Proposal Category: _X_Research ___Outreach

Proposal Status: _X_ New Proposal ___Previously funded by SRSFC; has been previously funded for ___ years

Title: The Effect of Methyl Jasmonate Treatment on Fruit Quality During Postharvest Storage in Blueberries

Name, Mailing and Email Address of Principal Investigator(s):

Principal Investigator	Co- Principal Investigator	Co- Principal Investigator
Savithri Nambeesan	Zilfina Rubio Ames	Harald Scherm
Assistant Research Scientist	Assistant Professor	Professor and Dept. Head
Department of Horticulture	Department of Horticulture	Department of Plant Pathology
1111 Miller Plant Sciences	2360 Rainwater Road	2311 Miller Plant Sciences
University of Georgia	University of Georgia	University of Georgia
Athens, GA 30602	Tifton, GA 31793	Athens, GA 30602
Email:sunamb@uga.edu	zilfina.rubioames@uga.edu	scherm@uga.edu
Phone: 706-542-0777	229-386-3424	706-542-1258

Objectives: To determine the effects of postharvest methyl jasmonate treatment on blueberry fruit quality

Justification and Description:

Blueberries are considered “super fruits” because of their numerous health benefits and high antioxidant content (Neto, 2007). As acreage in blueberry production has increased, bottlenecks related to fruit quality and postharvest storage have become more critical. Blueberries generally have a shelf-life of about 1 to 6 weeks after harvest depending on the cultivar, harvest method and storage regime (Sun et al., 2014; Abugoch et al., 2015). The primary factors that lead to decline in fruit quality after harvest are water loss, fruit softening, and susceptibly to pathogens.

In ripe fruit, about 80-96% of fresh weight is associated with water content. Fruits continue to lose water after harvest, leading to symptoms such as wilting and shriveling. Subsequent changes often include decrease in weight, skin and flesh browning, altered texture, as well as decrease in flavor. Postharvest water loss can lead to softening and decline in blueberry fruit quality (Paniagua et al., 2013). Many fruits, including blueberries, have cuticular and wax layers covering the outer epidermal cells. The cuticular layer is composed of cutin and waxes and is thought to limit water loss through the fruit surface. In addition, water loss in blueberries can occur via the stem end, the scar end, and through microscopic cracks and openings on the fruit surface. A previous study indicated that water loss through the scar end was important during low-temperature storage, but at room temperature, the majority of the water was lost via the cuticle (Moggia et al., 2017). In Chile, the permissible blueberry weight (water) loss is around 5–7% at 0 °C for about 3 weeks (Paniagua et al., 2013; Moggia et al., 2017). However due to the disruption of the optimal temperatures during the supply chain there may be many instances of

greater water loss. Despite water loss and shriveling being an important concern for fruit quality in blueberries, this process has not been well studied.

Cold storage is recommended for blueberries and many other fruits to minimize water loss, delay fruit softening and growth of postharvest pathogens. However, storage of fruit at low temperature can result in chilling injury. The symptoms of chilling injury vary among different fruits and are dependent on the temperature and duration of storage. For example, in fruits such as peaches and nectarines storage at 2-5 °C results in flesh and internal browning within 1 to 2 weeks of storage. Other symptoms also include dry and mealy fruit texture (Lurie and Crisosto et al., 2005). In banana, chilling symptoms can occur within 2 days of storage at 8 °C with darkening of the peel. Slightly severe symptoms include uneven ripening, fruit brittleness, and darkening of the internal placental and pulp tissues (Hewage et al., 1996). There is very little information on chilling injury in blueberries. In the cultivar Duke, chilling injury for fruit stored at 0 °C for 30 days was characterized as pitting at the pedicel end (Zhang et al., 2020). However chilling symptoms and threshold temperatures have not been characterized in other blueberry cultivars.

Jasmonates and their derivate methyl jasmonate (MeJA) are plant hormones that can influence various processes involved in plant growth and development including flowering, fruit ripening and abscission. In addition, MeJA also regulates responses during abiotic and biotic stress including chilling injury. Application of MeJA in peaches harvested at commercial maturity decreased internal browning during storage, and fruit exhibited higher membrane stability by changes in their fatty acid composition (Chen et al., 2019). MeJA applications reduced chilling injury and postharvest water loss in bananas (Elbagoury et al., 2020). Similarly, application of MeJA alleviated chilling injury in orange (Rehman et al., 2018), pineapple (Boonyaritthongchai and Supapvanich, 2017) and several other fruits. In strawberries however, preharvest application of MeJA promoted ripening and softening (Han et al., 2019). A recent study has indicated that hand-harvested rabbiteye blueberry (cv. Beauty) fruit treated with MeJA showed lower decline in fruit firmness and displayed reduced expression of cell wall-degrading enzymes (Wang et al., 2019; Wang et al., 2021). Another study found that MeJA applications increased individual anthocyanins in northern highbush blueberry (cv. Elliott) during cold storage (Huang et al., 2014). These results suggest a positive effect of MeJA application on blueberry fruit quality. However, these studies did not measure the effect of MeJA on postharvest water loss and chilling injury symptoms during postharvest storage. Also, the effect of MeJA on southern highbush blueberry fruit have not yet been tested. Hence, here investigated the effect of MeJA treatment on three southern highbush blueberry cultivars and its effect on postharvest fruit quality attributes including water loss and chilling injury.

Significance:
Fruit quality in blueberries is important for consumer satisfaction. Studying water loss and chilling injury will be beneficial in understanding the importance of these factors in determining blueberry fruit quality during storage. Further, MeJA has been demonstrated to alleviate chilling injury symptoms in many fruits, and the compound is known to induce

host resistance against pathogens and herbivores in many plants, including *Vaccinium* species (Benevenuto et al. 2019). Thus, evaluation of the effects of MeJA on southern highbush blueberry will be useful. If MeJA treatments are effective to minimize water loss, chilling injury, and postharvest fruit infection, then future studies will be focused on developing this as a management tool to maintain blueberry postharvest fruit quality.

Experimental plan:

Plant Material: Fruit were harvested from three southern highbush cultivars: 'Rebel,' 'Star,' and 'Legacy.' The three cultivars were harvested on May 5th ('Rebel'), 13th ('Star'), and 17th ('Legacy') of 2022. 'Rebel' and 'Star' were harvested from a commercial farm in Bacon County, GA, and 'Legacy' was harvested from a commercial farm in Coffee County, GA. Fully ripe fruit were hand harvested either by our research team ('Rebel' and 'Star') or by workers employed by the commercial farm ('Legacy'). Fruit were collected from multiple rows of plants and each row was used as a replicate. Fruit were collected in clamshells and transported to the University of Georgia, Athens campus and placed in a walk-in cooler set at 4 °C. The walk-in cooler at our facility is set to maintain relative humidity at >90%. The following day, fruit were sorted to remove any damaged fruit and only defect-free fruit were used for treatments.

Treatments: Three concentrations of MeJA (Sigma-Aldrich) (50 µM, 100 µM, and 250 µM) were applied to the fruit, along with an untreated control. The concentrations of MeJA were based on previous studies from other fruit crops where positive effects on fruit quality were noted (Wang et al., 2019; Wang et al., 2021). Fruit were placed in an airtight one-gallon jar in approximately a single layer by placing the jar sideways. Each replicate was treated separately with similar number of fruits based on fruit weight. Applications were performed by pipetting appropriate amounts of MeJA to achieve each treatment level onto filter paper held by a plastic cup inside the jar. The jars were then sealed. The untreated control was treated similarly. Untreated fruit were sealed inside the same type of jar, but no MeJA was applied to the filter paper. Air was circulated in the jars of 'Star' and 'Legacy' fruit during treatment, however technical issues prevented us from doing so during the treatment of 'Rebel.' The fruit were stored at 20 °C for 12 hours in the dark in an environmental chamber during the treatment. After the treatments were performed, fruit were allowed to vent at room temperature for 2 hours. Subsequently, fruit were re-sorted into clamshells and stored at 4 °C at 90% RH.

To measure chilling injury symptoms, 50 fruit were placed in a clamshell for each replicate. These measurements were destructive, hence a separate clamshell of 50 fruit was used at every time point. At the three-time points the fruit were visually inspected for any bruising or damage. Pitting of the fruit tissue at the stem end was recorded. If pitting is observed in other areas of the fruit, that was recorded as well. Subsequently, fruit were cut into two halves and internal tissues were examined for symptoms of chilling injury such as browning or water-soaked areas. Fruit were scanned using a scanner set at 600 dpi to visually record symptoms of chilling injury. This data has yet to be analyzed and is not included in the results section of the paper.

To measure water loss during postharvest storage, 50 fruit were placed in a clamshell for each replicate. Prior to placing the fruit in a clamshell, the weight of the clamshell was recorded. The clamshells containing the fruit were subsequently weighed at four time-points during postharvest storage (Post-harvest (PH) 7/9, PH 14/16, PH 18/19, and PH18+2, PH19+2). The initial weight of the clamshell was subtracted to obtain 50 fruit weight. Ten empty clamshells were weighed initially and placed in the cooler to account for any differences in clamshell weight loss during storage. Percent weight loss was calculated from these measurements. In summer of 2021, we were able to successfully measure water loss during storage by using this method.

Fruit firmness measurements (puncture and compression) were performed using a Fruit Texture Analyzer (Model GS-15, Güss Manufacturing Ltd., Strand, South Africa).

Electrolyte leakage was measured by cutting 8 disks (approximately 7mm round by 2mm thick) obtained from the pericarp of the fruit (excluding skin and seeds). These disks were then rinsed by swirling 6 times in a solution of 0.2M mannitol, drained, and placed in flasks containing 30 ml of 0.2M mannitol. Initial electroconductivity (EC) readings (E_0) were taken using a Hanna Instruments Edge EC Meter on 7 ml aliquots of the mannitol solution. Subsequently two more EC readings were taken: after being placed in a shaker at 100 rpm for 1.5 hours (E_1), and after autoclaving (E_{final}). After every measurement, the 7 ml of mannitol aliquots were returned to the sample. Electrolyte leakage was calculated using the formula: $EL = (E_1 - E_0)/ (E_{final} - E_0) *100$. A larger EL reading indicates increased membrane leakage. This protocol was adapted to blueberries from a protocol in Saltveit, M. E. (2002).

Carbon dioxide was measured using a Quantek Instruments Oxygen/Carbon Dioxide Analyzer Model 902P. Fruit were incubated in airtight jars for one hour prior to measurement. 60 mL of air was drawn using a needle and syringe from each jar via a septum and injected into the analyzer for each reading. Percent CO_2 was converted to µL CO_2 produced per gram of fruit per hour using jar size, fruit weight, and incubation time.

For measuring total soluble solids (TSS) and titratable acidity (TA), 20 g of fruit were juiced using a blender and centrifuged using a bench top micro-centrifuge. The supernatant was used to determine TSS using a digital handheld refractometer (Atago USA, Belleveue, WA) and TA using an automatic mini titrator (Hanna Instruments, Woonsocket, RI).

Pathogens were identified visually and/or based on isolation from the fruit as described previously (Mehra et al. 2013). Pathogens were assessed by Dr. Harald Scherm in the Department of Plant Pathology at the University of Georgia.

Statistical analysis was performed using one-way ANOVA (analysis of variance) for each time point after treatment within a cultivar using R version 4.1.1 (The R Foundation for Statistical Computing). Means separation was performed using Tukey's Honest Significant Difference (HSD) test ($\alpha = 0.05$).

Results:

Weight loss and fruit quality assessment

Weight loss increased over time as the experiment progressed and sharply increased when the fruit was removed from storage and placed in room temperature for two days (Fig. 1). This was expected. There were no significant differences between the treatments at any timepoint for any of the three cultivars (Fig. 1).

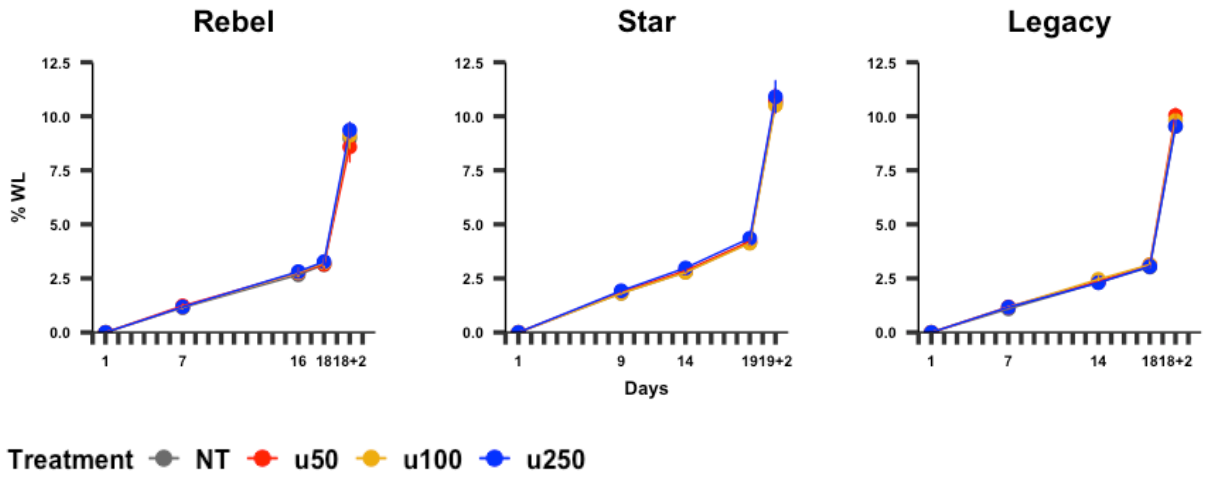

Figure 1: Treatment effects on weight loss during postharvest storage

Puncture and compression are shown in the graphs below in Fig. 2. The dotted grey lines represent reading taken on untreated fruit at day zero. Legacy compression is missing due to technical issues that occurred while taking measurements. Any significant differences between the treatment at each timepoint for each cultivar are displayed in the Table 1. Overall no consistent effects were observed among cultivars. Only for compression, 'Star' fruit displayed lower compression values after MeJA treatments, especially at 250 µM (Fig. 2 and Table 1).

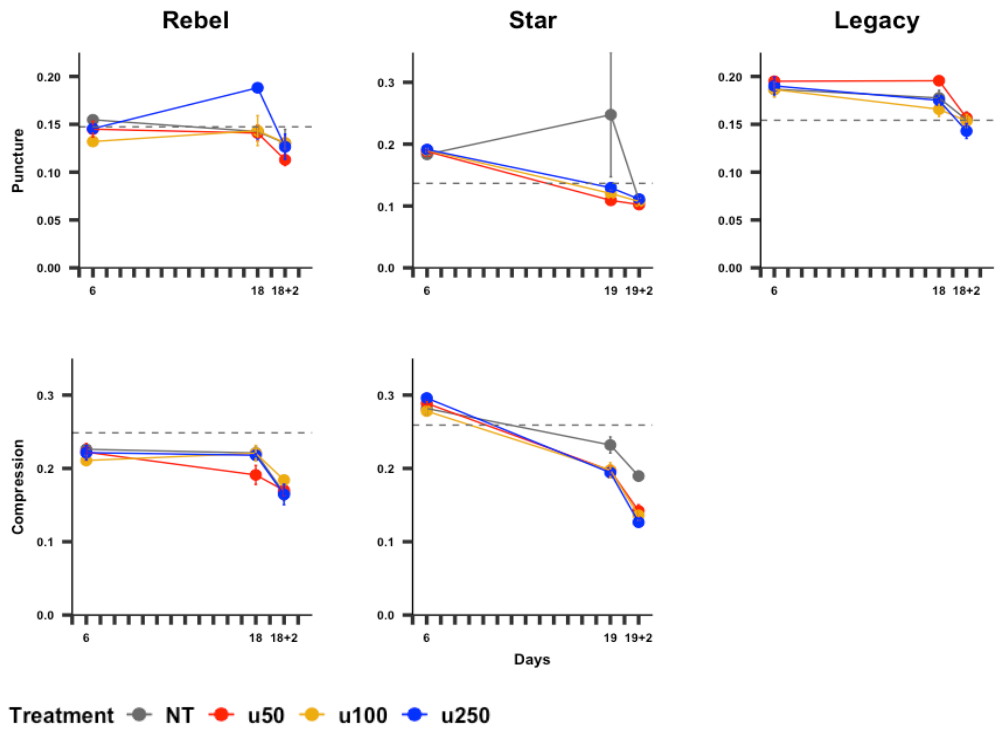

Figure 2: Treatment effects on puncture and compression during postharvest storage

Table 1: Statistical analysis one-way ANOVA (analysis of variance) for each time point after treatment within a cultivar for puncture and compression. Means separation was performed using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

Puncture and Compression Significance Table		Treatments	Rebel	Star	Legacy
Puncture	D6	NT	a	ns	ns
		u50	ab		
		u100	b		
		u250	ab		
	D18/19	NT	ns	ns	ab
		u50			a
		u100			b
		u250			ab
	D18+2/19+2	NT	ns	ns	ns
		u50			
		u100			
		u250			
Compression	D6	NT	ns	ns	N/a
		u50			
		u100			
		u250			
	D18/19	NT	ns	a	
		u50		ab	
		u100		ab	
		u250		b	
	D18+2/19+2	NT	ns	a	
		u50		b	
		u100		b	
		u250		b	

Electrolyte leakage showed unexpected results. It was expected that the measurements would decrease as the membranes became more damaged over time. Our results are shown in the graphs below (Figure 3). The dotted grey lines represent reading taken on untreated fruit at day zero. The only significant difference occurred on 'Rebel' day 17 (Table 2). The 100 µM readings were significantly higher than those of the untreated fruit indicating possible increased membrane damage compared to the untreated fruit.

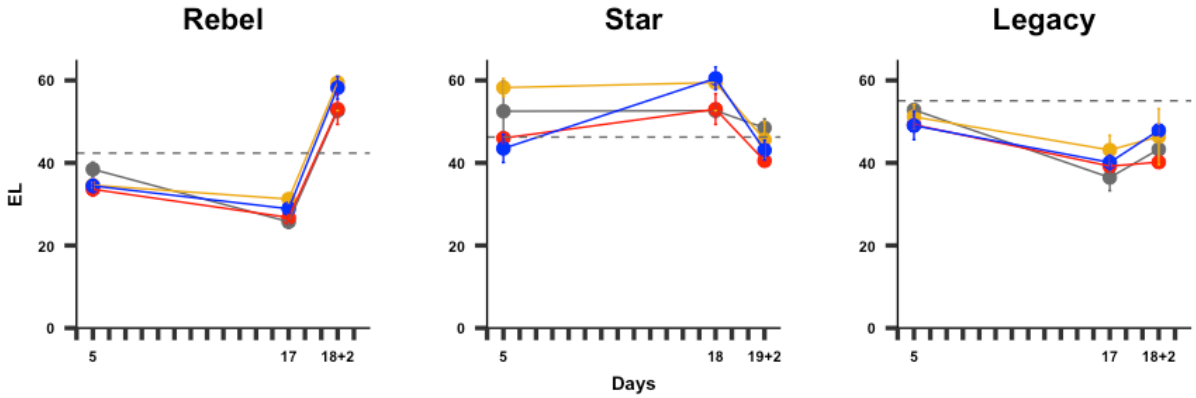

Figure 3: Treatment effects on electrolyte leakage during postharvest storage

Table 2: Statistical analysis one-way ANOVA (analysis of variance) for each time point after treatment within a cultivar for electrolyte leakage. Means separation was performed using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

EL Significance Table	Treatments	Rebel	Star	Legacy
D17/18	NT	b	ns	ns
	u50	ab		
	u100	a		
	u250	ab		

Carbon dioxide

CO_2 results for each cultivar are shown in the graphs below (Fig. 4). The dotted grey lines represent readings taken on untreated fruit at day zero. There were no significant differences between the treatments in CO_2 produced by the fruit at either timepoint in any of the cultivars. The treatments do not appear to have effected respiration (Fig. 4).

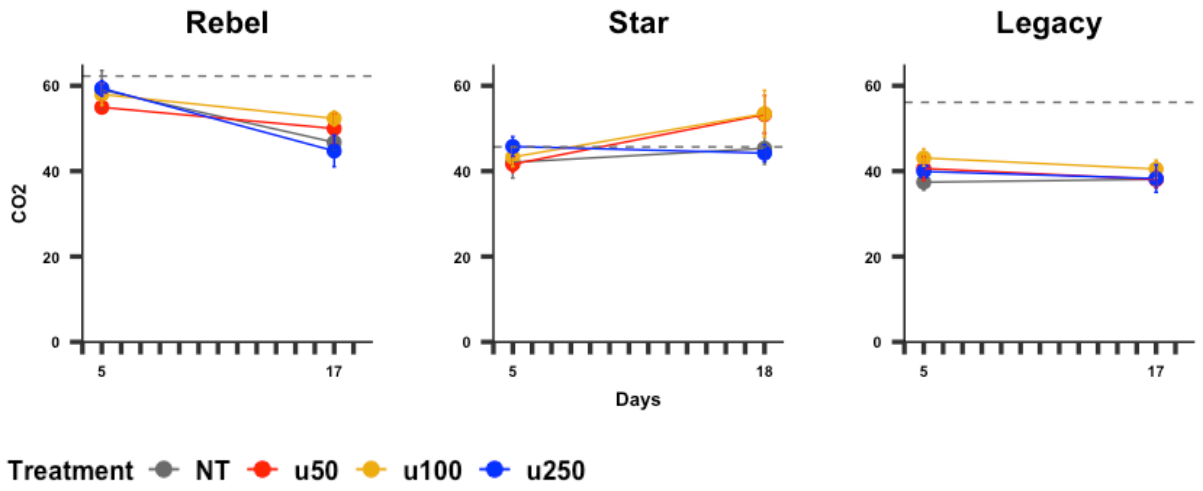

Figure 4: Treatment effects on carbon dioxide evolution during postharvest storage

Total soluble solids and titratable acidity

The results for TSS and TA are shown in the graphs below in Fig. 5. The dotted grey lines represent readings taken on untreated fruit at day zero. No significant differences in TSS were found between any of the treatments at any time point. TA was only significantly different on day 19+2 in Star (Table 3). TA was higher in the 50 µM treatment compared to the 100 µM treatment at this time (Fig. 5 and Table 3).

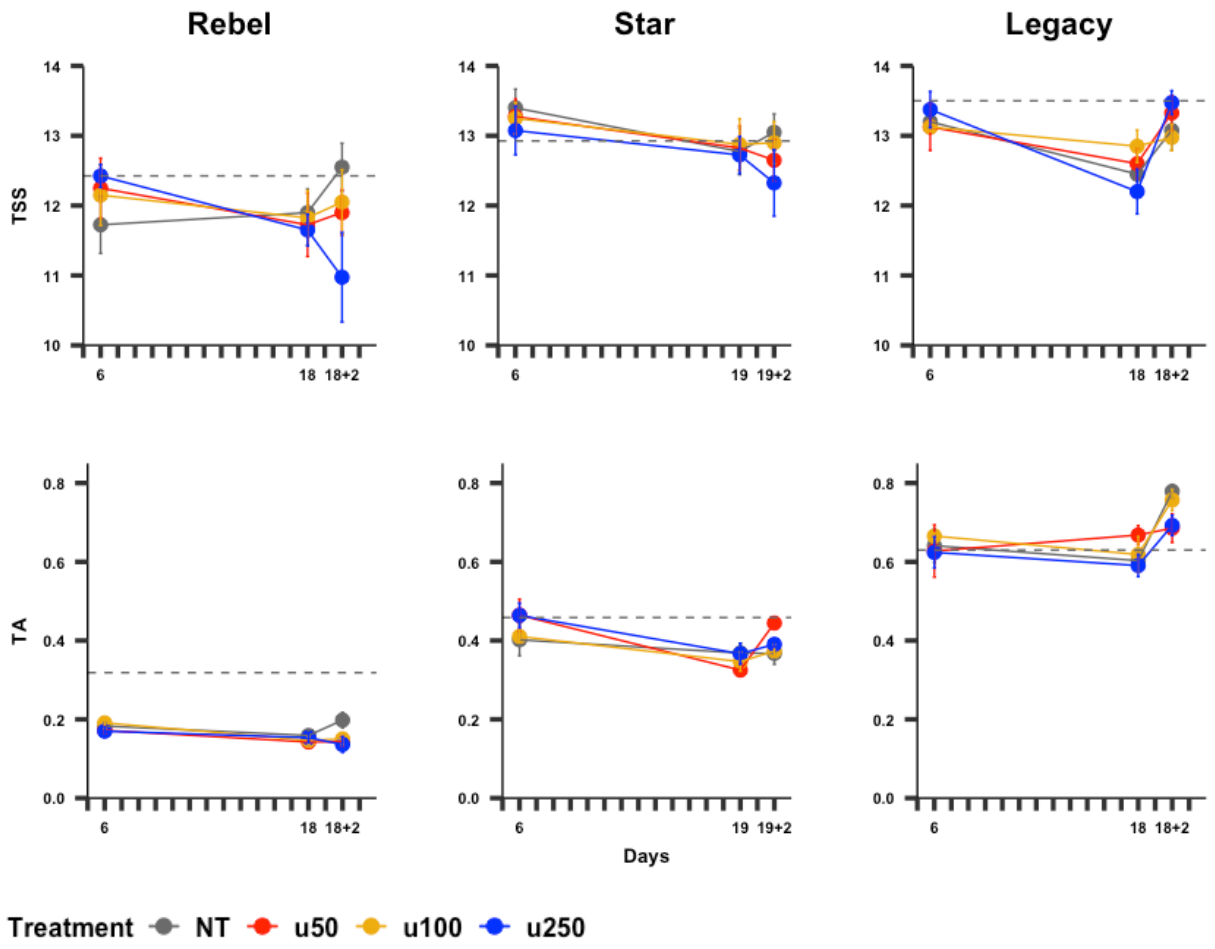

Figure 5: Treatment effects on TSS and TA during postharvest storage

Table 3: Statistical analysis one-way ANOVA (analysis of variance) for each time point after treatment within a cultivar for TSS and TA. Means separation was performed using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

TSS and TA Significance Table		Treatments	Rebel	Star	Legacy
TA	D18+2/19+2	NT	ns	ab	ns
		u50		a	
		u100		b	
		u250		ab	

Pathogen assessment

Alteraria, *Colletotrichum*, and *Botrytis* were detected on all cultivars, with *Alteraria* being the most abundant across all cultivars. The treatments did not affect the total incidence of pathogens. No significant differences were found between the treatments (Fig. 6).

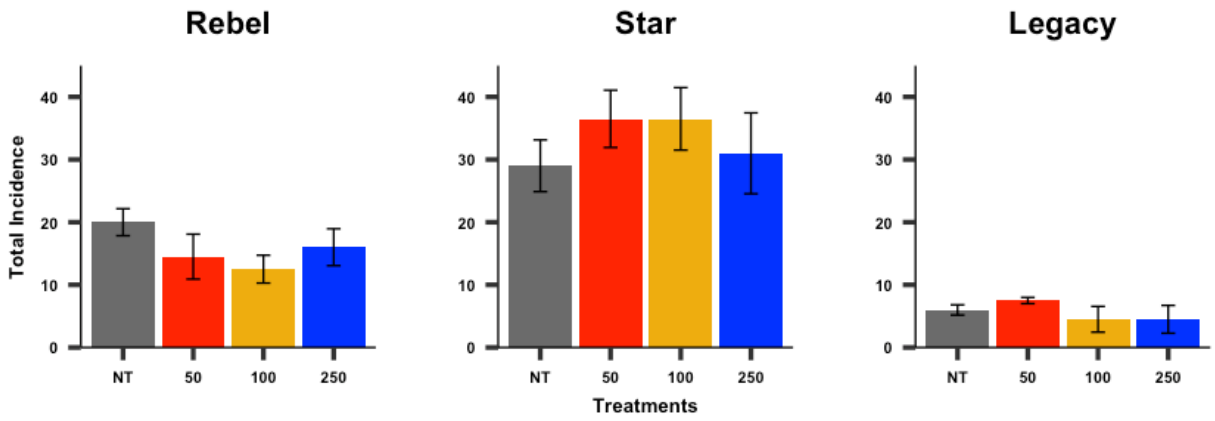

Figure 6: Treatment effects on disease incidence during postharvest storage

Discussion:

In previous studies, Methyl Jasmonate applications have been shown to have positive effects of fruit firmness, weight loss, TSS, TA, and surface color in *Vaccinium corymbosum* cv. Elliott and *Vaccinium ashei* cv. Beauty (Huang et al., 2014; Wang et al., 2019; Wang et al., 2021). We did not see the same effects in the southern highbush cultivars used in this study. Instead, the data suggested minimal treatment effects and inconsistent effects among cultivars. In this study, methyl jasmonate did not appear offer any advantages to maintain fruit quality during postharvest storage. MeJA effect on phenotypic analysis remains be evaluated.

References:

Abugoch, L., Tapia, C., Plasencia, D., Pastor, A., Castro-Mandujano, O., Lopez, L., et al. (2015) Shelf-life of fresh blueberries coated with quinoa protein/chitosan/ sunflower oil edible film. *J. Sci. Food Agric.* 96: 619-626.

Benevenuto, R.F., Seldal, T., Hegland, S.J. Rodriguez-Saona, C., Kawash, J., Polashock J. (2019). Transcriptional profiling of methyl jasmonate-induced defense responses in bilberry (*Vaccinium myrtillus* L.). *BMC Plant Biol.* 19: 70.

Boonyaritthongchai, P., Supapvanich, S. (2017) Effects of methyl jasmonate on physicochemical qualities and internal browning of 'queen' pineapple fruit during cold storage. *Hortic. Environ. Biotechnol.* 58: 479–487.

Chen, M.S., Guo, H.M., Chen, S.Q., Li, T.T., Li, M.Q., Rashid, A., Xu, C.J., Wang, K. (2019) Methyl jasmonate promotes phospholipid remodeling and jasmonic acid signaling to alleviate chilling injury in peach fruit. *J. Agric. Food Chem.* 67: 9958–9966.

Elbagoury, M.M., Turoop, L., Runo, S., Sila, D.N. (2020) Regulatory influences of methyl jasmonate and calcium chloride on chilling injury of banana fruit during cold storage and ripening. *Food Sci. Nutr.* 9:929–942.

Han, Y.L., Chen, C., Yan, Z.M., Li, J., Wang, Y.H. (2019) The methyl jasmonate accelerates the strawberry fruits ripening process. *Sci. Hortic.* 249:250–256.

Hewage, K. S., Wainwright, H., Wijeratnam, R.S.W. (1996) Quantitative assessment of chilling injury in bananas using a colorimeter. *J. Hort. Sci.* 71:135-139.

Huang, X., Li, J., Shang, H., Meng, X. (2015) Effect of methyl jasmonate on the anthocyanin content and antioxidant activity of blueberries during cold storage. *J. Sci. Food Agric.* 95: 337–343.

Lurie, S., Crisosto, C.H. (2005) Chilling injury in peach and nectarine. *Postharvest Biol Technol.* 37:195–208.

Mehra, L.K., MacLean, D.D., Savelle, A.T., Scherm, H. (2013) Postharvest disease development on southern highbush blueberry fruit in relation to berry flesh type and harvest method. *Plant Dis.* 97:213-221.

Moggia, C., Beaudry, R. M., Retamales, J. B., and Lobos, G. A. (2017). Variation in the impact of stem scar and cuticle on water loss in highbush blueberry fruit argue for the use of water permeance as a selection criterion in breeding. *Postharvest Biol. Technol.* 132, 88–96.

Neto, C.C. (2007) Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. *Mol. Nutr. Food Res.* 51:652–664.

Paniagua, A.C., East, R., Hindmarsh, J.P., Heyes, J.A., (2013) Moisture loss is the major cause of firmness change during postharvest storage of blueberry. *Postharvest Biol. Technol.* 79:13–19.

Rehman, M., Singh, Z., Khurshid, T. (2018). Methyl jasmonate alleviates chilling injury and regulate fruit quality in Midknight Valencia sweet orange. *Postharvest Biol. Technol.* 141: 58–62.

Saltveit, M. E. (2002). The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. *Postharvest Biology and Technology*, *26*(3), 295-304.

Sun, X., Narciso, J., Wang, Z., Ference, C., Bai, J., Zhou, K. (2014) Effects of chitosan-essential oil coatings on safety and quality of fresh blueberries. *J. Food Sci.* 79: M955–M960.

Zhang, F., Ji, S., Wei, B., Cheng, S., Wang, Y., Hao, J., Wang, S., Zhou, Q. (2020). Transcriptome analysis of postharvest blueberries (*Vaccinium corymbosum* 'Duke') in response to cold stress. *BMC Plant Biol.* 20: 80.

Wang, H., Cheng, X., Wu, C., Fan, G., Li, T., & Dong, C. (2021). Retardation of postharvest softening of blueberry fruit by methyl jasmonate is correlated with altered cell wall modification and energy metabolism. *Scientia Horticulturae*, *276*, 109752.

Wang, H., Wu, Y., Yu, R., Wu, C., Fan, G., & Li, T. (2019). Effects of postharvest application of methyl jasmonate on physicochemical characteristics and antioxidant system of the blueberry fruit. *Scientia Horticulturae*, *258*, 108785.