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Title: Postharvest Ethylene Treatment on Blueberry Fruit Quality Attributes During Storage

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Objectives: To determine the effects of ethylene treatment on blueberry fruit quality during postharvest storage

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 increases, 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 (Abugoch et al., 2016; Sun et al., 2014). The effect of postharvest ethylene application during storage of blueberry fruit remains to be investigated.

During ripening and postharvest storage, softening of fruit and susceptibility to pathogens are primary factors that lead to decline in fruit quality. Ethylene is an important plant hormone that influences fruit ripening and quality after harvest. Based on ethylene metabolism during ripening, fruits can be classified as exhibiting climacteric or non-climacteric physiology. Fruits such as tomatoes and bananas are classified as climacteric fruit. These fruits show a peak in respiration and ethylene production during ripening and can be harvested when they attain physiologically maturity. These fruits can be harvested at the mature green stage during fruit development and will continue to ripen after being detached from the plant. Further, in climacteric fruits ethylene production is autocatalytic due to the positive regulation of ethylene on its own biosynthesis. On the other hand, non-climacteric fruits, in general do not exhibit a discernable respiratory peak and the role of ethylene during ripening and postharvest storage is not clearly understood. However recent evidence suggests that non-climacteric fruits may produce small amounts of ethylene during ripening.

In climacteric fruits exposure to ethylene can hasten softening and deterioration of fruit quality. Fruit softening mainly occurs due to re-organization and disassembly of the cell wall and middle lamella. The coordinated action of multiple enzymes is involved in cell wall degradation during fruit ripening. Ethylene can induce the expression of cell wall loosening enzymes, such as polygalacturonase in tomatoes and bananas (Lobo et al., 2005; Qasid et al., 2021). In non-climacteric fruit, such as grapes, postharvest treatment with 500 mg/L of ethylene did not affect

fruit firmness, total soluble solids, and titratable acidity for up to two weeks of storage. However, metabolites involved in volatile production such as terpenes, esters and alcohol increased after ethylene treatments (Bellincontro et al., 2006). Treatment with 100 ppm of ethylene increased the tendency of browning in grape rachis (Li et al., 2015). Continuous ethylene treatment at 50 ppm in strawberries showed increased respiration and sucrose breakdown compared to control. This study suggested that ethylene can decrease postharvest shelf-life in strawberries (Elmi et al., 2016). However, studies summarized in Li et al. (2016), suggested that overall the effect ethylene in postharvest fruit quality in non-climacteric fruit seems to be minimal and only affect certain aspects depending on the commodity.

Whether blueberries are climacteric or non-climacteric has been controversial for many decades. My previous research has demonstrated that southern highbush and rabbiteye blueberry exhibit atypical climacteric ripening physiology with an increase in respiration and ethylene similar to climacteric fruits. However, ethylene production is not autocatalytic at the level of ethylene biosynthesis separating blueberry ripening physiology from other typical climacteric fruits (Wang et al., 2022). This addresses a long-standing question in the field. Further my research has shown that application of preharvest ethylene-related plant growth regulators (PGRs), ethephon and 1-aminocyclopropane 1-carboxylic acid (ACC) can accelerate fruit ripening by 22-37% after 10 days of application in both southern highbush and rabbiteye blueberries. However, preharvest ethephon treatments did not consistently affect fruit quality traits such as total soluble solids, titratable acidity, compression, puncture at harvest and during postharvest storage. In this proposal we wanted to follow-up on this work and determine the effect of ethylene gas on fruit treated postharvest. Interestingly, one previous study in northern highbush blueberry cultivar 'Duke' indicated that postharvest treatment of 10 ppm ethylene increased blueberry softening as well as positively influenced enzymes related to cell wall softening (Wang et al., 2020). However, this was a one-time study with only one cultivar. Therefore, there is merit in performing follow-up studies to confirm this finding and to determine differences in response among cultivars. A recent study indicated a substantial variation in ethylene production among cultivars during blueberry storage, with accessions displaying higher ethylene production showing more postharvest decay. This study indicates that although the effect of ethylene on postharvest fruit quality cannot be generalized, certain cultivars may exhibit more sensitivity (Farneti et al., 2022). Contrary to postharvest ethylene application, blocking ethylene perception by postharvest 1-MCP treatments in rabbiteye blueberry cultivars 'Austin,' 'Brightwell,' and 'Premier' resulted in higher ethylene production, and did not affect TSS and TA levels. Only 'Brightwell' showed an increased loss in fruit firmness compared to the control treatments, suggesting a cultivar-specific effect (MacLean & NeSmith, 2011). If increased ethylene production after 1-MCP treatment in 'Brightwell' led to increased fruit firmness warrants further studies. Thus, this proposal addresses the effect of postharvest ethylene exposure on fruit quality.

Significance: Fruit quality in blueberries is important for consumer satisfaction. Previously we have determined fruit quality of store brought berries. In general, fruit sampled from various supermarket stores were soft in texture and poor in quality. This indicates a potential for improvement in postharvest handling and maintenance of fruit quality after harvest. If exposure to ethylene has a negative effect on postharvest fruit quality of blueberries, this data will be useful for growers, packers, and marketers to better understand how they should handle varieties in time and space to offer consumers the best fruit quality and shelf-life possible.

Experimental plan: Objective: To assess the effect of the postharvest application of ethylene on fruit quality, water loss, chilling injury, and pathogens in blueberries during storage.

Two rabbiteye cultivars ('Brightwell' and 'Premier') were used in this study. We selected the above cultivars because they exhibit differences in ethylene production during ripening and postharvest storage. Our previous data indicates that 'Brightwell' produces lower ethylene levels in comparison to 'Premier' during various ripening and postharvest stages (Wang et al., 2022; Rachel Itle, personal communication). Fruit were hand harvested from commercial farms in coordination with Dr. Deltsidis. Fruits were uniform in terms of ripeness due to picking from a commercial farm that harvested fruit at regular intervals. Fruits were packed directly into one-pint clamshells in the field and transported in a cooler to the main campus of the University of Georgia (Athens, GA). Upon arrival, the clamshells containing the fruit were stored overnight in a walk-in cooler (4 °C, >90% RH). Fruits were sorted the following day to remove any damaged fruits. The treatments were only applied to defect free fruits. Treatments were replicated in 'Premier' 5 times, and in 'Brightwell', 6 times.

Three treatments of 10 ppm ethylene, 100 ppm ethylene, and an untreated control were applied to the ripe fruit from each replicate. The ethylene concentrations reflect the physiological range of ethylene production during ripening in multiple climacteric fruits. For treatment, fruit were placed in one-gallon jars. The jars were placed on their side, and the fruit arranged in a single layer on the bottom side of each jar. Each jar lid was fitted with a septum, sealed with silicone to make it airtight. Based on the volume of the jars and the space taken up by the fruit, appropriate volumes of ethylene were injected into the jars via the septum using a needle to achieve the different treatment levels. The untreated control was treated in the same manner, only ethylene was not injected into the jars. Each replicate was treated separately and stored at 20 °C for 18 hours in an environmental chamber (Percival, IA). After treatment, fruit were removed from the jars and placed in a single layer on a lunch tray. They were allowed to vent at room temperature for 2 hours prior to being placed back in one-pint clamshells for storage back in the walk-in cooler (4 °C, >90% RH). Each treatment was allowed to vent in separate areas so that gases will not diffuse into adjacent trays.

Fruit at ripe (prior to treatments) and during postharvest storage were evaluated to determine the effect of the ethylene treatments on postharvest fruit quality. Fruit quality attributes were assessed at 3 timepoints after treatment: 7 days of storage (DAS), 14 DAS, and 21 DAS. Compression and puncture measurements were used to assess fruit firmness and texture. This was performed using a Fruit Texture Analyzer (Model GS-15, Güss Manufacturing Ltd., Strand, South Africa). Juice for total soluble solids (TSS) measurements was obtained from 30 grams of fruit. The juice was produced by blending, centrifuging, and straining the fruit through cheesecloth to remove pulp and seeds. TSS was determined using a digital handheld refractometer (ATAGO Palette Digital Refractometer, Belleveue, WA).

Fruit from each treatment were evaluated at each timepoint for evolution of CO_2. 10 g of fruit were placed into a 16 oz glass jar and capped with a lid fitted with a septum. After 1 h of incubation, 60 mL of head space gas was withdrawn using a syringe and injected into an infrared CO_2 analyzer (Quantek instruments, MA). Data obtained used to determine amounts of CO_2 produced during ripening and storage and if variation among cultivars exists. We also planned to measure ethylene evolution but could not due to technical difficulties with our gas chromatography instrument.

At each timepoint, another group of 30 fruit from each treatment and replicate were visually assessed for shriveling, softness, physical damage, and pathogen presence. Physical damage included dents and tears in the surface of the fruits.

Statistical analysis was performed using one-way ANOVA (analysis of variance) for each time point after treatment within a cultivar using JMP Pro 12 (SAS Institute, Cary, NC, USA). Means separation was performed using Tukey's Honest Significant Difference (HSD) test (α = 0.05).

Results: This study revealed no treatment effects during postharvest storage in any of the fruit quality attributes, with the exception of visual assessment in 'Brightwell' (Figures 1-5). On 14 DAS, a higher percentage of torn fruit were found in the 10 ppm treatment compare with the control and 100 ppm treatment (Figure 5E). On day 21 of storage, a higher percentage of torn fruit occurred in the 10 ppm treatment versus the 100 ppm treatment (Figure 5E).

There were no differences in compression across timepoints (Figure 1). Puncture was significantly lower in 'Premier' at 14 DAS compared to the other two days (Figure 2). In 'Brightwell', puncture was significantly lower at 21 DAS than on 7 DAS (Figure 2). TSS was significantly lower at 21 DAS compared to 7 DAS in 'Premier' but showed no variation across timepoints in 'Brightwell' (Figure 3). CO_2 evolution was significantly lower on 7 DAS than 14 DAS in 'Premier,' but 2, 14, and 21 DAS did not differ (Figure 4). In 'Brightwell', CO_2 evolution was lower on 7 and 14 DAS compared to 2 and 21 DAS (Figure 4).

Many of the visual assessment paraments varied across storage times (Figure 5). In both cultivars, the percentage of damaged, shriveled, soft, dented, and infected fruit were all significantly higher on 21 DAS compared to 7 DAS. The percentage of torn fruit was also greater in 'Premier' on 21 DAS versus 7 DAS but did not differ in 'Brightwell'.

Compression:

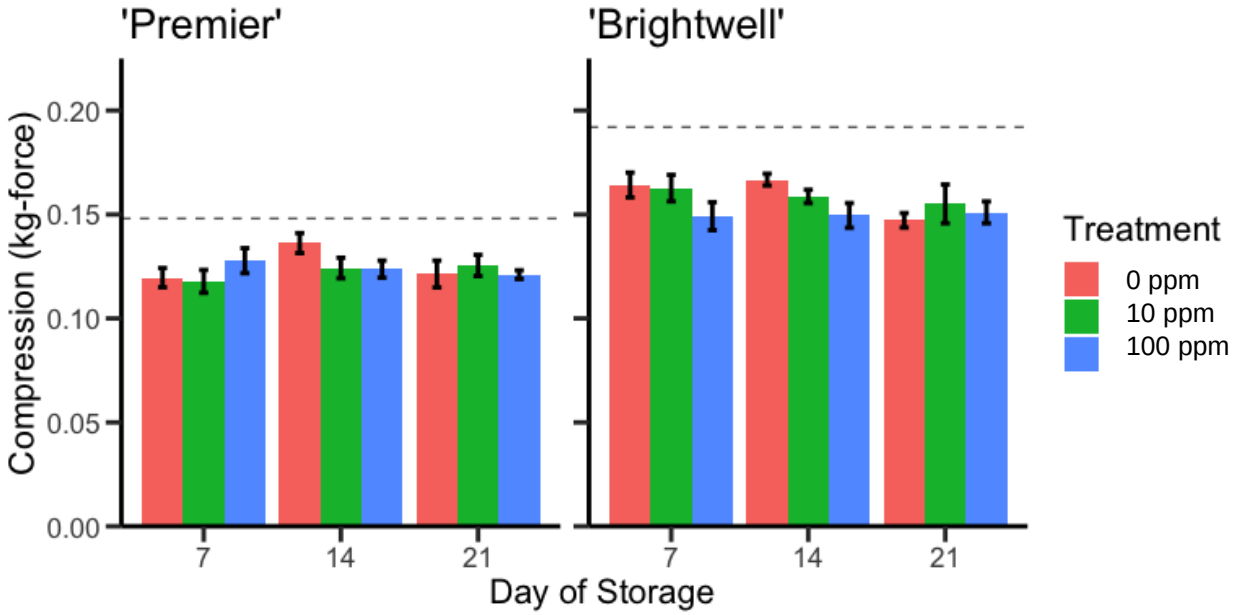

Figure 1 Compression results: No significant treatment effect or change over time was observed regarding compression. The gray line indicates the mean compression of untreated fruit on day 0 of the experiment. Means of treatments across times and within days of storage were compared using a two-way analysis of variance (α = 0.05).

Puncture:

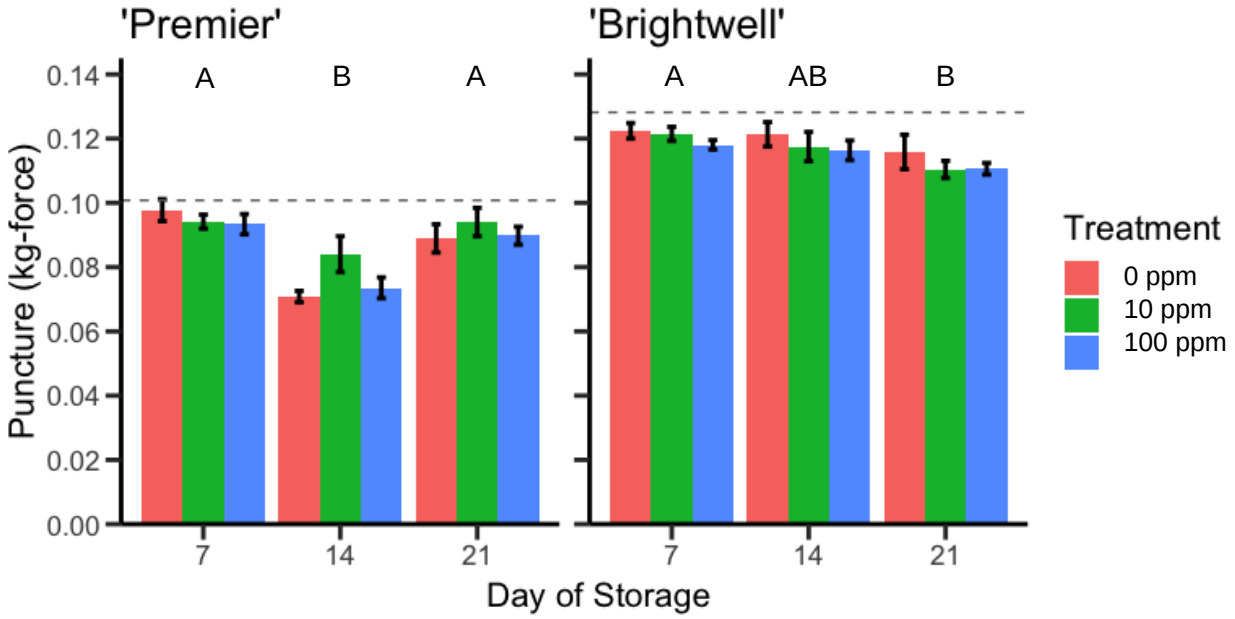

Figure 2 Puncture results: Significant differences between timepoints are indicated by capital letters at the top of the graphs. No significant differences in treatment were observed. The gray line indicates the mean puncture of untreated fruit on day 0 of the experiment. Means of treatments across times and within days of storage were compared using a two-way analysis of variance (α = 0.05).

Total soluble solids (TSS):

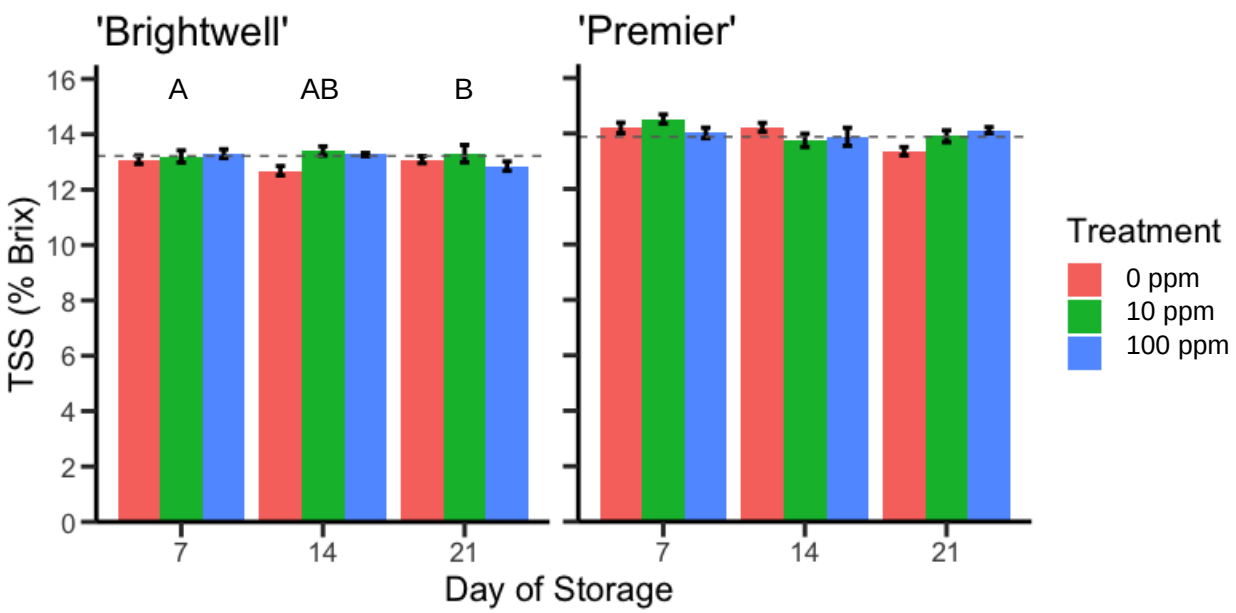

Figure 3 TSS results: Significant differences between timepoints are indicated by capital letters at the top of the graphs. Significant differences between timepoints were only observes in 'Premier.' No significant differences in treatment were observed. The gray line indicates the mean TSS of untreated fruit on day 0 of the experiment. Means of treatments across times and within days of storage were compared using a two-way analysis of variance (α = 0.05).

CO_2 evolution:

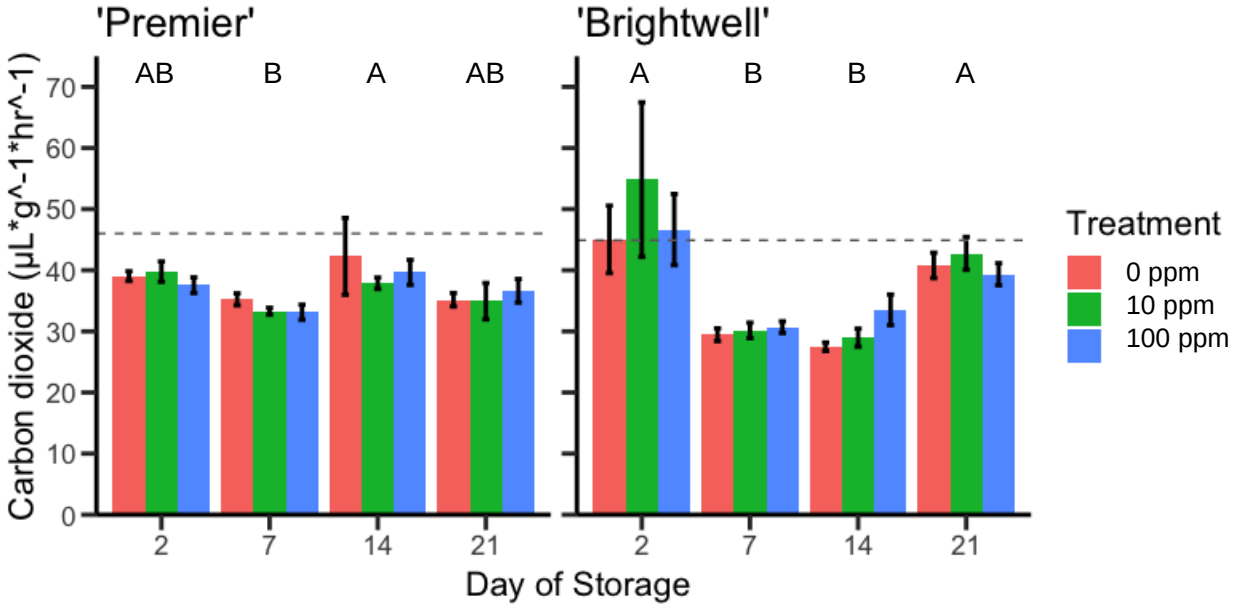

Figure 4 CO_2 evolution results: Significant differences between timepoints are indicated by capital letters at the top of the graphs. No significant differences in treatment were observed. The gray line indicates the mean CO_2 evolution of untreated fruit on day 0 of the experiment. Means of treatments across times and within days of storage were compared using a two-way analysis of variance (α = 0.05).

Visual assessment:

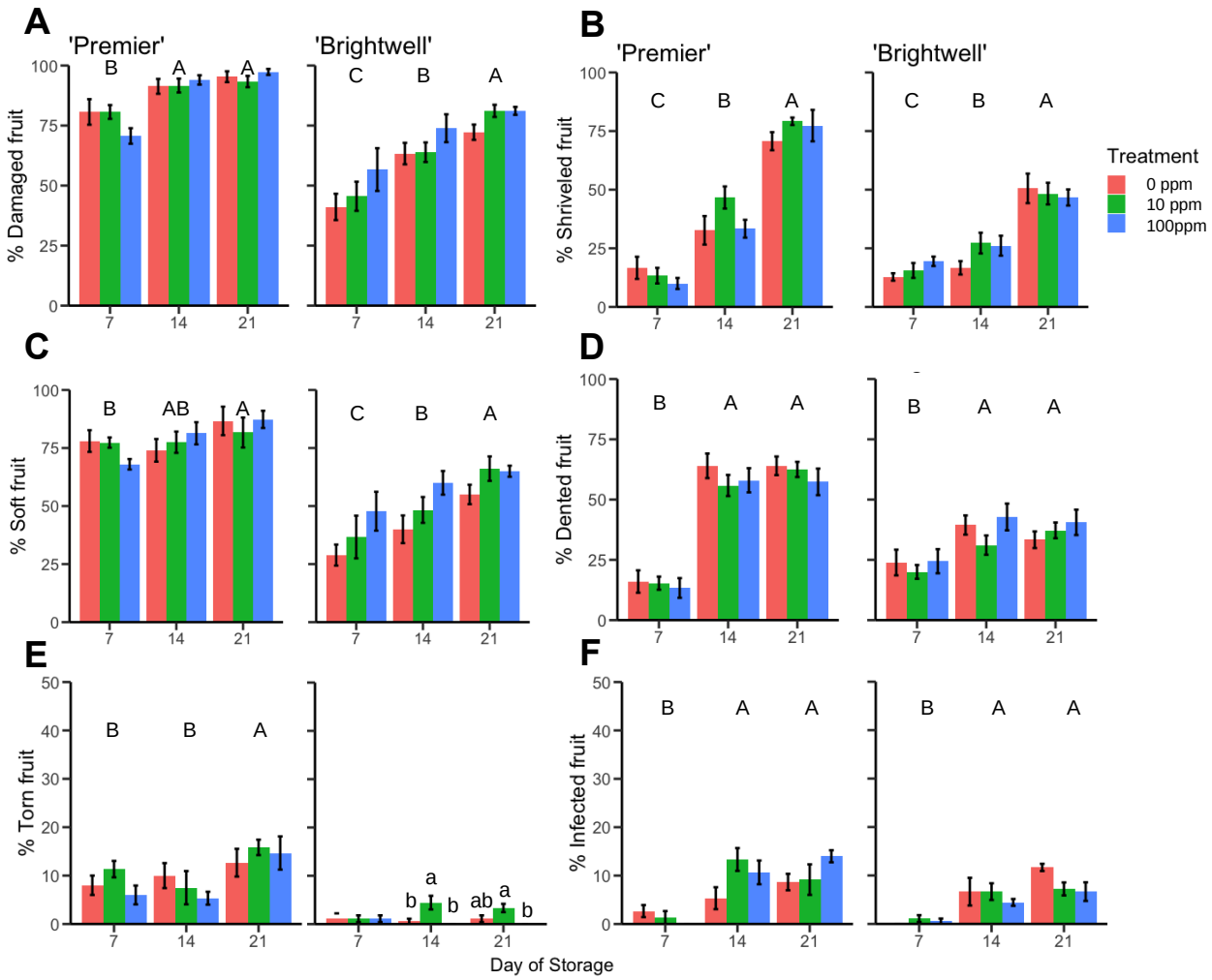

Figure 5 Visual assessment results: Significant differences between timepoints are indicated by capital letters at the top of the graphs. Significant differences between treatments are indicated by lower case letters above the bars. A - % Damaged fruits, B - % Shriveled fruits, C - % Soft fruit, D - % Damaged fruit, E - % Torn fruit, F - % Infected fruit (visible pathogenic infection). Means of treatments across times and within days of storage were compared using a two-way analysis of variance (α = 0.05).

Discussion: Overall, this study did not indicate any changes in fruit quality attributes after ethylene treatments during storage. The lack of treatment effects suggests that postharvest ethylene exposure has minimal influence on postharvest quality in 'Premier' and 'Brightwell' cultivars. In this study, the initial fruit quality of 'Premier' was poor, as evident by the low initial compression as well as the higher percentage of soft fruit and damaged fruits on 7 DAS. In this cultivar, it is possible that treatment effects may have been masked and minimized. Further studies using multiple cultivars and detailed evaluation of fruit quality parameters is needed to conclusively understand the postharvest effects of ethylene exposure on blueberries.

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