Title: Effects of abscisic acid and methyl jasmonate on blueberry ripening, postharvest storage and fruit disease incidence

Progress report

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Research Proposal

Name, Mailing and Email Address of Principal Investigator(s):
Principal Investigator:
Savithri Nambeesan
Assistant Research Scientist
Department of Horticulture
1111 Miller Plant Sciences, University of Georgia, Athens, GA 30602
Email: sunamb@uga.edu; Ph: 706-542-0777

Co- Principal Investigator:
Anish Malladi
Associate Professor
1121 Miller Plant Sciences
Department of Horticulture, University of Georgia, Athens, GA 30602
Email: malladi@uga.edu; Phone: 706-542-0783

Co- Principal Investigator:
Harald Scherm
Professor and Assistant Dean for Research
2105 Miller Plant Sciences
Department of Plant Pathology, University of Georgia, Athens, GA 30602
Email: scherm@uga.edu; Phone: 706-542-1258

Objectives: To evaluate the effects of abscisic acid (ABA) and methyl jasmonate (MJA) on blueberry ripening, postharvest fruit quality and disease incidence

Although initially we planned to determine the effect of only ABA and MJA treatments on ripening, we also included ethephon (ETP) as an additional treatment to look at the effect of ethylene.

Justification and Description:
Blueberries are a major fruit crop in the Southeast with over 28,000 acres under production in Georgia alone. As acreage and production have expanded, bottlenecks associated with fruit harvesting and postharvest storage have become more acute. During ripening, individual berries on a shoot do not ripen uniformly requiring them to be harvested multiple times during the growing season (Suzuki et al., 1997). This greatly increases the cost of harvest. Identification and use of plant growth regulators (PGRs) that can accelerate and/or synchronize ripening would greatly reduce harvesting costs. Upon harvest, blueberries do not store well due to loss of firmness and/or and infections from plant pathogens such as *Colletotrichum*, *Botrytis* and

Alternaria species, among others. Physiological changes during storage such as fruit softening and increased sugar accumulation can also increase susceptibility to pathogens thereby leading to deterioration in fruit quality. Some PGRs such as MJA can affect pathogen responses in plants. Use of PGRs that not only promote ripening but also maintain postharvest fruit quality can greatly enhance the profitability of blueberry production.

A common growth regulator often used to accelerate ripening is the ethylene-releasing compound, ethephon. However, the role of ethylene in blueberry fruit development is largely unclear. Also, blueberry fruit do not always display ripening-related responses to exogenous ethylene (Frenkel, 1972; Janes et al., 1978; El-Agamy et al., 1982; Suzuki et al., 1997). In fact, treatment of mature fruit with 1-methyl cyclopropane (1-MCP), an ethylene perception inhibitor, enhanced ethylene production and accelerated loss of fruit firmness (Maclean and NeSmith, 2011). Little information is available regarding other growth regulators that may aid in uniform ripening in blueberries.

Recent work has provided evidence for the role of ABA and MJA during fruit ripening in many fruits such as tomatoes (Zhang et al., 2009; Fan et al., 1998), apples (Buesa et al., 1994; Fan et al., 1998; Kondo et al., 2005), oranges (Rodrigo et al., 2003), cherries (Kondo and Inoue, 1997), strawberries (Jia et al., 2011) and also bilberries which are closely related to blueberries (Karppinen et al., 2013). In blueberries, too, ABA may promote flavonoid biosynthesis during ripening (Zifkin et al., 2012). In grape, the application of ABA improved red color and has been suggested as an alternative to ethephon (Jeong et al., 2004; Peppi et al., 2006; Cantín et al., 2007). It also helped in early harvest without affecting postharvest quality attributes such as firmness, and fruit weight (Cantín et al., 2007). One study with southern highbush blueberries showed that ABA applications increase fruit firmness which was interpreted as delayed ripening (Buran et al., 2012). However, in this study percent ripe fruit were not affected by ABA treatments except at one picking for one cultivar, indicating that some of these responses may be cultivar-specific and need more detailed analysis. MJA plays an important role in regulating abscission responses in blueberries (Malladi et al., 2012; Vashisth and Malladi, 2013; Vashisth and Malladi, 2014). These data suggest that blueberry is responsive to MJA applications. However, the roles of MJA and other jasmonates in regulating blueberry ripening are largely unknown. Pre-harvest and postharvest applications of MJA have been shown not only to improve fruit quality but also to have a protective role in limiting pathogen growth in crops such as strawberries and peaches (reviewed in Rohwer and Erwin, 2008). We hypothesize that applications of ABA and MJA can result in more uniform ripening in blueberries and improve postharvest fruit quality by decreasing disease incidence.

Materials and Methods:

Plant Materials and Plant growth regulators:

Two RE varieties, Premier and Powderblue, approximately 5 years old and located at the Durham Horticulture Farm in Watkinsville, GA, were used for this study. All applications were performed when 30-40% of overall berries on the plant were ripe. Whole plants were sprayed using a hand-held sprayer. For Premier, 0.5 μM of MJA, 600 ppm of ABA, and 500 ppm of Ethephon along with an adjuvant (0.15% Latron B-1956) were applied. All applications were in the evening close to sunset to prevent photo-destruction of ABA and potential volatilization losses of MJA. For Powderblue, the concentration of MJA and ABA were changed to 1 μM of

MJA and 1000 ppm of ABA. Due to overcast sky with chance of showers in the evening, all the applications were performed early in the morning. Four replicates were used for every treatment.

Objective 1: Evaluate the effects of ABA and MJA applications on blueberry ripening

Before application, three branches consisting of 100-150 berries were tagged. Small berries and ripe berries were removed from all the tagged branches. The proportions of green, pink and ripe berries were counted. After application the proportions of green, pink and ripe berries were determined for each replicate at regular intervals until approximately 10 days (in Premier) to 2 weeks (in Powderblue) after spraying. The percentages of green, pink and ripe berries were determined.

Objective 2: Evaluate the role of ABA and MJA and ETP on postharvest fruit quality and disease incidence

To study the effect of postharvest fruit quality and disease incidence, two additional branches containing at least 300 berries in total were tagged on the same plant that contained tagged branches to evaluate progression of ripening. As with fruit ripening, small and ripe berries at the time of application were removed. Ripe fruit were harvested approximately 2 weeks after application of PGRs and split into three groups for postharvest fruit quality analyses. These groups were randomly assigned to one of the following time periods for postharvest evaluation: PH+3; PH+14; and PH+21 days. For every time period ~40 berries were used for fruit quality evaluation and ~60 berries for disease incidence. For fruit quality measurements, a visual evaluation of fruit quality, weight, texture, titratable acidity (TA), total soluble solids (TSS) content, and berry color were determined. For visual evaluation of fruit quality 30 berries per replicate were scored for any symptoms of bruising such as tear, dent, leakiness, or appearance of mold. Fruit weight was measured using a balance (Quintix® Precision Balance, Sartorius, Bohemia, NY). Firmness measurements were performed using a Fruit Texture Analyzer (GS-15, Güss Manufacturing (Pty) Ltd., Strand, South Africa). Two tests, compression and puncture were performed on 12 berries per replicate. For compression and puncture, berries were oriented on the equatorial plane and placed upright. For the compression test a 1.5-cm diameter plate was used with parameters set at forward speed of 6 mm/s; measure speed of 5 mm/s and measure distance of 1.00 mm. For the puncture test a 1.5-mm tip was used with parameters set at forward speed of 10 mm/s; measure speed of 5 mm/s and measure distance of 3.00 mm. For measuring TA and TSS, juice from 30 g of fruit was extracted using a Magic Bullet blender (https://www.getmagicbullet.com/) and centrifuged for 10 min at 4500 rpm using a benchtop centrifuge (Allegra® X-22, Beckman Coulter Life Sciences, Indianapolis, IN). The supernatant was filtered through a cheese cloth, and 300 µl of supernatant was used to determine TSS using a digital handheld refractometer (Atago USA, Belleveue, WA). To determine TA, the supernatant was titrated using an automatic mini titrator (Hanna Instruments, Woonsocket, RI). Fruit color was determined using a handheld colorimeter (3nh Technology Co., Shenzhen, China).

To determine natural postharvest disease incidence, fruit were maintained at 23-25°C for 4 days after removing them from cold storage at the three intervals described above. Fruit showing signs or symptoms of disease were recorded from approximately 60 fruits/replicate, and the associated pathogens were identified microscopically (Mehra et al., 2013).

RESULTS:

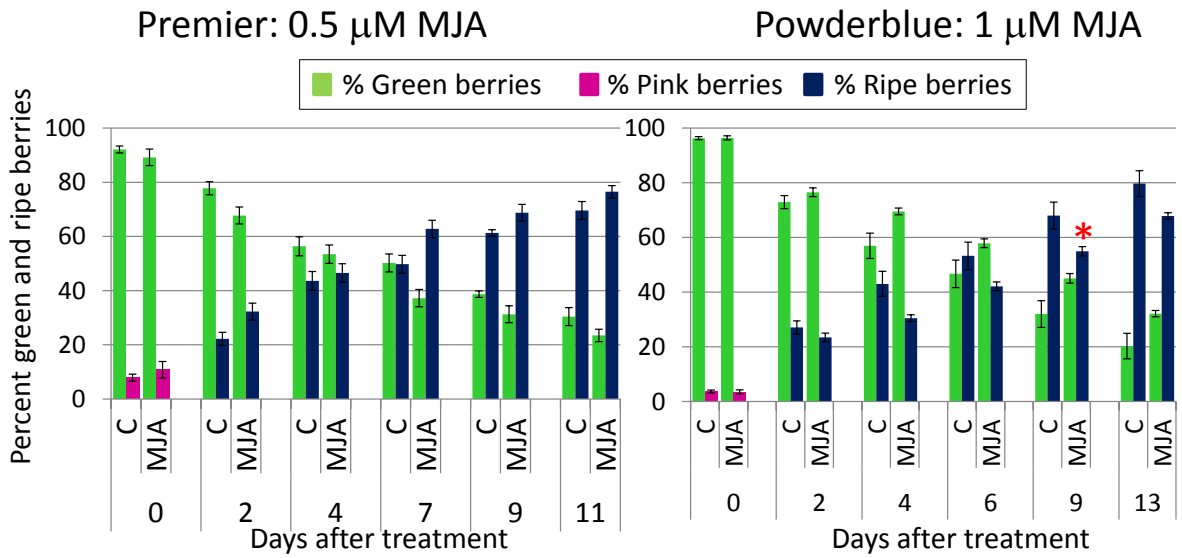
Effect of various treatments on rate of ripening

To determine ripening, percent green, pink and blue berries were determined under various treatments and control at regular intervals. At the day of application (day 0) all ripe berries were removed (explained in methods) and therefore data shown for day 0 represents % green and % pink berries. At all the other time points % green and % ripe berries are shown (% ripe berries includes ripe and pink berries). This applies for Figures 1, 2 and 3.

Effect of MJA treatment on ripening of blueberries

No effect of MJA (0.5 µM) on ripening of Premier blueberries were observed. Since we did not see a ripening effect for Premier we used a higher concentration of 1 µM for Powderblue. For Powderblue, 9 days after MJA treatment the percentage of ripe berries was significantly lower than the control, but at all the other times there were no difference in ripening rate due to MJA treatment (Figure 1). Overall no effect of MJA on ripening was observed with both varieties.

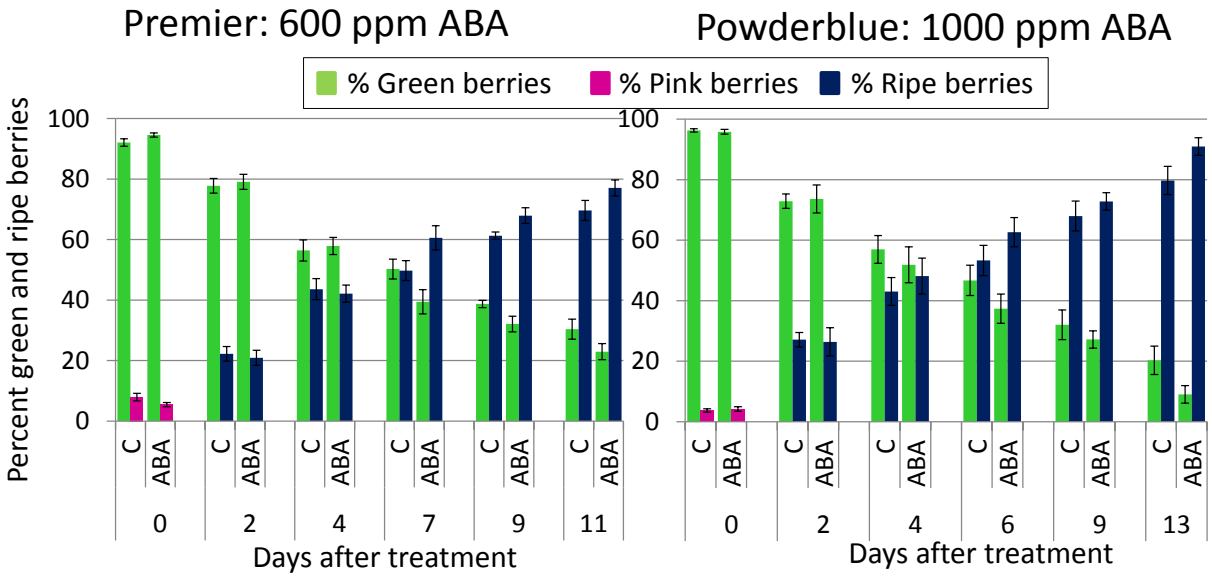
Figure 1: Effect of MJA treatment on ripening of blueberries



Effect of ABA treatment on ripening of blueberries

No differences in rate of ripening were observed after ABA treatment (600 ppm) with Premier berries. We increased the concentration to 1000 ppm for Powderblue and still did not see an effect on ripening (Figure 2). With 1000 ppm some phytotoxicity symptoms were observed on the leaves.

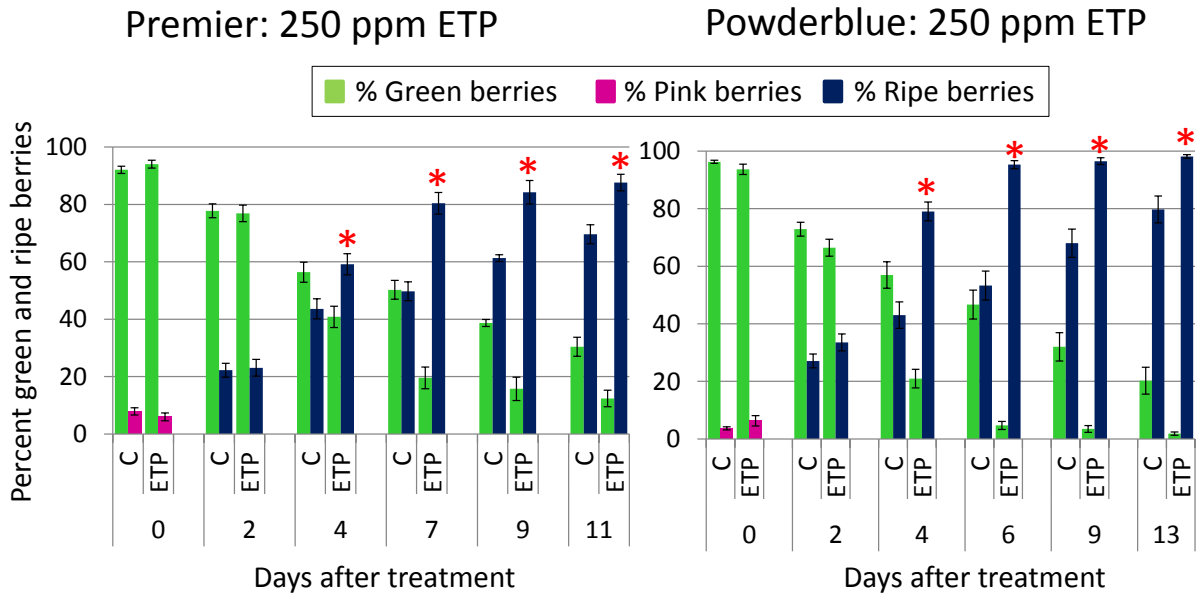
Figure 2: Effect of ABA treatment on ripening of blueberries



Effect of ETP treatment on ripening of blueberries

Treatment with ETP (250 ppm) accelerated ripening in both Premier and Powderblue (Figure 3). In Premier, within 7 days after treatment about 80% berries had ripened, whereas in the control only about 50% of the berries had ripened. The trend continued throughout ripening. In Powderblue, within 4 days after treatment about 80% berries had ripened, whereas in the control only about 50% of the berries had ripened. This trend continued throughout the remaining period of the study. The higher rate of ripening in Powderblue compared with Premier could be a varietal effect or be due to time of spray (morning application in Powderblue compared with evening application in Premier).

Figure 3: Effect of ETP treatment on ripening of blueberries



Effect of treatment on fruit quality attributes during storage in Premier

Other than some minor differences, there was no significant effect on fruit quality attributes that included, compression, puncture, weight, total soluble solids (TSS), titratable acidity (TA) and pH due to various treatments at various times during storage (Table 1). Also there was no difference in color of the berries after harvest (Table 2).

Table 1: Fruit quality attributes determined at various times during storage in Premier following in-field applications of growth regulators

Days after harvest	Treatment[a]	Compression	Puncture	Weight	TSS[b]	TA[c]	pH
1	C	0.22	0.15	0.81 ab	11.2	0.40	3.48
	MJA	0.23	0.15	0.70 b	10.5	0.44	3.43
	ABA	0.20	0.14	0.86 a	10.9	0.37	3.53
	ETP	0.23	0.15	0.77 ab	9.7	0.46	3.47
	Prob>F	*ns*	*ns*	*0.0229*	*Ns*	*ns*	*ns*
15	C	0.20	0.15	0.82 ab	10.6	0.34	3.60
	MJA	0.21	0.16	0.72 b	9.8	0.36	3.58
	ABA	0.19	0.14	0.88 a	9.6	0.34	3.60
	ETP	0.20	0.15	0.8 ab	9.8	0.37	3.60
	Prob>F	*ns*	*ns*	*0.0067*	*Ns*	*ns*	*ns*
29	C	0.18 a	0.12	0.81	10.6	0.27	3.70
	MJA	0.19 a	0.13	0.70	9.9	0.30	3.70
	ABA	0.15 b	0.11	0.79	9.9	0.29	3.60
	ETP	0.19 a	0.12	0.76	9.4	0.35	3.53
	Prob>F	*0.0048*	*ns*	*ns*	*Ns*	*ns*	*ns*

[a] The various treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP). Significance is set at $P < 0.05$ amongst all treatments for a given storage time. A different letter indicates significant differences between values. Non-significant values are denoted by ns.
[b] Total soluble solids (TSS); [c] Titratable acidity (TA)

Table 2: Fruit color values estimated after harvest in Premier following in-field applications of growth regulators

Treatment	*L*[a]*	*a**	*b**	*c**	*h**
C	*38.0*	*-1.1*	*-6.3*	*6.4*	*260.0*
MJA	*38.1*	*-1.0*	*-6.3*	*6.5*	*260.9*
ABA	*37.9*	*-1.0*	*-5.9*	*6.1*	*260.2*
ETP	*38.3*	*-1.0*	*-6.3*	*6.4*	*261.2*
Prob>F	*ns*	*ns*	*ns*	*ns*	*ns*

[a] The various treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP). Significance is set at $P < 0.05$ amongst all treatments for a given storage time. A different letter indicates significant differences between values. Non-significant values are denoted by ns.

Effect of treatment on fruit quality attributes during storage in Powderblue

Other than some minor differences, there was no significant effect on fruit quality attributes that included, compression, puncture, weight, total soluble solids (TSS), titratable acidity (TA) and pH due to various treatments at various times during storage (Table 3). Also there was no difference in color of the berries after harvest (Table 4).

Table 3: Fruit quality attributes determined at various times during storage in Powderblue following in-field applications of growth regulators

Days after harvest	Treatment[a]	Compression	Puncture	Weight	TSS[b]	TA[c]	pH
1	C	0.23	0.19	0.87	12.7	0.48	3.5 a
	MJA	0.24	0.19	0.80	12.1	0.6	3.4 b
	ABA	0.24	0.18	0.84	11.4	0.49	3.4 ab
	ETP	0.26	0.18	0.64	12.0	0.56	3.4 b
	Prob>F	*ns*	*ns*	*Ns*	*Ns*	*ns*	*0.015*
15	C	0.19 b	0.15	0.83	13.1 a	0.45 b	3.5 a
	MJA	0.21 ab	0.16	0.77	13.0 a	0.53 ab	3.4 a
	ABA	0.2 ab	0.15	0.83	11.6 b	0.5 ab	3.4 a
	ETP	0.22 a	0.15	0.68	12.1 ab	0.54 a	3.4 a
	Prob>F	*0.0077*	*ns*	*Ns*	*0.0166*	*0.049*	*0.0486*
29	C	0.19 ab	0.14	0.82	13.2	0.36	3.5
	MJA	0.17 b	0.15	0.78	13.2	0.41	3.4
	ABA	0.18 b	0.14	0.89	12.4	0.36	3.5
	ETP	0.21 a	0.15	0.69	12.6	0.41	3.5
	Prob>F	*0.012*	*ns*	*Ns*	*Ns*	*ns*	*ns*

[a] The various treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP). Significance is set at $P < 0.05$ amongst all treatments for a given storage time. A different letter indicates significant differences between values. Non-significant values are denoted by ns.
[b] Total soluble solids (TSS); [c] Titratable acidity (TA)

T*able 4: Fruit color values estimated after harvest in Powderblue following in-field applications of growth regulators*

Treatments	*L*[a]*	*a**	*b**	*c**	*h**
C	40.9 b	-1.2	-6.4 a	6.6 ab	260.5
MJA	44.0 a	-1.4	-6.8 b	6.9 a	258.6
ABA	40.7 b	-1.1	-6.2 a	6.3 b	260.1
ETP	43.2 ab	-1.3	-6.4 ab	6.6 ab	258.1
Prob>F	*0.0078*	*ns*	*0.0066*	*0.0063*	*ns*

[a] The various treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP). Significance is set at $P < 0.05$ amongst all treatments for a given storage time. A different letter indicates significant differences between values. Non-significant values are denoted by ns.

Postharvest disease incidence for various treatments during storage

In general disease incidence was low and did not appear to differ amongst various treatments at different time intervals during storage. The major postharvest pathogens recorded were anthracnose, Phomopsis, Botrytis, Alternaria and Pestalotia. Due to low pathogen count, percent disease incidence was calculated on overall disease incidence rather than for individual pathogens (Tables 5 and 6).

Table 5: *Percent disease data determined at various times during storage in Premier following in-field applications of growth regulators*

Treatment[a]	% Disease incidence days after harvest[b]		
	1	15	29
C	3.6	1.7	0.4
MJA	3.4	1.1	0
ABA	0.8	0.8	0.4
ETP	1.8	3.1	1.2

[a] Treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP).
[b]Disease incidence calculated included four pathogens, Anthracnose, Phomopsis, Botrytis and Alternaria

Table 6: *Percent disease data determined at various times during storage in Powderblue following in-field applications of growth regulators*

Treatment[a]	% Disease incidence days after harvest[b]		
	1	15	29
C	8.2	0.4	4.3
MJA	1.7	0	11
ABA	5.7	2.9	9.5
ETP	5.4	1.7	5.5

[a] Treatments include control (C); methyl jasmonate (MJA); abscisic acid (ABA); and Ethephon (ETP).
[b]Disease incidence calculated included five diseases, anthracnose, Phomopsis, Botrytis, Alternaria and Pestalotia.

CONCLUSIONS:

We saw a significant effect of ethephon in accelerating ripening in blueberries compared with the untreated control. There were no significant differences with ABA or MJA on rate of ripening compared with the control. We did not see any significant differences in all three treatments in various fruit quality attributes and disease incidence measured at various times during postharvest storage.

Impact Statement

This work has been important in demonstrating that ethephon can accelerate ripening in Premier and Powderblue rabbiteye blueberries. This is a preliminary study, and the role of ethephon in accelerating ripening of southern highbush and other rabbiteye varieties needs to be established.

There may be a potential to use ethephon as a ripening aid to accelerate ripening if further studies show it to be effective in other varieties. If confirmed, this could be an important plant growth regulator to promote more uniform ripening and may reduce picking times, saving on labor.

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