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Effect of Electronic Cold-Pasteurization™ (ECP™) on Fruit Quality and Postharvest Diseases during Blueberry Storage

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Abstract: With the growing popularity of blueberries and the associated increase in blueberry imports and exports worldwide, delivering fruit with high quality, longer shelf-life, and meeting phytosanitary requirements has become increasingly important. The objective of this study was to determine the effects of electron beam irradiation using a new Electronic Cold-Pasteurization™ (ECP™) technology on fruit quality, microbial safety, and postharvest disease development in two southern highbush blueberry cultivars, 'Farthing' and 'Rebel'. Fruit packed in clamshells were subjected to four levels of ECP™ irradiation (0, 0.15, 0.5, and 1.0 kGy) and evaluated for fruit quality attributes, surface microbial load, and postharvest disease incidence during various storage times after treatment and cold storage. Overall, there was no effect of irradiation on visual fruit quality in either cultivar. Fruit firmness and skin toughness in 'Farthing' was reduced following irradiation at 1.0 kGy, but no such effect was observed in 'Rebel'. Other fruit quality characteristics such as fruit weight, total soluble solids content, or titratable acidity were not affected. Irradiation at 1.0 kGy significantly reduced total aerobic bacteria and yeast on the fruit surface, and in the case of 'Rebel', also levels of total coliform bacteria. There was no significant effect of irradiation on postharvest disease incidence in these trials. Overall, data from this study suggests that an irradiation dose lower than 1.0 kGy using ECP™ can be useful for phytosanitary treatment in blueberry fruit while avoiding undesirable effects on fruit quality in a cultivar-dependent manner.

Keywords: electron beam irradiation; fruit texture; postharvest rot

1. Introduction

Blueberries (*Vaccinium* spp.) are becoming increasingly popular due to the rising awareness of the health benefits of consuming blueberry fruit, which include decreased risk of cardiovascular diseases, improved cognitive performance, and decrease in aging-related damage [1,2]. Commercially important blueberry species include lowbush (*Vaccinium angustifolium* Ait.) and northern highbush (*Vaccinium corymbosum* L.) mainly cultivated in the northern parts of the United States, and rabbiteye (*V. virgatum* Ait.) and southern highbush (hybrids of *V. corymbosum*, *V. virgatum*, and *V. darrowii* Camp.) grown mostly in the southern states [3,4]. Recently, production of blueberries has expanded to 27 countries (in 2011) compared with only ten countries in 1990 [5]. The United States is the largest producer of blueberries globally [5], supplying 347.7 million kg of cultivated and wild blueberries in

2016 [6]. The United States also plays an important role in the import and export trade of blueberries [7]. In 2016, the United States exported 31.7 million kg of fresh and 25.4 million kg of frozen blueberries and imported 149 million kg of fresh and 75.6 million kg of frozen fruit [8].

As global production and trade continues to rise, it becomes increasingly important to maintain fruit quality, nutrient content, phytosanitary safety, and eliminate pests and diseases in blueberries during storage to ensure that this fast-growing export and import market is not negatively impacted. Postharvest losses in fruits can vary from 10 to 40% [9]. After harvest, blueberries have a shelf-life of approximately 7 to 40 days depending on the genotype, method of harvest, and storage regime [9,10]. During postharvest storage, blueberry fruit quality can decline due to fruit softening [11]. Other contributing factors in loss of fruit quality are postharvest diseases caused primarily by fungal plant pathogens such as *Colletotrichum* spp. (ripe rot), *Alternaria* spp. (Alternaria fruit rot), and *Botrytis cinerea* (gray mold), among others [12–15]. In addition to postharvest disease-causing organisms, it is important to eliminate foodborne pathogens or associated indicator organisms [16–18]. Although outbreaks of foodborne illnesses associated with consumption of blueberry fruit have been relatively rare, produce brokers and buyers have begun to apply rigid (and typically proprietary) microbial standards to frozen blueberries destined for the processing market [19]. Although similar standards currently are not in place for the fresh-market, reducing microbial risk remains a key consideration for fresh-market production as well [20]. Finally, in order to export blueberries to other countries, they are required to be certified free of certain insect pests such as Mediterranean fruit fly (*Ceratitis capitata*), South American fruit fly (*Anastrepha fraterculus*), European grapevine moth (*Lobesia botrana*), blueberry maggot (*Rhagoletis mendax*), and plum curculio (*Conotrachelus nenuphar*) [21,22].

Fumigation of export goods with methyl bromide was the most commonly used phytosanitary treatment for elimination of pests, but has been phased out in the United States, with the exception of a few critical uses [23,24]. Methyl bromide also requires the produce temperature to be increased in order to be effective, thereby breaking the cold-chain and potentially having an adverse effect on quality. Interruption of cold-chain can decrease shelf-life considerably by increasing undesirable fruit metabolism [25]. Irradiation using gamma rays, X-rays, or electron beams could be an alternative to fumigation in eliminating pests and in preserving quality by reducing decay organisms and plant and human pathogens [23,24,26]. Previous work supported the use of electron beam and gamma irradiation to maintain shelf-life and fruit quality attributes in blueberry fruit [27–30]. In the United States, regulatory approval has been obtained for the use of irradiation on fresh fruits and vegetables up to 1 kGy [31]. Previous studies suggested an irradiation dose of 0.4 kGy to be effective against most insect pests, 0.2–0.8 kGy to cause a 1-log reduction in surface bacterial pathogens causing foodborne illness, and higher doses of 1–3 kGy for postharvest disease-causing fungi [22,32–34].

The objective of this study was to determine the effect of irradiating postharvest blueberry fruit using a new form of electron beam technology, Electronic Cold-Pasteurization^{TM} (ECP^{TM}) developed by ScanTech Sciences (Norcross, GA, USA) at their Research and Development (R&D) facility at Idaho State University (ISU). This R&D facility is a small-scale version of a commercial ECP^{TM} food treatment facility, which is currently being constructed by ScanTech in McAllen, TX and will be operational in the fourth quarter of 2018. This technology employs a highly focused beam of electrons, treating samples for only milliseconds on a high-speed conveyor while maintaining cold-chain integrity. A key advantage of electron beam irradiation over gamma rays (from nuclear sources such as Cobalt-60) or X-rays is the ability to deliver extremely high dose rates with improved accuracy since the beam dynamics can be more precisely controlled. These high dose rates equate to significantly less time for treatment and, consequently, potential for higher quality produce. The ECP^{TM} treatment can treat an entire truckload (around 60,000 clamshells) of blueberries in a little over 30 min, whereas gamma rays can take several hours for the same quantity (C. Starns, unpublished observations). This is the first study to investigate the effect of irradiation on fruit quality attributes, postharvest disease incidence, and surface microbes of food safety concern in two southern highbush blueberry cultivars treated with ECP^{TM} prior to cold storage.

2. Materials and Methods

2.1. Fruit Collection and Irradiation

Two trials were conducted with hand-harvested fruit from southern highbush blueberry cultivars 'Farthing' and 'Rebel' in Alma, GA. In trial 1 (April 2016), 'Farthing' fruit were obtained from a commercial packing facility, where fruit had already been prepacked into pint-size clamshell containers (473 mL). In trial 2 (May 2016), 'Rebel' fruit were obtained from a different packing facility, also already prepacked in pint-size clamshells. In addition, trial 2 included 'Farthing' fruit hand-harvested by the investigators from a commercial blueberry farm and packed into pint-size clamshells.

A subsample of clamshells in each trial was taken directly to the University of Georgia, Athens, GA, USA (330-km transit in refrigerated cooler) to serve as an unshipped control (not transported to and from the irradiation facility). Initial fruit quality attributes and postharvest disease incidence were recorded from this unshipped control. The remaining fruit in clamshells were arranged on standard flats (12 clamshells/flat), placed in a styrofoam cooler with ice packs, and shipped overnight from Alma, GA to ISU, Pocatello, ID. A foam sheet was placed on the inner side of the lid of each clamshell and in between clamshells to minimize fruit injury during shipment.

At ISU, fruit in clamshells were subjected to electron beam irradiation treatment at ScanTech's R&D facility using proprietary ECP^{TM} technology. A 10-MeV electron beam, driven by an advanced high-energy electron accelerator, is magnetically focused through a scanning horn which delivers precision dose control. At the R&D facility, clamshells containing fruit were subjected to four levels of irradiation, 0, 0.15, 0.5, and 1.0 kGy; the treatments were completed in less than a second per clamshell. The respective doses were achieved using the National Institute of Standards and Technology (NIST)-traceable alanine pellets with extensive dose mapping on various blueberry configurations prior to the experimental fruit being shipped to the facility. Hundreds of data points were obtained and measured on a Bruker Bio-spin Electron Paramagnetic Resonance spectrometer, all of which are NIST traceable and International Organization for Standardization/American Section of the International Association for Testing Materials compliant. Treatments were replicated four times (i.e., four clamshells/irradiation level/postharvest storage period/cultivar), with a few exceptions where fewer replicate clamshells were available. The 0-kGy treatment served as an untreated control wherein fruit were shipped but not irradiated. After irradiation, fruit were shipped back by overnight courier to the University of Georgia where they were placed in a walk-in cooler at 2 to 4 °C under high relative humidity (>85%) until further assessment. The entire shipping and treatment process (from Alma to the treatment facility at ISU and to Athens for cold-storage and evaluation) took between 6 to 7 days. The unshipped control clamshells were stored in a 2 to 4 °C walk-in cooler until further evaluation. Fruit were removed from cold storage and evaluated for postharvest fruit quality attributes at 6, 13, and 25 days after irradiation treatment; microbial load on the fruit surface at 6 days after treatment; and postharvest disease incidence at 6 and 13 days after treatment followed by 4 days at room temperature. Fruit quality, microbial load and postharvest disease incidence analyses at a given time-point were performed using four replicates; for every replicate, fruit from a separate clamshell were used and divided for the above analyses.

2.2. Evaluation of Fruit Quality Attributes

For evaluation of fruit quality, visual assessment as well as measurement of fruit weight, texture, titratable acidity (TA), and total soluble solids (TSS) content were performed. For visual assessment, 30 fruit per replicate were scored for symptoms of bruising such as tears, dents, leakiness, or signs of mold. Fruit were examined by eye for visual defects and percent sound fruit were calculated. For fruit texture, two variables, fruit compression and skin puncture force, were measured on 12 fruit per replicate using a fruit texture analyzer (GS-15, Güss Manufacturing, Strand, South Africa); fruit were oriented on the equatorial plane for this assessment. For compression measurements, a 1.5-cm diameter plate was used with parameters set at forward speed 6 mm/s, measure speed 5 mm/s,

and measure distance 1.00 mm. For skin puncture force measurements, a 1.5-mm flat-tip probe was used with parameters set at a forward speed 10 mm/s, measure speed 5 mm/s, and measure distance 3.00 mm. Fruit weight was recorded on 20 individual fruit per replicate using a balance (Quintix Precision Balance, Sartorius, Bohemia, NY, USA).

For TA and TSS measurement, juice was extracted from ~40 g of fruit per replicate using a household blender and centrifuged for 10 min at 3901X *g* using a benchtop centrifuge (Allegra X-22, Beckman Coulter Life Sciences, Indianapolis, IN, USA). The resulting supernatant was filtered through two layers of cheesecloth. To measure TSS, 300 µL of supernatant was tested using a digital handheld refractometer (Atago USA, Belleveue, WA, USA). For TA, the supernatant was titrated using an automatic mini titrator (Hanna Instruments, Woonsocket, RI, USA) and values were reported as percent citric acid (CA). Statistical analysis (one-way analysis of variance for a completely randomized design) was performed separately for each trial and cultivar using JMP Pro 12 (SAS Institute, Cary, NC, USA). Means were separated using Tukey's Honest Significant Difference (HSD) test ($\alpha = 0.05$).

2.3. Evaluation of Fruit Surface Contaminants

Microbial loads on the fruit surface were determined 6 days after treatment following the protocol described in Mehra et al. [35]. One 50-g fruit sample (~30 berries) per replicate was placed in a 0.5-L flask with 50 mL of sterile phosphate buffer (pH 7.2), and the flask was agitated on a wrist action shaker (Burrell, Pittsburg, PA, USA) at medium speed for 15 min. Aliquots of the wash buffer and 1:20 and 1:100 dilutions were plated in triplicate onto plate count agar (PCA), dichloran rose bengal chloramphenicol agar (DRBC), and Petrifilms (3M Microbiology, St. Paul, MN, USA) for enumeration of aerobic bacteria, total yeasts and molds, and *E. coli* and coliforms, respectively. PCA and DRBC dishes were incubated at room temperature and evaluated after 3 and 5 days, respectively. Petrifilms were incubated at 35 °C and colony counts made after 2 days. Colony-forming units (CFU) per gram of fruit were log-transformed and subjected to one-way analysis of variance using PROC GLM in SAS version 9.4 (SAS Institute, Cary, NC, UAS) followed by means separation using Tukey's test.

2.4. Assessment of Postharvest Disease

An initial postharvest disease assessment was made on the unshipped control following 4 days of storage at room temperature (23–25 °C) to allow latent infections to manifest themselves [35]. Subsequently, on fruit subjected to ECP^{TM} treatment, fruit samples (60 berries per replicate) were removed from postharvest storage 6 days (trials 1 and 2) and 13 days (trial 1 only) after treatment, and similarly incubated at room temperature for 4 days. The 13-day assessment was not included in trial 2 as poor fruit quality of 'Rebel' in that trial resulted in near 100% decay after cold storage and subsequent room temperature incubation. For each assessment date and replicate, the number of fruit with symptoms and signs of postharvest decay was counted following examination of fruit samples with a stereo microscope. Fungal pathogens associated with diseased fruit were identified macroscopically and microscopically (utilizing both stereo- and compound microscopes) based on characteristic symptoms and signs [36,37]. Based on the number of fruit with disease symptoms and pathogen signs, postharvest disease incidence was calculated and arcsine-square root transformed for analysis by one-way analysis of variance using PROC GLM followed by means separation using Tukey's test.

3. Results

3.1. Fruit Visual Quality and Texture

To determine the effect of ECP^{TM} treatment on fruit quality and texture, qualitative visual assessment to determine percent sound fruit, and quantitative measurements on fruit compression and skin puncture were performed (Figures 1–3). Since fruit were shipped from the site of harvest in Alma, GA, to the irradiation facility in Pocatello, ID, an unshipped control was included along with the shipped but untreated control (shipped to the treatment facility but receiving 0 kGy irradiation) to

compare changes in fruit quality associated with shipping. In general, shipping did not affect fruit visual quality and texture characteristics (Figures 1–3). There were no significant effects of ECP^{TM} on visual quality in 'Farthing' in both trials compared with the control (Figures 1A and 2A).

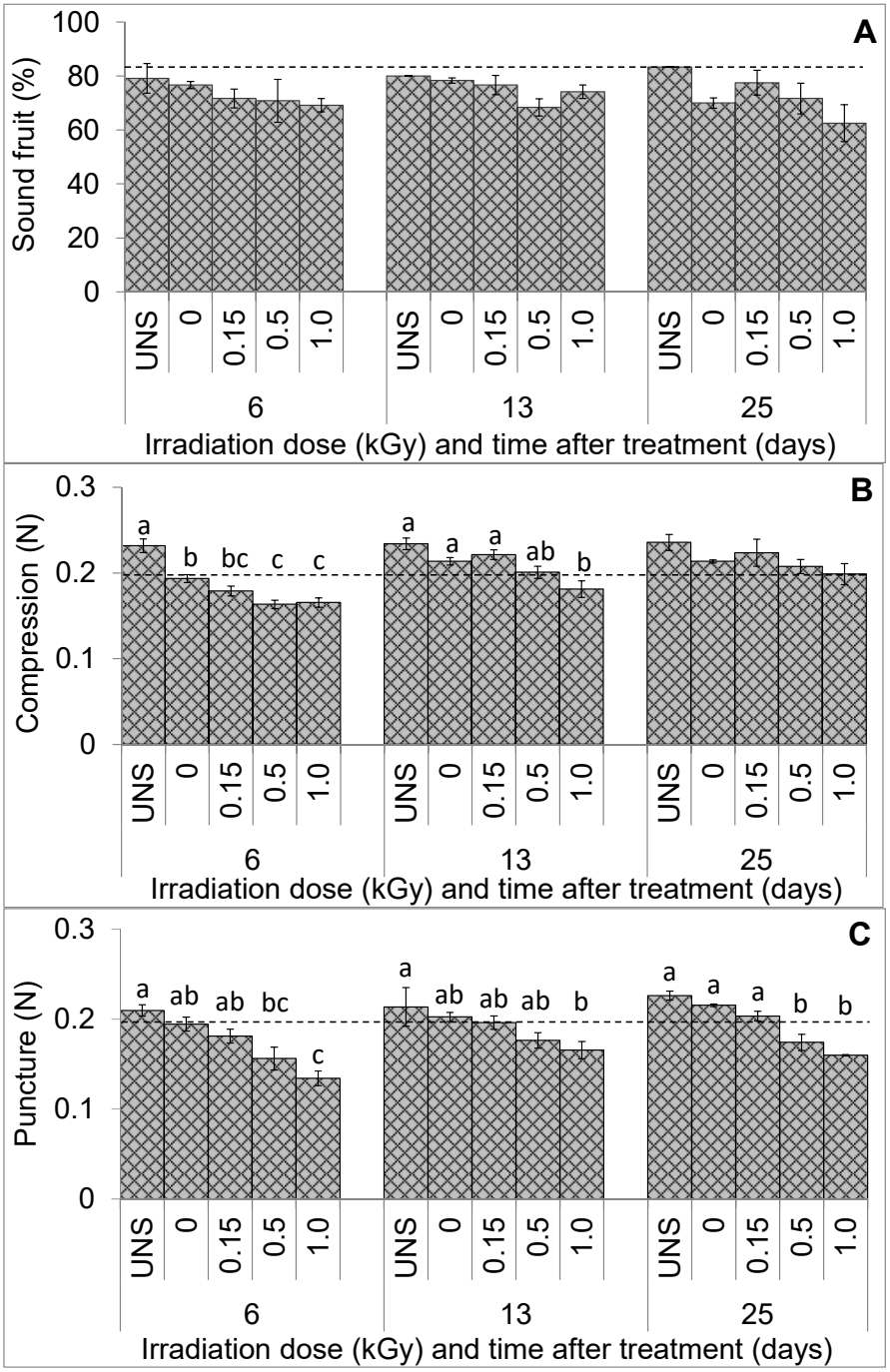

Figure 1. Effect of Electronic Cold-Pasteurization^{TM} on percent sound fruit (**A**), compression (**B**), and puncture (**C**) for 'Farthing' blueberries in trial 1. Treatments included an unshipped control (UNS; not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Evaluations were conducted 6, 13, and 25 days after irradiation treatment. Fruit were stored at 2 to 4 °C under high relative humidity until assessments were performed. An initial fruit quality assessment was performed after harvest shown as a horizontal dashed line. Means within the same storage times after treatment followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$).

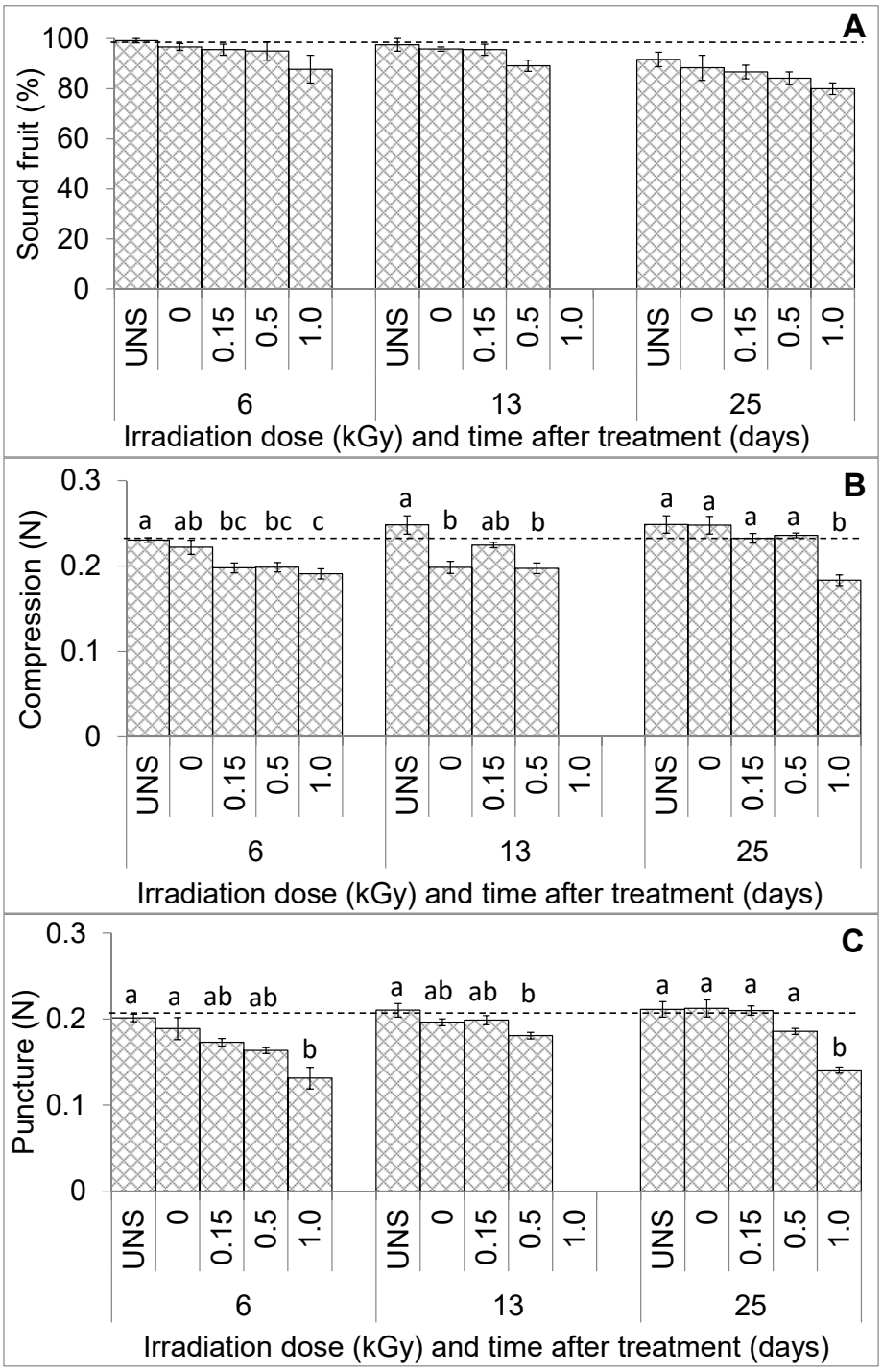

Figure 2. Effect of Electronic Cold-Pasteurization™ on percent sound fruit (**A**), compression (**B**), and puncture (**C**) for 'Farthing' blueberries in trial 2. Treatments included an unshipped control (UNS; not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Evaluations were conducted 6, 13, and 25 days after irradiation treatment. Fruit were stored at 2 to 4 °C under high relative humidity until assessments were performed. An initial fruit quality assessment was performed after harvest shown as a horizontal dashed line. Due to low number of fruit, measurements were not performed for fruit treated at 1 kGy at 13 days after treatment. Means within the same storage times after treatment followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$).

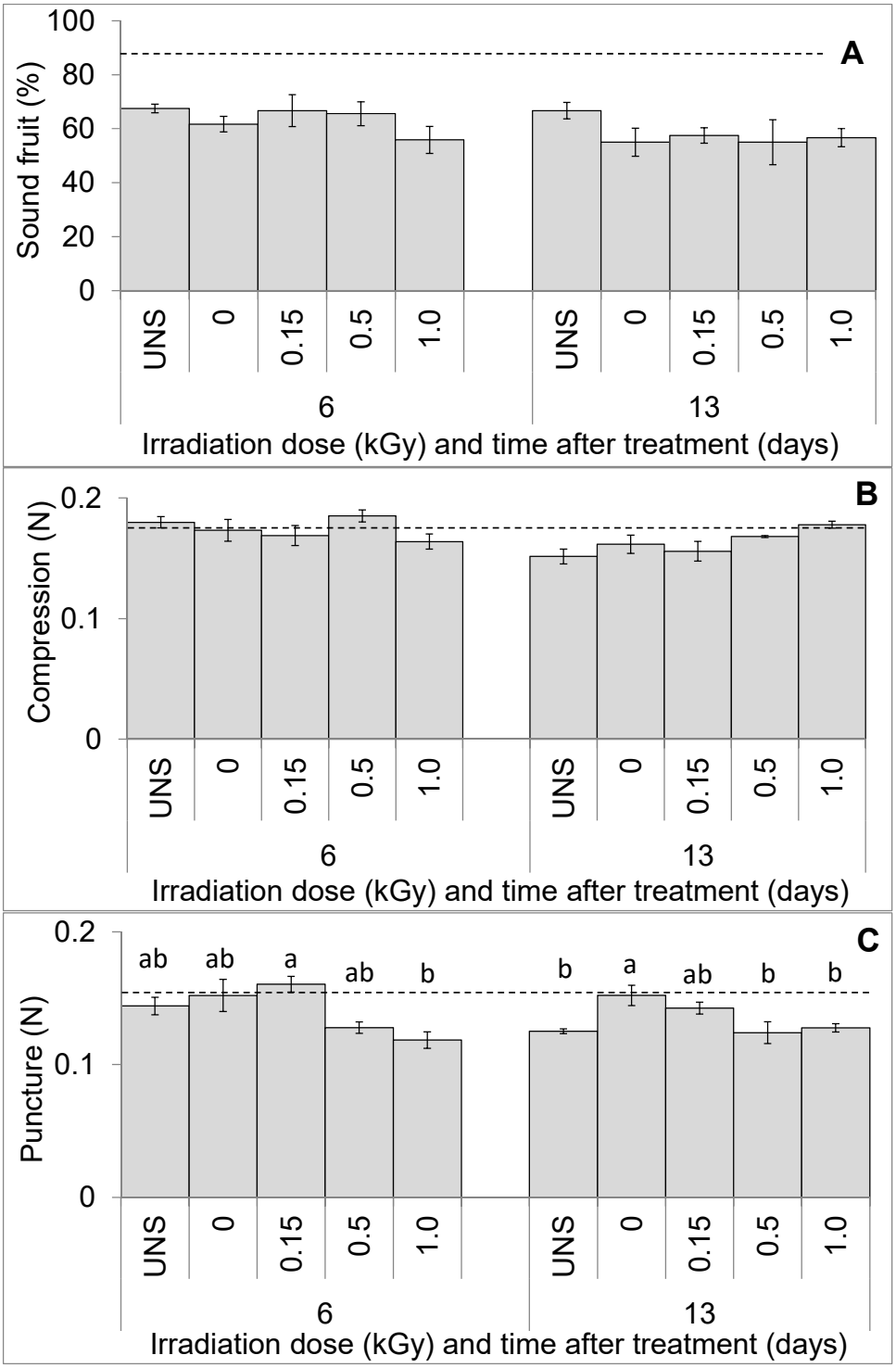

Figure 3. Effect of Electronic Cold-Pasteurization™ on percent sound fruit (**A**), compression (**B**), and puncture (**C**) for 'Rebel' blueberries in trial 2. Treatments included an unshipped control (UNS; not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Evaluations were conducted 6 and 13 days after irradiation treatment. Fruit were stored at 2 to 4 °C under high relative humidity until assessments were performed. An initial fruit quality assessment was performed after harvest shown as a horizontal dashed line. Means within the same storage times after treatment followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$).

Fruit texture, measured using compression, indicated that a higher dose of irradiation resulted in a loss of firmness in 'Farthing' in both trials at various times after treatment (Figures 1B and 2B). Compared with unshipped and 0-kGy controls, a decrease in firmness was small but statistically significant with the 1.0-kGy treatment. Compared with the 0-kGy control, there was a 0.03 N decrease in firmness in the 1.0-kGy treatment at 6 and 13 days after treatment in trial 1; trial 2 showed a 0.03 N and 0.06 N at 6 and 25 days after treatment, respectively. Similarly, irradiation at 1.0 kGy resulted in a decrease in skin toughness, measured by the skin puncture force, relative to the controls in 'Farthing' in both trials. Compared with the 0-kGy control, there was a 0.04 to 0.06 N decrease in skin puncture force in the 1.0-kGy treatment at 6, 13, and 25 days after storage; trial 2 showed a 0.07 N decrease in skin puncture force at 6 and 25 days after treatment. (Figures 1C and 2C).

Comparison of fruit texture between varieties in the unshipped control at the initial and later time-points during postharvest storage indicated that 'Rebel' exhibited lower firmness and skin puncture force than 'Farthing'. 'Rebel' fruit could not be evaluated for postharvest quality attributes at 25 days after treatment due to poor quality. Visual quality of ECP^{TM} -treated fruit of 'Rebel' did not differ from that in the control treatments (Figure 3A). There were no significant differences in fruit compression among treatments at both time points during postharvest storage (Figure 3B). Skin toughness was not different among treatments at 6 days after irradiation (Figure 3C). At 13 days after treatment, fruit irradiated at 0.5 and 1.0 kGy had lower values than the 0-kGy control, but were not different from the unshipped control suggesting that skin toughness did not change due to ECP^{TM} in 'Rebel'.

3.2. Total Soluble Solids Content, Titratable Acidity, and Weight

There were no effects of irradiation on total soluble solids content or titratable acidity in 'Farthing' and 'Rebel' at various times after treatment (Table 1). In general, fruit weight did not change during postharvest storage. Similarly, no significant change in fruit weight was observed at various times after irradiation treatment compared with both unshipped and the 0-kGy controls in 'Farthing' and 'Rebel' (Table 2).

Table 1. Total soluble solids (TSS) content and titratable acidity (TA) of 'Farthing' and 'Rebel' blueberry fruit subjected to Electronic Cold-Pasteurization^{TM} followed by different cold storage periods.

Days after Treatment	Treatment [a]	Farthing Trial 1		Farthing Trial 2		Rebel Trial 2	
		TSS	TA	TSS	TA	TSS	TA
	(kGy)	(% Brix)	(% CA)	(% Brix)	(% CA)	(% Brix)	(% CA)
0	UNS	13.0	0.64	13.0	0.68	8.3	0.21
6	UNS	12.4	0.51	12.2	0.56	7.9	0.20
	0	12.6	0.59	12.0	0.56	8.2	0.20
	0.15	12.7	0.54	13.0	0.45	8.2	0.23
	0.5	12.9	0.51	12.2	0.47	8.4	0.20
	1.0	13.0	0.51	12.1	0.53	8.0	0.21
13	UNS	12.6	0.51	12.8	0.53	8.0	0.20
	0	12.7	0.57	12.9	0.53	8.0	0.21
	0.15	12.8	0.54	12.0	0.51	8.1	0.21
	0.5	12.7	0.52	12.3	0.53	8.1	0.20
	1.0	12.9	0.51	-	-	8.1	0.21
25	UNS	12.8	0.45	13.0	0.38	-	-
	0	12.8	0.47	12.4	0.41	-	-
	0.15	12.7	0.47	12.8	0.42	-	-
	0.5	12.6	0.48	12.4	0.33	-	-
	1.0	12.9	0.44	12.6	0.30	-	-

[a] Treatments included an unshipped control (UNS, not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Fruit were stored at 2 to 4 °C under high relative humidity until TSS and TA measurements were performed. An initial fruit quality assessment was done after harvest (day 0). Due to low number of 'Farthing' fruit in trial 2, no assessment was performed at 13 days after irradiation for fruit treated at 1.0 kGy. In case of Rebel, almost 100% decay in fruit resulted in no assessment at 25 days after treatment. One-way analysis of variance indicated no significant differences among irradiation levels within a given storage period after treatment in each trial ($\alpha = 0.05$).

Table 2. Weight of 'Farthing' and 'Rebel' blueberry fruit subjected to Electronic Cold-Pasteurization™ followed by different cold storage periods.

Days after Treatment	Treatment [a] (kGy)	Farthing Trial 1	Farthing Trial 2	Rebel Trial 2
		Weight (g)	Weight (g)	Weight (g)
0	UNS	1.8	2.1	1.6
6	UNS	1.8	1.8 b	1.7
	0	1.9	2.1 ab	1.6
	0.15	2.0	2.3 a	1.7
	0.5	1.9	2.1 ab	1.7
	1.0	2.0	2.1 ab	1.7
	Prob > *F*	ns	0.0484	Ns
13	UNS	2.1 a	2.0	1.6
	0	1.9 ab	2.0	1.6
	0.15	1.9 ab	2.0	1.6
	0.5	1.9 ab	2.0	1.6
	1.0	1.8 b	-	1.6
	Prob > *F*	0.0694	ns	Ns
25	UNS	1.7	2.1	-
	0	1.8	1.9	-
	0.15	1.8	2.1	-
	0.5	1.7	1.9	-
	1.0	1.8	2.2	-
	Prob > *F*	ns	ns	

[a] Treatments included an unshipped control (UNS, not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Fruit were stored at 2 to 4 °C under high relative humidity until weight measurements were performed. An initial fruit quality assessment was done after harvest (day 0). Due to low number of 'Farthing' fruit in trial 2, no assessment was performed at 13 days after irradiation for fruit treated at 1.0 kGy. In case of Rebel, almost 100% decay in fruit resulted in no assessment at 25 days after treatment. For every trial, means within the same storage times after treatment followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$). Nonsignificant values are denoted by ns.

3.3. Microbial Load on Fruit after Treatment

Microbial loads on the fruit surface were determined for samples collected 6 days after ECP™ treatment. Microbial population densities were highest for total aerobic bacteria and total yeasts (up to ~10^5 CFU/g of fruit), followed by total molds; colony counts were lowest for coliforms (Table 3). Only a single sample of 'Rebel' showed presence of *E. coli* (at 2.7 CFU/g fruit), and therefore no statistical analysis was possible for *E. coli*. Microbial loads were similar across the two trials of 'Farthing', but were considerably higher for 'Rebel', which had very soft fruit and also the highest microbial counts (Table 3).

ECP™ significantly reduced total aerobic bacteria (by between 0.5 and 1 log units) in each of the three cultivar × trial combinations, but typically only at the 1.0-kGy irradiation level (Table 3). Yeast counts were similarly reduced in all cases, but again significant only for the 1.0-kGy level. Total surface mold counts were not reduced by irradiation in any of the cases. Population densities of coliform bacteria were not impacted on 'Farthing', but were reduced significantly and by over 2 log units on 'Rebel' (Table 3), which had the highest microbial loads in general.

Table 3. Surface microbial load, in log (colony-forming units per g of fruit), on 'Farthing' and 'Rebel' blueberry fruit subjected to Electronic Cold-Pasteurization™ 6 days after treatment.

Treatment [a] (kGy)	Aerobic Bacteria	Yeasts	Molds	Coliforms
Farthing Trial 1				
UNS	3.83 a	4.09 a	1.82	1.15
0	3.94 a	3.99 a	1.07	0.89
0.15	3.83 a	4.00 a	1.11	0.88
0.5	3.59 ab	3.77 ab	1.26	0.52
1.0	3.14 b	3.48 b	1.41	0.19
Prob > F	0.0226	0.0119	ns	ns
Farthing Trial 2				
UNS	3.15 a	3.15 a	2.31	0.73
0	3.00 a	2.98 a	1.43	0.41
0.15	3.25 a	3.21 a	1.78	0.47
0.5	3.06 a	3.03 a	1.44	0.34
1.0	2.34 b	2.47 b	1.94	0.06
Prob > F	0.0182	0.0169	ns	ns
Rebel Trial 2				
UNS	5.03 a	4.75 a	4.28	2.90 a
0	4.82 a	4.74 a	3.98	3.05 a
0.15	4.28 b	4.10 b	3.85	2.43 ab
0.5	4.10 b	4.20 ab	3.70	1.40 bc
1.0	3.93 b	3.96 b	3.38	0.85 c
Prob > F	0.0003	0.0295	ns	0.0106

[a] Treatments included an unshipped control (UNS, not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Fruit were stored at 2 to 4 °C under high relative humidity until wash platings were performed. Means within the same trial and column followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$). Nonsignificant values are denoted by ns.

3.4. Postharvest Disease Incidence on Fruit after Treatment

Postharvest disease incidence was determined at 6 and 13 days after treatment in trial 1 and for the 6-day post-treatment period in trial 2, each followed by a 4-day fruit exposure at room temperature to allow infections to develop. Anthracnose (caused by *Colletotrichum acutatum*), *Botrytis cinerea*, *Alternaria* sp., *Aureobasidium pullulans*, *Phomopsis vaccinii*, and *Cladosporium* sp. were observed on postharvest fruit; no significant effects of ECP™ on disease incidence were observed, neither at low decay incidence levels (<5% as observed with 'Farthing'), nor at high levels (~15% as observed with 'Rebel') (Table 4).

Table 4. Postharvest disease incidence, in percent, on 'Farthing' and 'Rebel' blueberry fruit subjected to Electronic Cold-Pasteurization™ 6 or 13 days after treatment plus 4 days at room temperature.

Days after Harvest	Treatment [a] (kGy)	Farthing Trial 1	Farthing Trial 2	Rebel Trial 2
0	UNS	0.75	1.4	17.5
6	UNS	0.83	0.42	28.3
	0	4.2	1.3	17.1
	0.15	4.6	0.63	15.4
	0.5	4.6	0.42	16.7
	1	4.2	0	14.2
	Prob > *F*	ns	ns	ns
13	UNS	0 b	-	-
	0	4.2 a	-	-
	150	3.8 a	-	-
	500	5.4 a	-	-
	1000	2.8 a	-	-
	Prob > *F*	0.011		

[a] Treatments included an unshipped control (UNS, not shipped to the irradiation facility) and four levels of irradiation; no irradiation control (0), 0.15, 0.5, and 1.0 kGy. Fruit were stored at 2 to 4 °C under high relative humidity, followed by 4 days at room temperature, until disease assessments were performed. An initial fruit quality assessment was done after harvest (day 0). The 13-day assessment was not included in trial 2 due to low number of fruit in Farthing and nearly 100% decay in 'Rebel'. Means within the same trial and column followed by the same letter are not significantly different from each other based on one-way analysis of variance ($\alpha = 0.05$). Nonsignificant values are denoted by ns.

4. Discussion

The objective of this study was to determine the effect of ECP™ on fruit quality attributes, surface microbial load, and postharvest diseases on two southern highbush cultivars. ECP™ treatment resulted in a cultivar-specific response on fruit quality. In 'Rebel', ECP™ had no effect on visual appearance, fruit firmness, and skin toughness. In 'Farthing', however, ECP™ at 1.0 kGy, resulted in a reduction in fruit firmness and skin toughness but did not affect the visual appearance of the fruit, which was assessed based on the presence of bruises and defects such as leakiness or dents. The differential cultivar response to irradiation could be due to inherent differences in fruit firmness between the two cultivars. 'Rebel' was softer and had lower firmness and skin puncture force than 'Farthing'. Thus, irradiation may not have decreased firmness further in 'Rebel'. Similar results with differences in responses of blueberry cultivars varying in fruit texture have been observed using previous irradiation studies with various radiation sources [21,27,38]. Cultivars with firmer texture were softened after irradiation, whereas the effect of irradiation on two softer-textured cultivars varied; irradiation further softened fruit of one of the cultivars but had no effect on the other [38]. These data indicate that fruit having inherently firmer texture may be softened by irradiation, whereas the texture of fruit with lower fruit firmness may not be affected.

In this study, fruit softening and a decrease in skin toughness in 'Farthing' occurred only at the highest irradiation dose of 1.0 kGy. These results are consistent with other studies that report a dose-dependent response to irradiation with higher doses resulting in a decrease in firmness in blueberry fruit regardless of the method of irradiation. When conventional electron beam irradiation was used to treat blueberries, doses of 1.1 kGy and higher affected fruit texture resulted in softening [28]. Other studies using gamma irradiation around 0.75 kGy and higher reported increased softening in blueberries [21,27,39]. The effect of higher doses of irradiation on fruit softening has also been observed with other fruits such as raspberries [40], peaches [23,41,42], apricots [23], and grapes [43].

In spite of changes in fruit firmness, irradiation did not change other fruit quality attributes such as total soluble solids content, titratable acidity, and weight. Apart from a few minor differences, our results are consistent with other studies that indicate no effect of irradiation on fruit quality characteristics related to flavor [21,27,28,38]. The overall effect of irradiation on fruit firmness and

quality in terms of consumer acceptability is an important consideration. In this study we did not perform sensory evaluations; only few other studies have conducted post-irradiation sensory analyses, and have shown mixed results related to irradiation induced softening and consumer acceptability [21,28,42] in peaches and blueberries.

In addition to fruit quality attributes, it is important to understand the effect of irradiation on the presence of fruit surface organisms that may cause foodborne illness. Blueberries are produced in open fields and can harbor various human pathogens by route of animal waste, irrigation water, and handling by farm workers. After harvest, blueberries for the fresh market are not washed nor treated for surface pathogens [20,44]. Therefore, it would be an added benefit if irradiation could reduce or eliminate such surface organisms. ECP^{TM} treatment was effective in reducing surface microbial load in both 'Rebel' and 'Farthing'. In 'Rebel' irradiation at smaller doses was more effective in reducing surface pathogen load than in 'Farthing'. This was likely because 'Rebel' harbored a higher load of microbes on the fruit surface than 'Farthing'. In 'Rebel', aerobic bacteria and yeasts were reduced by 0.6–0.7 log units and coliforms by 2 log units at 1.0 kGy irradiation. In 'Farthing', similar reductions were observed for aerobic bacteria and yeasts, but not for coliforms. These results are partially consistent with previous studies suggesting irradiation doses between 0.2–0.8 kGy are sufficient to cause a 1-log reduction in surface bacterial pathogens such as *E. coli* 0157:H7, *Salmonella*, and *Listeria* [32,33]. In another study with blueberries, 0.4-kGy irradiation resulted in a 1-log reduction in *Salmonella* and *Listeria* [34], but those specific taxa were not investigated in the present study. The authors concluded, and we concur, that this level of reduction may reduce risk but not guarantee safety.

Blueberries are affected by various postharvest diseases caused mainly by plant-pathogenic fungi [21,45,46]. In this study, some of the common postharvest pathogens *B. cinerea*, *Alternaria* spp., *Colletotrichum* spp., as well as *Aurebasidium*, *Phomopsis*, and *Cladosporium* were identified after postharvest storage. However, in our study ECP^{TM} treatment did not affect the incidence of symptoms and signs associated with postharvest pathogens. Compared with microbes located on the fruit surface, a much higher dose of irradiation, typically at 1–3 kGy, is necessary to eliminate plant-pathogenic fungi [32]. Further, sensitivity of irradiation also can differ among various plant pathogens. Using an in vitro assay, inactivation of *B. cinerea*, *Penicillium expansum*, and *Rhizopus stolonifer* was observed at irradiation doses of 3–4 kGy and 1–2 kGy, respectively [47]. The maximum dose of irradiation of 1.0 kGy in our study may not have been sufficient to decrease postharvest decay pathogens. In addition, 'Farthing' had an inherently low prevalence of postharvest pathogens; hence, irradiation did not further reduce postharvest disease incidence.

Data from this study with the new ECP^{TM} approach is in agreement with previous research which recommends a dose between 0.5 and 1.0 kGy for blueberry fruit to avoid undesirable effects on fruit quality [21,28]. While irradiation at this dose may provide protection from insect pests (not tested in this study) and some reduction in surface microbial load, more research is needed on its potential to reduce postharvest rots. In apples, mangoes, peaches, and carrots, irradiation combined with other postharvest treatments, such as cold, heat, fungicides, $CaCl_2$ treatment, or modified atmosphere offered greater benefits in controlling postharvest diseases and maintaining higher fruit quality [48–52]. Importantly, the above studies demonstrate that lower doses of irradiation are more effective when used in combination with other treatments than using irradiation alone. Blueberries are generally not treated after harvest, therefore future studies should focus on preharvest applications such as fungicides or calcium treatments in combination with irradiation and storage with modified atmosphere.

ECP^{TM} is attractive because the method's high dose rates allow the desired irradiation dose to be obtained in a considerably shorter period of time, reducing treatment bottlenecks during operation and potentially improving produce quality through shorter treatment times outside of the cold-chain. However, direct side-by-side comparisons of ECP^{TM} with gamma rays or X-rays at identical irradiation doses (but varying dose rates as dictated by the method) have not been conducted previously, pointing to an important research need. Future research also should address one of the

limitations of our study, the need to ship the fruit to and from the treatment facility after harvest and before postharvest storage, which could have impacted treatment efficacy.

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