents. Fruit color was determined on 20 fruit using a handheld colorimeter (3nh Technology Co., Shenzhen, China).

To determine natural postharvest disease incidence, fruits were maintained at 23–25 °C for 4 days after removing them from cold storage at the three postharvest intervals described above. Fruit displaying symptoms of disease and/or signs of plant pathogens were recorded using around 60 fruit per replicate. The associated pathogens were identified microscopically as described in Mehra et al. [48].

Statistical analysis (one-way analysis of variance for a randomized complete block design) was performed separately for every time-point after harvest using JMP Pro 12 (SAS Institute, Cary, NC, USA). Means were separated using Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$).

3. Results

3.1. Effect of PGR Application on Fruit Ripening

In both cultivars, the proportion of green fruit decreased while that of ripe fruit increased over the duration of the experiment (Figure 1). In Premier, ethephon-treated fruit had a lower proportion of green fruit than that in the control from 4 days after treatment (Figure 1A). At this stage, the proportion of pink fruit was higher in ethephon-treated fruit (Figure 1B). The proportion of ripe fruit was significantly higher in the ethephon treatment from 7 days after treatment compared with the control

(Figure 1C). At 7 days after treatment, 42% of the fruit were ripe in the control compared with 61% in the ethephon treatment. In contrast, treatment with ABA did not affect the proportion of green or ripe fruit but transiently increased the proportion of pink fruit at 7 days after treatment, compared with the control (Figure 1B). Similarly, treatment with MeJA did not alter the proportion of green and ripe fruit compared with the control, but increased the proportion of pink fruit at 2 days and 7 days after treatment (Figure 1B).

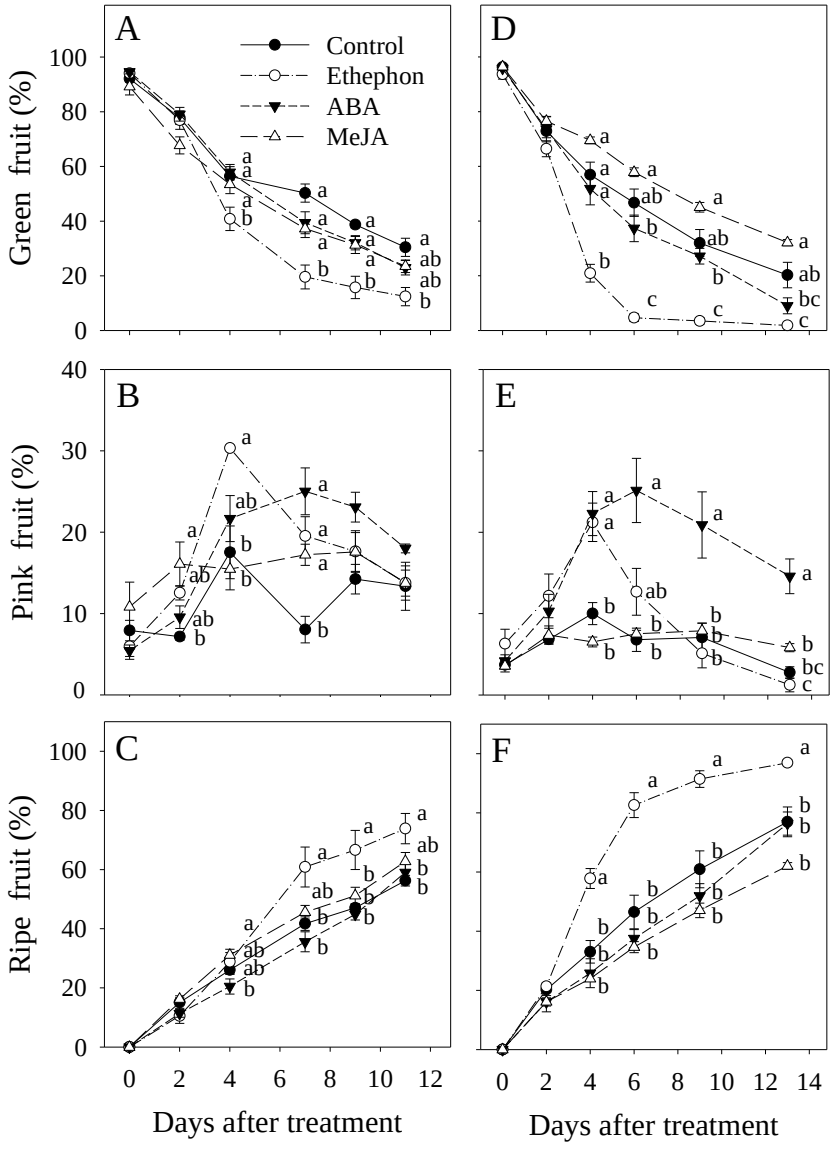

Figure 1. Effect of preharvest treatments with water (contol), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA) on ripening of rabbiteye blueberry, Premier (**A**–**C**) and Powderblue (**D**–**F**). Values are means and standard errors of four replicates. Within each assessment period, means with the same letter are not significantly different according to ANOVA and Tukey's HSD ($\alpha = 0.05$).

In Powderblue, the doses of application of ABA and MeJA were higher (see Section 2) as these PGRs did not appear to affect ripening in Premier at lower doses of application. The PGR applications generally resulted in similar effects on Powderblue fruit ripening as in Premier, with a few exceptions (Figure 1D–F). The proportion of green fruit was lower (Figure 1D), and ripe fruit was higher (Figure 1F) than in the control in ethephon-treated fruit starting from 4 days after treatment until the end of evaluation; and, the proportion of pink fruit was higher at 4 days after treatment (Figure 1E). At 4

days after treatment, while only 33% of the fruit were ripe on the control plants, around 58% were ripe in response to the ethephon treatment (Figure 1F). Application of ABA did not affect the proportion of green or ripe fruit compared with the control but increased the proportion of pink fruit from 4 days after application (Figure 1E). At the rate of ABA used in this study (1000 mg L^{-1}), phytotoxicity symptoms were observed in leaves (data not shown). Application of MeJA did not alter the proportion of green, pink, or ripe fruit at any stage after treatment in comparison with the control (Figure 1D–F).

3.2. Effect of PGR Application on Fruit Color

None of the fruit color-related parameters were significantly different among the PGR treatments in Premier at 1 day after harvest (Table 1). In Powderblue, treatment with ethephon and ABA also did not alter any of the fruit color-related parameters with respect to the control treatment at 1 day after harvest. In response to MeJA treatment, however, the parameters L*, which measures the lightness, and b*, which measures yellow/blue color, were higher and lower respectively, indicating lighter and greater blue fruit color than in the control (Table 1).

Table 1. Effect of preharvest treatment with water (control), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA) on fruit color after 1 day of cold storage at 4 °C in Premier and Powderblue blueberry.

Cultivar/Treatment [z]	L *	a *	b *	c *	h *
Premier					
Control	38.0	−1.1	−6.3	6.4	260.0
Ethephon	38.3	−1.0	−6.3	6.4	261.2
ABA	37.9	−1.0	−5.9	6.1	260.2
MeJA	38.1	−1.0	−6.3	6.5	260.9
Significance	NS	NS	NS	NS	NS
Powderblue					
Control	40.9b	−1.2	−6.37a	6.6ab	260.5
Ethephon	43.3ab	−1.3	−6.44ab	6.6ab	258.1
ABA	40.7b	−1.1	−6.16a	6.3b	260.1
MeJA	44.0a	−1.4	−6.77b	6.9a	258.6
Significance	0.0078	NS	0.0066	0.0063	NS

[z] Means followed by the same letter within a column are not significantly different, according to Tukey's HSD ($\alpha = 0.05$).

3.3. Effect of PGR Application on Fruit Quality during Postharvest Storage

Visual assessment of postharvest fruit quality using variables such as bruises, dents, and mold incidence during postharvest storage indicated a 20% increase in the percentage of defective fruit from 1 day until 29 days after storage in Premier (Table 2). There were no significant effects of the PGR treatments until 29 days after harvest in Premier (Table 2). At 29 days after harvest, ABA application resulted in a higher proportion whereas ethephon application resulted in a lower proportion of defective fruit compared with the control. In Powderblue the percentage of defective fruit increased by 15% from 1 day until 29 days after storage in control fruit; none of the PGR applications significantly affected the visually assessed variables for fruit quality (Table 2).

In Premier, fruit compression and puncture declined in the control by 18 and 20%, respectively, at 29 days after storage compared with 1 day after storage (Table 3). In Premier, ABA applications reduced the force required for fruit compression at 29 days of postharvest storage by ~17%, suggesting a decrease in fruit firmness relative to the control (Table 3). None of the other treatments affected fruit texture characteristics or the other fruit quality characteristics such as fruit weight, TSS, TA, and juice pH, evaluated during postharvest storage with respect to the control (Tables 3 and 4). In Powderblue, fruit compression and puncture declined in the control by 17% and 26%, respectively, at 29 days after storage compared with 1 day after storage (Table 3). In Powderblue, fruit firmness as measured by compression was higher by 16% in ethephon-treated fruit compared with the control at 15 days of

postharvest storage (Table 3). Fruit weight did not differ among various treatments during postharvest storage with respect to the control (Table 3). Ethephon treatment resulted in higher TA values than that in the control at various times after storage, although this was significant only at 15 days of postharvest storage (by 21%) (Table 4). TSS was lower in the ABA treatment than in the control at 15 days after harvest by ~11%. Also, juice pH was lower in response to ethephon and MeJA treatments than in the control at 1 day after harvest (Table 4).

Table 2. Percent defective fruit determined at various times after harvest in Premier and Powderblue blueberry following preharvest treatment with water (control), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA).

	Defective Fruit (%) [z]		
Cultivar/Treatment	**1 Day**	**15 Days**	**29 Days**
Premier			
Control	3.3	19.2	23.3b
Ethephon	2.2	7.8	10.0c
ABA	3.3	16.7	43.3a
MeJA	11.7	15.0	28.3ab
Significance	NS	NS	0.0003
Powderblue			
Control	5.0	13.3	20.0
Ethephon	5.0	6.7	19.2
ABA	4.2	8.3	18.3
MeJA	5.8	11.7	21.7
Significance	NS	NS	NS

[z] Means followed by the same letter within a column for a given time-point after storage are not significantly different, according to Tukey's HSD ($\alpha = 0.05$).

Table 3. Effect of preharvest treatment with water (control), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA) on fruit texture and weight sampled at 1, 15, and 29 days of cold storage at 4 °C in Premier and Powderblue blueberry.

	Berry Texture [z]								
	Compression (kgF)			Pressure (kgF)			Berry Weight (g) [z]		
Cultivar/Treatment	**Initial**	**15 d**	**29 d**	**1 d**	**15 d**	**29 d**	**1 d**	**15 d**	**29 d**
Premier									
Control	0.22	0.20	0.18a	0.15	0.15	0.12	0.81ab	0.82ab	0.81
Ethephon	0.23	0.20	0.19a	0.15	0.15	0.12	0.77ab	0.80ab	0.76
ABA	0.20	0.19	0.15b	0.14	0.14	0.11	0.86a	0.88a	0.79
MeJA	0.23	0.21	0.19a	0.15	0.16	0.13	0.70b	0.72b	0.70
Significance	NS	NS	0.0166	NS	NS	NS	0.0229	0.0067	NS
Powderblue									
Control	0.23	0.19b	0.19ab	0.19	0.15	0.14	0.87	0.83	0.82
Ethephon	0.26	0.22a	0.21a	0.18	0.15	0.15	0.64	0.68	0.69
ABA	0.24	0.20b	0.18b	0.18	0.15	0.14	0.84	0.83	0.89
MeJA	0.24	0.21ab	0.17b	0.19	0.16	0.15	0.80	0.77	0.78
Significance	NS	0.0077	0.0120	NS	NS	NS	NS	NS	NS

[z] Means followed by the same letter within a column for a given time-point after storage are not significantly different, according to Tukey's HSD ($\alpha = 0.05$).

Table 4. Effect of preharvest treatment with water (control), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA) on fruit quality sampled after 1, 15, and 29 days of cold storage at 4 °C in Premier and Powderblue blueberry.

	Total Soluble Solids (Brix) [z]			Titratable Acidity (%) [z]			Juice pH [z]		
Cultivar/Treatment	**1 d**	**15 d**	**29 d**	**1 d**	**15 d**	**29 d**	**1 d**	**15 d**	**29 d**
Premier									
Control	11.2	10.6	10.6	0.40	0.34	0.27	3.48	3.60	3.70
Ethephon	9.7	9.8	9.4	0.46	0.37	0.35	3.47	3.60	3.53
ABA	10.9	9.6	9.9	0.37	0.34	0.29	3.53	3.60	3.60
MeJA	10.5	9.8	9.9	0.44	0.36	0.30	3.43	3.58	3.70
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Powderblue									
Control	12.7	13.1a [z]	13.2	0.48	0.45b	0.36	3.48a	3.48a	3.45
Ethephon	12.0	12.1ab	12.6	0.56	0.54a	0.41	3.35b	3.38a	3.45
ABA	11.4	11.6b	12.4	0.49	0.50ab	0.36	3.40ab	3.40a	3.48
MeJA	12.1	13.0a	13.2	0.60	0.53ab	0.41	3.35b	3.38a	3.43
Significance	NS	0.0166	NS	NS	0.0490	NS	0.0150	0.0486	NS

[z] Means followed by the same letter within a column for a given time-point after storage are not significantly different, according to Tukey's HSD ($\alpha = 0.05$).

3.4. Effect of PGR Application on Postharvest Disease Incidence During Storage

The major postharvest pathogens indicated by disease symptoms and signs in this study were *Colletotrichum acutatum* (causal agent of anthracnose fruit rot), *Phomopsis vaccinii*, *Botrytis cinerea* (gray mold), *Alternaria* spp., and *Pestalotia* spp. Postharvest disease incidence in both Premier (typically < 5%) and Powderblue (typically < 10%) was low, despite the 4-day incubation period at room temperature following various postharvest storage periods. Due to low pathogen counts, only overall postharvest disease incidence was analyzed; no significant differences among treatments at different time intervals of storage were observed (Figure 2).

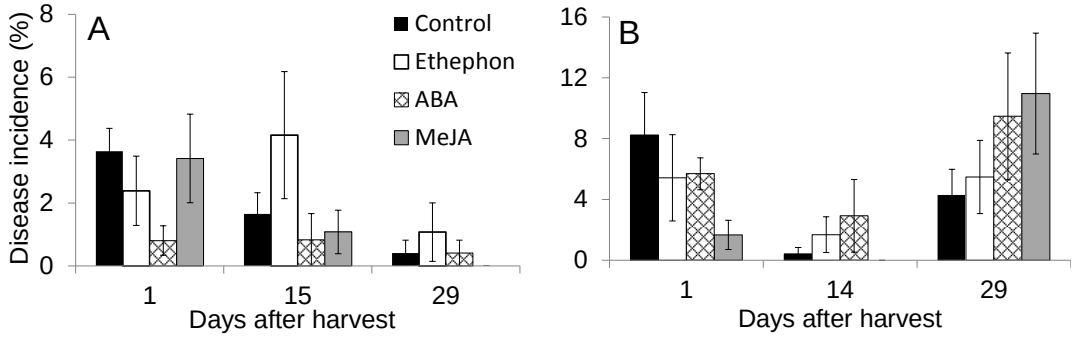

Figure 2. Percent postharvest disease incidence determined at various times after harvest in Premier (**A**) and Powderblue (**B**) rabbiteye blueberry following applications of water (control), ethephon, abscisic acid (ABA), and methyl jasmonate (MeJA). Values are means and standard errors of four replicates and 40 to 60 fruit per replicate. No significant differences ($\alpha = 0.05$) were detected among the treatments.

4. Discussion

Data from this study clearly indicate that both of the rabbiteye blueberry cultivars responded rapidly to ethephon applications. The time taken for 50% of fruit to ripen was advanced by up to 3 days after ethephon treatment compared with the control (Figure 1). Ethephon also increased the number of ripe fruit; ripe fruit increased from 42% to 61% in Premier at 7 days after application and 46% to 83% in Powderblue 6 days after treatment, indicating that the application of this PGR can accelerate

the progression of ripening and reduce the time to harvest in blueberry. Several previous studies demonstrated that ethephon accelerates ripening and can reduce the number of required harvests in blueberry [19–21,49]. Results from the current study further expand these findings of acceleration of fruit ripening by ethephon to rabbiteye blueberry.

For a PGR to be effective as a ripening aid, in addition to accelerating ripening it should display minimal negative effects on postharvest fruit quality. Ethephon treatment resulted in a substantial decrease in the proportion of defective fruit after postharvest storage (29 days), at least in Premier. However, fruit texture was not affected by ethephon application in Premier. In Powderblue, compression force at 15 days after ethephon treatment was slightly higher but was not different by 29 days after storage, indicating that ethephon had minimal and temporary effects on fruit firmness characteristics during postharvest storage. These data are generally consistent with those of Dekazos [20] who used rabbiteye blueberry. However, Ban et al. [21] reported substantial reduction in firmness in another rabbiteye blueberry cultivar, Tifblue, in response to ethephon. In that study, fruit slices were used for analysis of firmness rather than intact fruit as used in the current study as well as several others [20,50,51], which may explain the different observations. In the current study, no change in fruit weight in response to ethephon treatment was observed, consistent with results from a study on Tifblue treated with 200 mg L^{-1} ethephon [21], and in two highbush blueberry genotypes treated with similar doses of ethephon (240 mg L^{-1}) [19]. With similar or comparable (500 mg L^{-1}) doses of application, Eck [19] and Howell et al. [52] reported that ethephon did not affect TSS in highbush blueberry, consistent with results from the current study. In rabbiteye blueberry, Dekazos [20] reported no effect of ethephon on TSS even with repeated 500 mg L^{-1} applications or a single 1000 mg L^{-1} application. However, Ban et al. [21] reported an increase in TSS with ethephon applications at 8 days after treatment in Tifblue, although the effects of this application on postharvest storage were not evaluated in this study. Overall, it appears that ethephon applications do not generally alter TSS content in blueberry fruit during postharvest storage. Several studies have reported a decrease in TA after ethephon applications (ranging from 200 to 3840 mg L^{-1}) in highbush and rabbiteye blueberry [19–21]. In the current study, TA levels were unaffected by ethephon in Premier and slightly increased in Powderblue during postharvest storage (15 days). It is possible that the genotypes used here responded differently for this ripening parameter. Juice pH was generally not affected by ethephon treatment as has been seen previously [19,20], except at 1 day after storage. Dekazos [20] reported changes in fruit color parameters in response to ethephon, in contrast to that reported here. As indicated above, Dekazos [20] used repeated and higher doses of ethephon, which may explain the different results observed. Overall, data from this study suggest that ethephon application at 250 mg L^{-1} may have minimal effects on rabbiteye blueberry fruit quality during postharvest storage.

The role of ethylene in regulating blueberry ripening and postharvest quality is not completely clear [50]. Although a peak in respiration and ethylene production has been observed in blueberry in some studies [6,17,18], this was not conclusive in others [22]. Treatment of mature fruit with the ethylene perception inhibitor 1-MCP enhanced ethylene production, accelerated loss of fruit firmness, and had little effect on fruit quality characteristics in rabbiteye blueberry cultivars [50], underlining the complex and unclear role of ethylene in regulating blueberry ripening. Recently, based on the analysis of transcriptomics data during various stages of fruit development in highbush blueberry, Gupta et al. [53] indicated that genes associated with ethylene biosynthesis were abundant during the initiation of ripening, suggesting that ethylene may in fact play specific role(s) in modulating the progression of this process in blueberry fruit. The data presented in the current study demonstrating the effect of an ethylene-releasing compound on the progression of ripening further support a potential role for ethylene in the regulation of the ripening program in rabbiteye blueberry. These data indicate that blueberry fruit are responsive to external ethylene. Further studies evaluating the climacteric/non-climacteric nature of blueberry ripening are needed to better understand the potential role of ethylene in the regulation of this fruit developmental process. This information will also be critical for fine-tuning the timing of ethephon application in relation to fruit development.

In the current study, ABA generally did not affect the rate of ripening in blueberry fruit, even when applied at a rate of 1000 mg L^{-1}, although it increased the proportion of pink fruit. It may be likely that ABA (1000 mg L^{-1}) was able to stimulate the synthesis of anthocyanin pigments associated with pink color in the fruit. Phytotoxicity symptoms were observed in leaves when ABA was applied at 1000 mg L^{-1} (data not shown). Application of ABA did not consistently affect any of the fruit quality characteristics measured across the two cultivars except for compression in Premier at 29 days and TSS in Powderblue at 15 days after harvest. Furthermore, ABA applications appeared to increase the proportion of defective fruit at 29 days after storage in Premier. Overall, external ABA applications did not influence the progression of ripening in rabbiteye blueberry, in contrast to some previous reports with highbush blueberry and closely-related bilberry, where ABA concentration was found to increase during ripening leading to the hypothesis that it may regulate anthocyanin biosynthesis and other ripening related characteristics [35,36]. It may be that rabbiteye blueberry is less responsive to ABA or that the genotypes studied exhibited limited ABA responsiveness. Further analysis involving comparison of different blueberry genotypes and species may be needed to clarify any potential roles of ABA in blueberry ripening.

While MeJA applications have been noted to alter the progression of ripening in several fruits such as strawberry, raspberry, peach, apple, and tomato [14,39,43], in the current study MeJA application did not result in any consistent effects on the progression of ripening even when the application doses were at 1 mM. Further evaluation may be required to determine whether higher doses of MeJA can affect ripening in blueberry. However, previous studies have indicated that MeJA application at 10 mM and higher accelerate fruit detachment and result in extensive fruit drop in blueberry [54–56]. Hence, if higher doses of MeJA are successful at accelerating the progression of ripening, this needs to be optimized such that fruit detachment responses are not induced. Additionally, quantification of jasmonates during fruit development and specifically during ripening may help provide further insights into their potential roles in blueberry ripening.

5. Conclusions

Data from this study indicated that ethephon applied at a relatively low dose of 250 mg L^{-1} accelerated the progression of ripening in rabbiteye blueberries without altering many of the fruit quality characteristics. The other two PGRs tested, ABA and MeJA, did not appear to alter the progression of ripening in these cultivars. Further studies are needed to determine whether ethephon can consistently alter the ripening process in other types of blueberry, particularly southern highbush blueberry. In such studies, it may be essential to evaluate multiple doses of application, stages of application in relation to fruit development, and the time of day of application to determine the optimum application parameters for this PGR. Additionally, considering the response to ethephon, mechanisms involved in ethylene-mediated alteration of fruit ripening warrant further evaluation in blueberry.

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