Proposal Category: **_X_Research** **___Outreach**

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

Title: Effects of postharvest blue light treatment on ripening, fruit quality and disease incidence in blueberries

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Objectives: To evaluate the postharvest effects of blue light on fruit quality, anthocyanin accumulation and disease incidence during postharvest storage

Justification and Description:

Blueberries are a major fruit crop in the Southeast with more than 28,000 acres under production in Georgia alone. As acreage and production have expanded, bottlenecks related to fruit harvesting and postharvest storage have become more critical. This project evaluated the effect of blue light on a) enhancing fruit quality including anthocyanins b) limiting/inhibiting the development of postharvest disease in blueberries, and c) improving color of green spots in fruit of certain cultivars (e.g. Farthing) or due to Exobasidium fruit spot.

Physiological changes during ripening of blueberries include flesh softening, increase in size, increase in sugar/acid ratio, and a change in color from green to pink and then blue. The change in color to blue is due to the accumulation of anthocyanins, which have numerous documented health benefits. Blueberries for the fresh market are commercially hand harvested after the berry has completed its ripening on the bush as indicated by a deep blue color. But often under-ripe berries (comprising pink to blue transition stages) get picked during harvest. Some of these under-ripe berries get discarded during sorting in the packing line but many of the pinkish-blue berries are included with the ripe berries when being packed in the clamshell. This leads to a certain proportion of slightly under-ripe berries with poor color, and possibly lower anthocyanin content included in every clamshell. Treatment with blue light has been shown to promote coloration in certain fruits by increasing anthocyanins. If applicable to blueberries, treatment with blue light may increase anthocyanins which are health-promoting compounds. This will also improve color of under-ripe berries within a clamshell giving a more uniform appearance. These changes will lead to better fruit quality attributes and marketability.

Upon harvest, blueberries do not store well and have a shelf-life of 2-4 weeks. This loss in fruit quality occurs due to fruit softening and/or infections from plant pathogens such as species of *Colletotrichum*, *Botrytis* and *Alternaria*, among others. Commercially, postharvest decay can be controlled by the use preharvest fungicides. However, fungicides may offer limited protection depending on the dose applied, their specificity, and potential fungicide resistance (currently suspected for the pathogens causing anthracnose rot). Blue light has shown to inhibit growth of certain pathogens during postharvest storage and may improve the postharvest shelf-life in blueberry. This strategy may provide an alternative and/or supplement to the use fungicides.

In addition to fruit spoilage during postharvest storage, there are other ripening issues that can cause fruit to become unmarketable. Some cultivars, such as Farthing which have very good fruit quality attributes, sometimes retain a green spot at the stem end that cannot be easily picked up by the color sorter. These fruit are of inferior quality. Another emerging disease in the Southeast, Exobasidum fruit spot, causes green spots in fruits that do not ripen well (Brannen, 2013; Brewer et al., 2014). These green spots render the fruit unmarketable. Blue light may enhance marketability of green spotted fruit by influencing aspects of ripening including color development.

The role of light as an important environmental factor in agriculture and horticulture is obvious since light drives photosynthesis. However the role of light and it usefulness in maintaining postharvest shelf-life and fruit quality is less recognized. Low-intensity light can maintain texture and improve extended storage of spinach, lettuce, basil and delay senescence in broccoli compared with storage in dark. Many of these above studies have been performed mainly on green vegetables (Büchert et al., 2011; Costa et al., 2013; Braidot et al., 2014; Glowacz et al., 2014). In the visible range, treatment specifically with blue light has been effective in improving postharvest attributes due to 1) flavonoid accumulation (e.g. anthocyanins) and 2) limiting postharvest disease incidence. Blue light has a prominent effect on accumulation of anthocyanins and improves pigmentation in strawberries and grapes (Kadomura-Ishikawa et al., 2013; Kondo et al., 2014). In our study, we hypothesize that blue light increases the accumulation of anthocyanins and promotes uniform color development of the berries during storage. In addition it may also influence color development in green spotted fruit caused by ripening related disorders.

Blue light also has an inhibitory effect on the growth of certain postharvest disease fungi. In citrus, blue light suppressed mycelial growth by postharvest fungi, *Penicillium digitatum*, *P. italicum* and *P. citri* but had no effect on *Lasiodiplodia theobromae* or *C. gloeosporioides*. The role of blue light in inhibiting fungal growth could be a direct effect in inhibiting mycelial growth (Liao et al., 2013). Light could also suppress the activity fungal polygalacturonase to limit host tissue maceration (Barash and Angel, 1970; Barmore and Brown, 1979). In fruit tissue, blue light could trigger defense responses, which has been shown by the induction of a gene involved in a resistance phenotype (Alferez et al., 2012). Blue light increased the production of octanal, an oil volatile which plays a role in defense response to *Penicillium* (Liao et al., 2013). Blue light also inhibits development of gray mold, *B. cinerea* in leaves of tomato and grape (Kim et al., 2013; Ahn et al., 2015). In this study we hypothesize that blue light limits the development of postharvest pathogens and improve shelf-life of blueberries.

We propose to use light-emitting diodes (LEDs) that have been the source of blue light in some of the above referenced studies. LEDs offer several advantages over other lights (reviewed in Souza et al., 2015). They offer high emissions of monochromatic light and therefore can be used in a narrow bandwidth. Another advantage of LEDs is that they can be placed very close to the produce since LEDs emit little radiant heat and may have only minimal heating effect. This can allow for the use of LEDs in combination with cold storage methods. Other benefits are long life expectancies and compact design. Their increasing efficiency makes LEDs, very attractive in the food industry. We propose to test the effect of blue LEDs on accumulation of anthocyanins and inhibiting disease development during ripening and postharvest storage. If positive benefits are seen, strategies to use this technique during storage, transport and/or in retail will be explored.

Experimental Plan:
The effect of blue LED light was evaluated on 1) postharvest storage attributes that include accumulation of pigments 2) postharvest disease development and 3) improvement of color in green spotted fruits. Research was conducted on southern highbush (SHB) blueberry cultivar Star and rabbiteye cultivar Alapaha (Objectives 1 and 2), as well as SHB cultivar Farthing (Objective 3).

Set-up of LEDs as a light source:

LED light racks were set up in a walk-in cooler maintained at 2-4 °C. By placing humidifiers in the cooler, relative humidity was maintained above 90%. Harvested berries were placed under blue light illumination for 4 days under a 12 h blue light/12 h dark cycle. The peak wavelength of the blue light was around 430 nm at an intensity of 40 μmol m^{-2} s^{-1}. Two controls were used for this study. One set of control berries was placed under an alternating 12 h white light/12 h dark cycle (referred to as "white" henceforth) and the other set of control berries was placed in continuous dark. After 4 days, one subset of samples was used for assessment of postharvest fruit quality attributes and disease incidence (PH+4) described below. The samples for the remaining time points were held under the same light regime for subsequent sampling after 15 and 21 days.

Objective 1: Evaluate the effects of blue light on fruit quality attributes including anthocyanin accumulation and color development

Ripe fruit harvested at the Cornelius farm near Manor, GA, were split into three groups for postharvest fruit quality analyses including anthocyanin and color determination. These three groups were assigned randomly to one of the following time periods of postharvest storage under blue light treatment: 1) PH+4 days; 2) PH+15 days and 3) PH+21 days. For determining fruit quality, weight, texture, titratable acidity (TA), total soluble solids (TSS) content, total anthocyanins and berry color were quantified. Briefly, firmness measurements were performed using a Fruit Texture Analyzer (Model GS-15, Güss, Strand, South Africa). For measuring TA and TSS, juice from 40 g of fruit was extracted 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, Bellevue, WA). To determine TA, the supernatant was titrated using automatic mini titrator (Hanna, Woonsocket, RI). Total anthocyanins were extracted and measured using spectrophotometry. Fruit color was determined using a handheld colorimeter (3nh Technology Co., Shenzhen, China).

Objective 2: Evaluate the effect of blue light on postharvest disease development.

The effect on postharvest disease development was assayed using two approaches. The first approach was 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 any symptoms of disease were recorded from typically 40 to 70 fruit/replicate, and the associated pathogens were identified macro- and microscopically based on symptoms and signs (Mehra et al., 2013).

For the second assay type, fruit were artificially inoculated to determine the effect of blue light on disease incidence. Artificial inoculation was done with *A. tenuissima*, *B. cinerea* and *C. acutatum*, common postharvest pathogens in blueberry. After being held at 4 days under the light treatments described above, fruit were inoculated with a 20-µl drop of a spore suspension (1×10^5 conidia per ml) along with a water control on the stem end with the above pathogens and kept on moist filter paper at 23-25°C for 24 h followed by the three light treatments at 2-4°C for 9 days. Fruit were maintained for 4 days at 23-25°C after removal from cold storage, and disease incidence was recorded separately for each pathogen from 50 fruit/replicate (Mehra et al., 2013).

Objective 3: Evaluate color development on green spots in fruit of cultivar Farthing.
In a commercial blueberry planting near Alma, GA, ripe fruit of cultivar Farthing were collected, however these fruit retained green spots at the stem end. About 27-30 fruit per light treatment per replication, with the green areas positioned upward, were subjected to cold storage under the three light treatments described previously. A photograph of each group of fruit was taken on days 0, 4, 8, and 11 and analyzed using ImageJ software (National Institutes of Health, Rockville, MD) to quantify the green to blue color transition under the three light treatments. For each image, the analysis yielded a grayscale value on a scale from 0 (black) to 255 (white); hence, the lower the value, the more blue the spot on the berries.

Results:

Objective 1: Evaluate the effects of blue light on fruit quality attributes including anthocyanin accumulation and color development

I. Visual quality of the fruit

We determined the effect of blue light on percent defect-free fruit over time. In general, blue light did not affect the development of visual defects such as bruising and leakiness of fruit in 'Star" and 'Alapaha" (Fig. 1A and 1B). The percent of defect-free fruit in 'Star' was higher over time compared with 'Alapaha'. In Star, the percent of defect-free fruit on an average for all the various light treatments combined declined from 88% at PH4 to 83 % at PH15 and 81% at PH21; in case of 'Alapaha' the decline was from 73 % at PH4 to 24 % at PH14 and 20% at PH24.

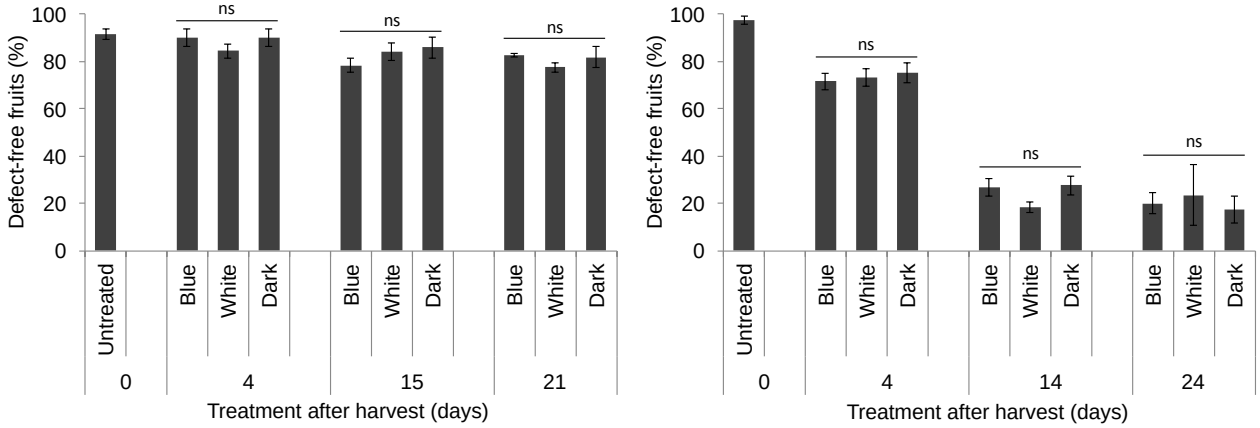

Figure 1. Percentage of defect-free fruit determined at various times after harvest in 'Star' (left) and 'Alapaha' (right). Significance set at $P < 0.05$ among treatments for a given storage time. Non-significant values are denoted by ns.

II. Compression and Puncture

We determined the effect of blue light on fruit firmness (compression) and skin toughness (puncture). In general 'Star' had higher compression and puncture values than 'Alapaha' (Tables 1 and 2). However, there was no effect of various light regimes on fruit softening and skin toughness at various times during postharvest storage in both cultivars.

Table 1: Fruit quality attributes determined at various times during storage in 'Star'

Days after harvest	Treatment	Compression (kg)	Puncture (kg)	Weight (g)	TSS[a] (^{o}Brix)	TA[b] (%)	pH
0	-	0.23	0.13	2.07	13.4	0.58	3.43
4	Blue	0.24	0.14	2.11	13.1 b	0.55	3.35
	White	0.23	0.14	2.14	14.1 a	0.41	3.53
	Dark	0.24	0.14	2.28	13.2 b	0.52	3.40
	Prob>*F*	ns	ns	ns	0.0134	ns	ns
15	Blue	0.27	0.13	2.17 ab	13.4	0.40	3.50 b
	White	0.25	0.12	2.02 b	13.5	0.42	3.50 b
	Dark	0.27	0.12	2.20 a	13.3	0.40	3.68 a
	Prob>*F*	ns	ns	0.0263	ns	ns	0.0282
21	Blue	0.27	0.11	2.08	13.0	0.39	3.63
	White	0.27	0.11	2.00	13.1	0.37	3.68
	Dark	0.28	0.12	2.11	12.7	0.41	3.53
	Prob>*F*	ns	ns	ns	ns	ns	ns

Significance set at $P < 0.05$ among treatments for a given storage time. Means followed by the same letter are not significantly different from each other. Non-significant values are denoted by ns.
[a] Total soluble solids (TSS); [b] Titratable acidity (TA)

Table 2: Fruit quality attributes determined at various times during storage in 'Alapaha'

Days after harvest	Treatment	Compression (kg)	Puncture (kg)	Weight (g)	TSS[a] (^{o}Brix)	TA[b] (%)	pH
0	-	0.15	0.11	1.13	14.0	0.41	3.30
4	Blue	0.15	0.10	1.08	14.0	0.35	3.33
	White	0.15	0.10	1.16	14.2	0.36	3.35
	Dark	0.16	0.10	1.08	13.7	0.37	3.38
	Prob>*F*	ns	ns	ns	ns	ns	ns
14	Blue	0.13	0.10	1.14	14.1	0.292	3.58 b
	White	0.15	0.11	1.06	14.2	0.295	3.63 ab
	Dark	0.15	0.11	1.10	14.1	0.298	3.75 a
	Prob>*F*	ns	ns	ns	ns	ns	0.0415
24	Blue	0.16	0.11	1.10	13.8	0.28	3.53
	White	0.17	0.12	1.13	13.9	0.27	3.48
	Dark	0.17	0.11	1.05	13.7	0.29	3.48
	Prob>*F*	ns	ns	ns	ns	ns	ns

Significance set at $P < 0.05$ among treatments for a given storage time. Means followed by the same letter are not significantly different from each other. Non-significant values are denoted by ns.
[a] Total soluble solids (TSS); [b] Titratable acidity (TA)

III. Fruit weight, total soluble solids (TSS), titratable acidity (TA) and pH

We determined the effect of blue light on various fruit quality attributes (Tables 1 and 2). Fruit weight of 'Alapaha' was lower than that of 'Star', but blue light did not change the weight of the fruit during postharvest storage. TSS was slightly higher and TA was lower in 'Alapaha' compared with 'Star'. However, blue light did not affect TSS, TA and pH during postharvest storage in both the cultivars.

1V. Fruit Anthocyanin content

We determined total anthocyanin content in "Alapaha' and 'Star' (Fig. 2). Blue light did not affect the levels of fruit anthocyanin content in either cultivars tested.

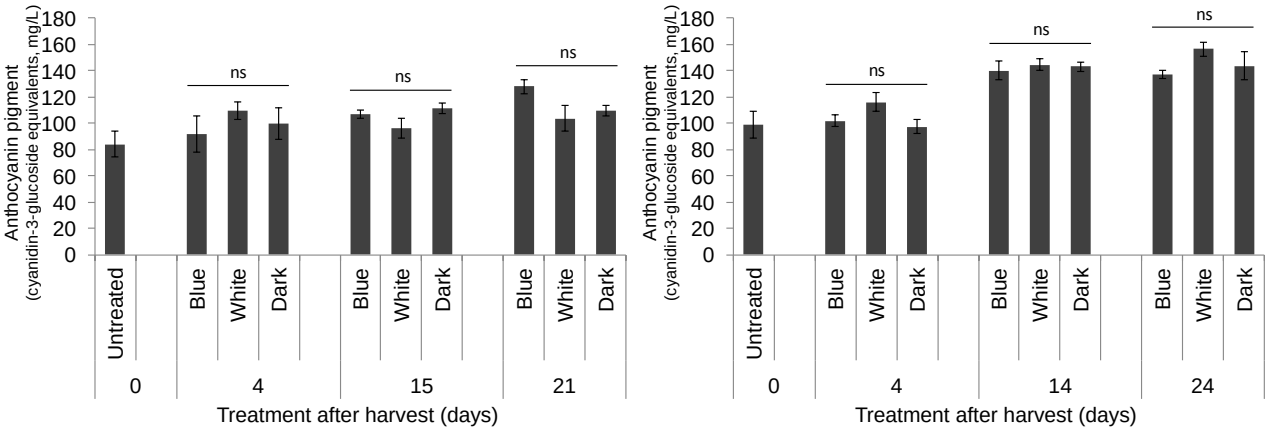

Figure 2. Anthocyanin pigment concentration determined at various times after harvest in 'Star' (left) and 'Alapaha' (right). Significance set at $P < 0.05$ among treatments for a given storage time. Non-significant values are denoted by ns.

V. Fruit color

We determined the effect of blue light on various aspects of fruit color and did not observe any significant difference due to the effect of blue light in 'Alapaha' and 'Star' during postharvest storage (Table 3).

Table 3: Fruit color values determined at various times during storage in 'Star'.

Days after treatment	Treatment	L*	a*	b*	c*	h*
0	-	29.37	-0.72	-3.60	3.71	258.71
4	Blue	30.23 a	-0.87	-4.01	4.12	258.52
	White	29.15 b	-0.64	-3.44	3.55	261.12
	Dark	30.19 ab	-0.90	-4.05	4.16	257.92
	Prob>*F*	0.0269	ns	ns	ns	ns
15	Blue	30.66	-0.89	-4.24	4.40	257.35
	White	29.79	-0.65	-3.86	4.07	258.62
	Dark	30.27	-0.81	-3.92	4.04	260.90
	Prob>*F*	ns	ns	ns	ns	ns
21	Blue	30.41	-1.00	-4.37	4.50	257.78
	White	30.07	-1.03	-4.22	4.36	256.47
	Dark	30.69	-1.04	-4.32	4.47	255.43
	Prob>*F*	ns	ns	ns	ns	ns

Significance set at $P < 0.05$ among treatments for a given storage time. Means followed by the same letter are not significantly different from each other. Non-significant values are denoted by ns.

Table 4: Fruit color values determined at various times during storage in 'Alapaha'.

Days after treatment	Treatment	L*	a*	b*	c*	h*
0	-	29.35	-0.92	-3.7	3.83	256.57
4	Blue	29.56	-0.97	-3.41	3.57	255.08
	White	29.59	-0.90	-3.28	3.41	255.13
	Dark	29.42	-0.93	-3.36	3.50	255.28
	Prob>*F*	ns	ns	ns	ns	ns
14	Blue	27.97	-0.79 b	-3.08	3.27	253.69
	White	28.35	-0.58 ab	-2.83	2.96	259.74
	Dark	27.85	-0.42 a	-2.74	2.87	261.24
	Prob>*F*	ns	0.0398	ns	ns	ns
24	Blue	29.02	-0.72	-2.95	3.11	255.15
	White	29.43	-0.89	-3.25	3.39	254.59
	Dark	28.84	-0.58	-2.55	2.83	256.67
	Prob>*F*	ns	ns	ns	ns	ns

Significance set at $P < 0.05$ among treatments for a given storage time. Means followed by the same letter are not significantly different from each other. Non-significant values are denoted by ns.

Objective 2: Evaluate the effect of blue light on postharvest disease development.

I. Natural disease development on fruit

Alternaria, Botrytis, and anthracnose (caused by *C. acutatum*) fruit rots were the most common naturally occurring postharvest diseases on both cultivars. Overall fruit disease incidence after the last postharvest storage period ranged from 44.8 to 56.2% on Alapaha and 17.0 to 26.3% on Star (Fig. 3). There was no significant effect of light treatment on fruit disease incidence for either cultivar ($P = 0.319$ and 0.139 for Alapaha and Star, respectively).

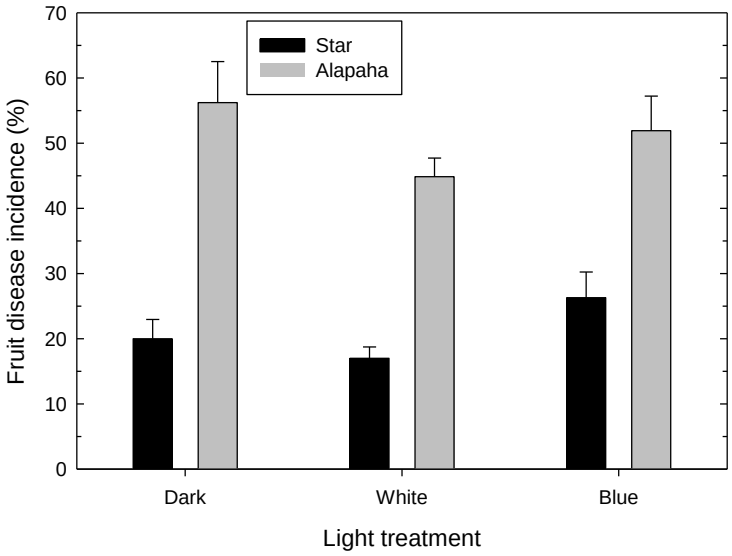

Figure 3. Natural postharvest disease incidence in 'Star' and 'Alapha' fruit under various light treatments after the last postharvest storage period.

II. Artificial inoculations

Artificial inoculations with *Alternaria* and *Colletotrichum* on cultivar Star yielded consistent fruit disease development, whereas the other pathogen-cultivar combinations did not; hence, only data for these two pathogens on Star were analyzed further. Mean postharvest fruit disease incidence among four replicates varied from 64.3 to 75.2% for *Alternaria* and from 44.8 to 68.7% for *Colletotrichum* (Fig. 4) and was not influenced by light treatment prior to inoculation and during fruit storage ($P > 0.173$).

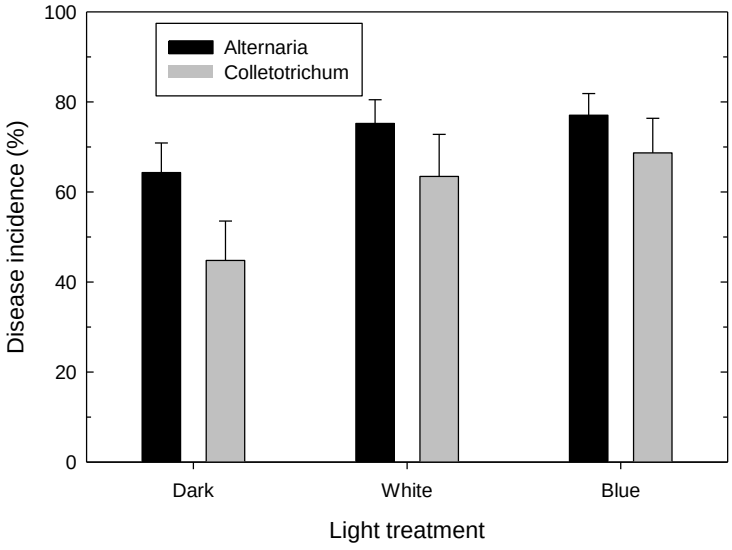

Figure 4. Percent postharvest disease incidence in 'Star' fruit inoculated with *A. tenuissima* and *C. acutatum* under various light treatments.

Objective 3: Evaluate color development on green spots in fruit of cultivar Farthing.

The grayscale values of the green spots on Farthing fruit, as determined by image analysis of photographs taken at 0, 4, 8, and 11 days of exposure to the three light treatments, decreased over time, documenting that the green spots became more blue (Fig. 5). However, there was no difference in the rate of ripening of the green spots among the dark, white, and blue light treatments.

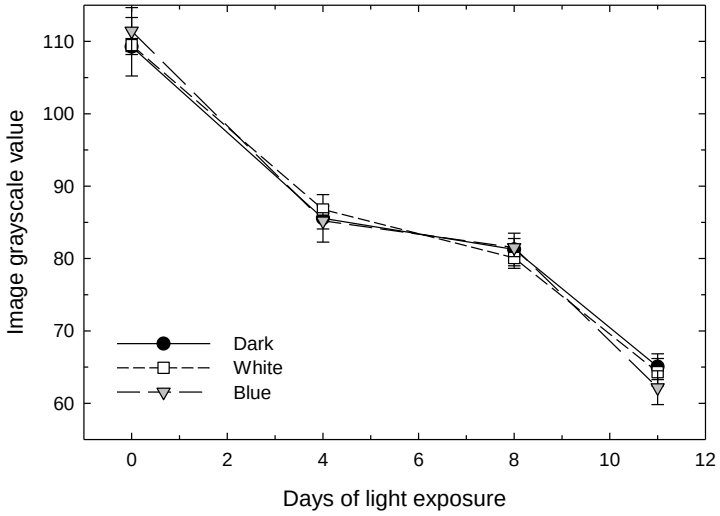

Figure 5. Ripening of green spots on 'Farthing' fruit under various light treatments in cold storage. Image analysis grayscale values are scaled between 0 (black) and 255 (white); hence, the lower the value, the more blue the spot on the berries.

Conclusions:

The research determined the effect of blue light on postharvest storage attributes including anthocyanin accumulation, postharvest disease development in 'Star' and "Alapaha' and color improvement in green spotted 'Farthing' fruits. We did not see any meaningful effect of blue light on postharvest shelf-life and anthocyanin accumulation. Further there were no statistically significant effects of the light treatments on postharvest fruit rot development following natural or artificial inoculation. Under the experimental conditions evaluated here, postharvest blue light treatment also did not accelerate coloration of immature green spots on Farthing fruit.

Impact Statement

The research proposed in this study determined the effect of blue light on blueberry postharvest fruit quality, anthocyanin accumulation, disease development and color improvement in green spotted fruit. Blue light did not have any significantly effect on postharvest fruit quality traits, anthocyanin content, postharvest disease development and did not hasten color development in green spotted 'Fathing' fruit compared to the control treatments. Further studies with increasing the quantity and duration of blue light may be required to generate maximal fruit response

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