



Pre-treatment with 1-methylcyclopropene alleviates methyl bromide-induced internal breakdown, softening and wall degradation in blueberry

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ABSTRACT

Methyl bromide (MeBr) fumigation is the most common quarantine treatment used to control fruit flies in blueberry. Recent studies suggest that the treatments may increase decay and softening during transport, distribution and retail. We evaluated whether the ethylene action inhibitor 1-methylcyclopropene (1-MCP) could counteract the detrimental effects caused by MeBr. 'Jewel' and 'Emerald' blueberries with 100% surface blue color were harvested and treated with 1-MCP (1 $\mu\text{L L}^{-1}$ 12 h, 4 °C), MeBr (32 g m^{-3} , 3 h, 21 °C), or 1-MCP followed by MeBr. Untreated berries were used as a control. Fruit was stored for 0, 7 or 14 d at 2 °C and internal breakdown, firmness, respiration, weight loss, color, soluble solids, acidity and the total ascorbic acid (AsA), anthocyanin and glutathione (GSH) concentrations were determined. We also assessed pectin solubility by sequential cell wall extraction and neutral sugar composition. MeBr exposure exacerbated internal breakdown and respiration after long-term storage. These effects were significantly reduced by pre-treatment with 1-MCP, indicating that MeBr-induced damage requires ethylene action. 1-MCP application prior to MeBr fumigation also prevented berry softening by delaying solubilization of cell wall uronic acids and galactose. The combination of 1-MCP followed by MeBr caused no detrimental effects on fruit surface color, anthocyanin, weight loss, soluble solids or acidity. MeBr fumigation reduced total GSH concentrations regardless of 1-MCP, indicating that the improved quality retention could not be attributed to the detoxification of the xenobiotic by this compound and was more likely due to inhibition of ethylene-dependent over-ripening and senescence symptoms. Pre-treatment with 1-MCP may be useful to alleviate MeBr-induced deterioration in blueberry.

1. Introduction

Blueberries produced in zones in which the Mediterranean fruit fly, *Ceratitis capitata*, or the South American fruit fly, *Anastrepha fraterculus*, are endemic must receive a decontamination treatment before entering a pest-free zone (Follett and Neven, 2006). Irradiation has been evaluated as a promising alternative (Miller and McDonald, 1996), but regulatory issues, logistic difficulties of implementation and slow consumer acceptance have limited their commercial adoption (Osterholm and Norgan, 2004). Other approved methods such as cold quarantine treatments (1.11 °C for 15 d or 1.67 °C for 17 d) must, in blueberries, be conducted during transit and are thus difficult to apply. Consequently, methyl bromide (MeBr) is still the most common quarantine treatment

used to control flies (TEAP, 2010).

MeBr has practical advantages that make it difficult to find good replacements. It has been used commercially for almost a century; it is versatile enough to control a wide spectrum of pests, including fungi, bacteria, soil-borne viruses, insects, mites, nematodes, rodents and weeds; it has good penetration; its action is usually sufficiently fast and it airs rapidly enough from treated systems to cause relatively little disruption to commerce (Anon., 1994; Heaps, 2006; TEAP, 2010). Moreover, MeBr fumigation meets most countries' export requirements. Unfortunately, MeBr is listed as an ozone-depleting substance scheduled to be phased out under the Montreal protocol and is extremely toxic to humans (Heaps, 2006). In addition, in some fruit species it can cause phytotoxic responses (Drake et al., 1988; Harman et al., 1990;

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Fields and White, 2002). MeBr applications approved as quarantine treatments in blueberries exacerbated deterioration and mold growth and resulted in sliminess symptoms during storage (Thang et al., 2016). The effects were only visible after long storage times and compromised shelf life. Unfortunately, the physiological basis for such responses is unknown. In animal systems, halogenated hydrocarbons such as MeBr can cause macromolecule alkylation and overproduction of free radicals (Hallier et al., 1990). In contrast, almost no studies have examined the mechanism of MeBr-induced damage in fruit.

Blueberries exhibit a typical climacteric ripening behavior when still attached to the plant (El-Agamy et al., 1982). However, there is still some debate about the role of ethylene has on ripening regulation in this species (Eck, 1970). Besides that and since blueberries accumulate sugars during late maturity it is not recommended to harvest until the berries have reached the full blue color stage (Song et al., 2003; Zheng et al., 2003). Therefore, the ethylene and carbon dioxide respiratory peaks occur prior to commercial harvest (Suzuki et al., 1997). Likely for this reason, attempts to manipulate blueberry quality and postharvest life through ethylene production or action inhibitors has been mostly unsuccessful (De Long et al., 2003; Blaker and Olmstead, 2014). However, the involvement of ethylene on blueberry postharvest deterioration is still disputed. Recent work by Wang et al. (2018) showed that ethylene absorbers improved the quality retention of long-term stored blueberries.

Besides its key regulatory function in ripening and senescence (Kader, 2005), ethylene concentrations increase dramatically after exposure to xenobiotic compounds (Thao et al., 2015). Its role in this response is largely unknown. In some species, ethylene perception was necessary to activate plant defense mechanisms, while in others; it triggered detrimental secondary effects (Khan et al., 2017). The ethylene action inhibitor 1-methylcyclopropene (1-MCP) tested initially to delay senescence and prevent ripening of climacteric fruit, has been used to identify novel roles of ethylene in other aspects of plant development (Blankenship and Dole, 2003; Watkins, 2006). In this work, we determined whether the ethylene action inhibitor 1-methylcyclopropene (1-MCP) could counter-act the detrimental effects caused by MeBr in stored blueberries. We hypothesized that inhibiting ethylene action through 1-MCP prior to MeBr stress would diminish cell wall disassembly, delay softening and reduce susceptibility to internal breakdown.

2. Materials and methods

2.1. Plant material and treatments

Blueberries (*Vaccinium corymbosum* L. x *Vaccinium darrowi* Camp, cvs. 'Jewel' and 'Emerald', kindly provided by Blueberries Argentina) were harvested at the end of November after reaching 100% surface blue color in an orchard in Concordia (Entre Ríos, Argentina). Fruit was transported immediately to the packinghouse, packed in perforated polyethylene-terephthalate (PET) clamshells. Fruit was randomly selected and divided into 96 clamshells (48 Jewel from and 48 from Emerald). The clamshells (10 × 10 × 4 cm containing 150 g fruit) were assigned to three sampling dates (harvest, one week and two weeks of refrigerated storage) and four treatments (Control, 1-MCP, methyl bromide and 1-MCP followed by methyl bromide). All fruit was refrigerated at 4 °C. Half of the clamshells (corresponding to end treatments 1-MCP and 1-MCP followed by MeBr) were treated with 1-MCP (Smartfresh, Röhm and Haas, USA) at 1 µL L⁻¹ and 4 °C for 12 h into a hermetic tarp under the same temperature conditions. After 12 h the fruit was removed from the cold storage and kept at 21 °C. Half of the clamshells (corresponding to end treatments MeBr and MCP followed by MeBr) were held for 3 h into a fumigation chamber and treated with MeBr at 32 g m⁻³ at 21 °C. When the treatments were finished, the chamber was opened and degassed (1 h) and after that, all the fruit was taken to the laboratory. During this time, the remaining treatments

(Control and 1-MCP) were kept at 21 °C in air to assure that all the fruit was subjected to similar temperature conditions. Fruit was subsequently stored for 14 d at 2 °C (85–90% RH). During the treatments and storage, the temperature, relative humidity and dew point were tracked with a data logger UX-100-003 (Onset Computer Corporation, Bourne MA, USA). After 7 and 14 d storage, samples were taken and either immediately used for quality evaluation. In order to assure that measurements were conducted at similar temperature at all sampling dates the berries were kept at 20 °C for 2 h until equilibration. In addition, tissue samples were frozen in liquid N₂ and stored at –80 °C until analysis.

2.2. Internal breakdown (IB) and weight loss

The percentage of fruit showing IB (fruit without visible mycelium, but having lost pulp integrity upon cutting) was determined. Four subsample clamshells containing at least 60 fruit each were evaluated for each treatment and storage time.

For weight loss evaluation fruit clamshells were weighed throughout the storage period. The weight of the clamshells was subtracted and fruit weight loss was calculated as: $WL = 100 \times (W_i - W_f) / W_i$, being W_i the initial fruit weight and W_f the final fruit weight. Results were expressed in percentage.

2.3. Respiration rate

Fruit respiration rate was determined using an infrared gas analyzer (Model GC, -2028 Lutron Electronic Enterprise Co. L.T.D., USA). Approximately 125 g fruit was placed in a 5.6 L hermetic glass chamber. The CO₂ concentration was determined every minute for 20 min and plotted on Cartesian axes. The slope of the regression line represented the respiration rate. Four subsamples were measured for each replicate. Results were expressed in ng CO₂ produced per kilogram fresh weight (FW) per second.

2.4. Firmness

Firmness was measured with a texture analyzer (Exponent Texture Analyzer TA.XT.PLUS from Stable Micro System, Goldalming, Surrey, UK) equipped with a three-mm diameter flat probe. Fruit was deformed 4.0 mm at a speed of 0.5 mm s⁻¹ and the maximum force during this assay was recorded and expressed in Newton. Subsamples of 60 fruit were evaluated for each measured for each cultivar, treatment and storage time.

2.5. Soluble solids (SSC), acidity (TA), anthocyanins and color

For SSC evaluation, 10 g fruit was ground with a mortar and pestle. Measurements were performed using a temperature-compensated refractometer (Milwaukee MA871, Rocky Mount, USA) and results were expressed as g SS per kg FW. Acidity (TA) was determined on 10 g fruit juice after titration with 0.1 mol L⁻¹ NaOH until pH 8.2 (AOAC, 1980). Results were expressed in grams citric acid per kg FW. For anthocyanin evaluation, 10 g of frozen fruit was ground in a mill and ~0.1 g of the resulting powder was added to 20 mL methanol containing 1% v/v HCl and vortexed. Samples were subsequently centrifuged at 10,000 × g and 4 °C for 10 min. The supernatant was used to evaluate anthocyanins as described by Angeletti et al. (2010); concentrations were expressed in g kg⁻¹ FW. Three subsamples replicate for SSC, acidity and anthocyanin evaluations. Color was measured with a chromameter (Minolta, CR-400, Osaka, Japan) to obtain the L*, a* and b* values. Sixty fruits were evaluated for each cultivar, treatment and storage time.

2.6. Cell wall isolation

Approximately 30 g frozen fruit was ground in a mill and the

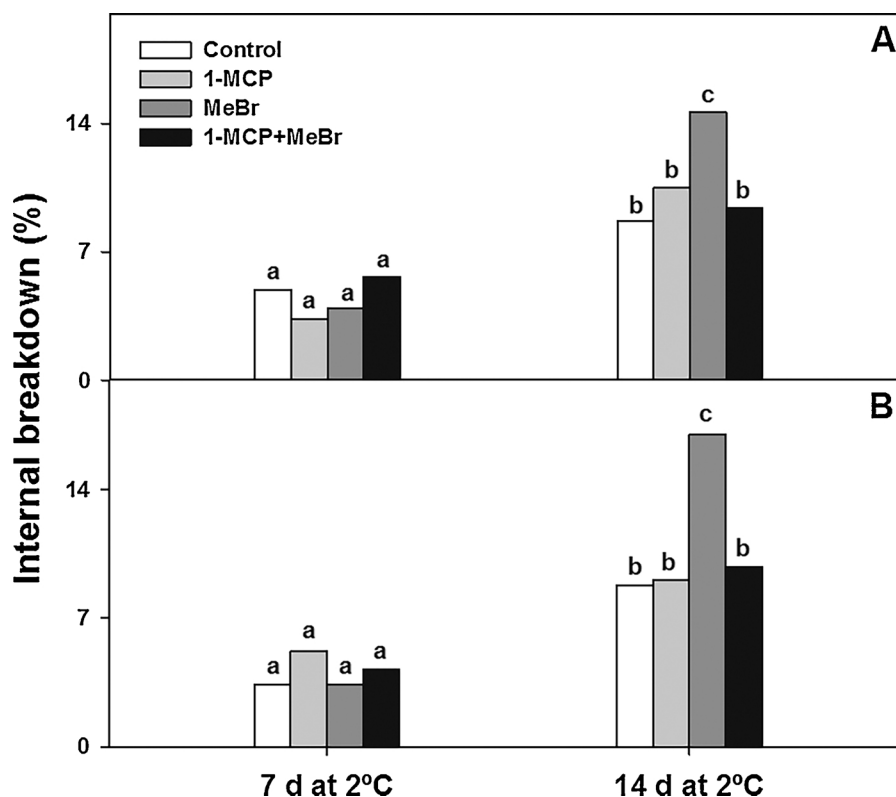


Fig. 1. Internal breakdown (%) in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) during storage at 2 °C for 7 or 14 d. Different letters indicate significant differences based on a Tukey test at a significance level of $P < 0.05$.

obtained powder was added to 75 mL of 96% v/v ethanol and boiled 45 min to ensure extraction of low molecular weight solutes and to prevent autolytic activity. The insoluble material was filtered through Miracloth (Calbiochem, USA) and sequentially washed with 150 mL boiling ethanol, 150 mL chloroform:methanol (1:1 v/v), and 150 mL acetone and dried at 25 °C, yielding a crude cell wall extract (alcohol-insoluble residue, AIR). The residue was dried overnight at 37 °C and weighed.

2.7. Cell wall neutral sugar gas chromatography (GC) analysis

Samples from the AIR were hydrolyzed with 2 mol L⁻¹ trifluoroacetic acid (Albersheim et al., 1967) and converted to alditol acetates (Blakeney et al., 1983) for GC neutral sugar analysis. Aliquots of the derivatized samples were injected into a GC fitted with a 30 m × 0.25 mm DB-23 capillary column (J&W Scientific, CA, USA) and a mass selective detector. The temperature in the injector was 250 °C and a linear temperature gradient (initial oven temperature 160 °C, oven increase at 4 °C per min to 250 °C) was used to improve separation. The different alditol acetates were identified based on their mass spectra and standards containing inositol, rhamnose (*Rha*), fucose (*Fuc*), arabinose (*Ara*), xylose (*Xyl*), mannose (*Man*), galactose (*Gal*) and glucose (*Glc*) were prepared and neutral sugar amount calculated relative to an inositol internal standard. Results were expressed in mol %. Measurements were done in duplicate.

2.8. Cell wall fractionation

Fractions of different cell wall components were obtained by sequential chemical extraction of the cell wall material (AIR). Approximately 100 mg AIR residue from each sample was suspended in 10 mL water and stirred 12 h at room temperature, then centrifuged at 6000 × g and 4 °C for 10 min. The supernatant was filtered through glass fiber filters (Whatman GF/C) and the pellet was washed with

water. The filtrate and water washings were combined and designated the water-soluble fraction (WSF). The residue was then extracted with 10 mL of 50 mM Na₂CO₃ containing 20 mM NaBH₄ at 1 °C for 12 h. After filtration, the extraction solution was designated the Na₂CO₃-soluble fraction (NSF) and saved and the pellet was extracted with 10 mL of 24% KOH containing 0.1% NaBH₄ at room temperature for 12 h, with shaking; this extracted solution was designated the KSF. This fraction was filtered, as above, neutralized with glacial acetic acid, and extensively dialyzed against water. Uronic acids (UA) were measured as described (Blumenkrantz and Asboe-Hansen, 1973). UA in the WSF, NSF and KSF were expressed in grams per kilogram of AIR. Three subsamples were analyzed replicate

2.9. Water soluble galactose

Samples from the WSF of fruit stored 14 d at 2 °C containing 100 mg neutral sugars were dried by placing the test tubes in a water bath and by N₂ flushing. Samples were hydrolyzed, derivatized and analyzed by GC as described in Section 2.8. Two subsamples were evaluated per replicate. Results were expressed in grams per kilogram of AIR.

2.10. Ascorbic acid (AsA) and glutathione (GSH)

AsA was determined by high performance liquid chromatography (HPLC) as described (Gergoff Grozeff et al., 2013). Total glutathione concentration was evaluated using an enzymatic procedure (Griffith, 1980). Three measurements were made for each cultivar, treatment and storage time. Results were expressed in μmol kg⁻¹ FW.

2.11. Statistical analysis

The experimental layout was factorial with the factors being the fumigation treatment and storage time. The whole experiment was repeated to generate an experimental design with two replicates. For each

quality attribute and measurement, the number of subsamples analyzed is indicated in the corresponding section. Data were analyzed by means of ANOVA. The means were compared with the Least Significant Difference (LSD) test at a significance level of 0.05. Statistical analysis was performed with Statistica 6.0 software from StatSoft.

3. Results and discussion

3.1. Internal breakdown (IB), respiration and weight loss

IB characterized by a loss of pulp tissue integrity in absence of exudate of visible symptoms or decay and excessive softening were the main symptoms of deterioration observed during storage. Although no differences were observed among treatments during the first week, after 14 d both ‘Jewel’ and ‘Emerald’ berries subjected to MeBr had a greater percentage of broken-down berries than the control (Fig. 1). Previous work by Thang et al. (2016) reported that MeBr induced sliminess symptoms and decreased blueberry storage potential. Increased incidence and severity of internal breakdown and the formation of brown sunken spots on the skin were reported in other species exposed to MeBr (Harman et al., 1990). Because ethylene is involved in responses to a wide range of stressors, we tested whether or not treatment with 1-MCP prior to fumigation, which competitively binds the ethylene receptors (Sisler and Serek, 1997), could reduce MeBr-induced damages. 1-MCP did not affect fruit deterioration in non-fumigated fruit, but markedly alleviated MeBr-induced IB (Fig. 1).

MeBr can also modulate fruit metabolic activity in a commodity- and treatment-dependent manner: it caused no appreciable damage in pre-climacteric tomato or apple (Drake et al., 1988; Brecht et al., 1986), but stimulated respiration in asparagus (Beever et al., 1985). In the present work, MeBr dramatically increased respiration after two weeks storage (Fig. 2). Remarkably the rise of fruit respiration induced by MeBr fumigation inhibited by 1-MCP pre-treatment. Overall, IB induced in blueberry by MeBr after 14 d of storage was alleviated by blocking ethylene perception. This differs from the protective mechanisms of

plants against some heavy metal xenobiotics, which require ethylene signaling (Asgher et al., 2014).

3.2. Weight loss, anthocyanin, color, soluble solids content and acidity

Weight loss (WL) is a primary factor in blueberry deterioration (Paniagua et al., 2013). Here, it increased with storage time, reaching ~1.5–1.9% at the last sampling date (Supplementary Tables 1 and 2). 1-MCP did not affect fruit dehydration, as has been reported in previous work (De Long et al., 2003; Chiabrando and Giacalone, 2011) even when the fruit was stored under high water vapor deficit (Deng et al., 2014). In accordance to Thang et al. (2016), MeBr treatment did not increase fruit susceptibility to dehydration.

No changes in anthocyanin concentration were observed in control fruit during refrigeration. Some increase in anthocyanin has been reported in ripe blueberries during storage, but this was primarily induced by water loss (Kalt and McDonald, 1996). No differences in anthocyanin concentrations and surface color was found in all treatments (Supplementary Tables 1 and 2). In contrast, MeBr accelerated anthocyanin degradation in orchid flowers (Pumnuan et al., 2015). These discrepancies may be due to variations among plant organs in susceptibility to MeBr and/or to the distinctly different surface to volume ratio between fruits and flowers, which strongly affects the ease of gas diffusion from and to the tissues. Fruit soluble solids content (SSC) and titratable acidity (TA) were unaffected by MeBr fumigation as well (Supplementary Tables 1 and 2).

3.3. Firmness, cell wall composition, uronic acid and galactose solubilization

Blueberry quality declines rapidly after harvest and, with excessive softening, is one of the most critical causes of deterioration (Kader, 2005). Thus, any technology that helps maintain firmness after harvest would be of great benefit to the blueberry industry. The two cultivars studied in this work showed contrasting behavior: control ‘Jewel’ fruit

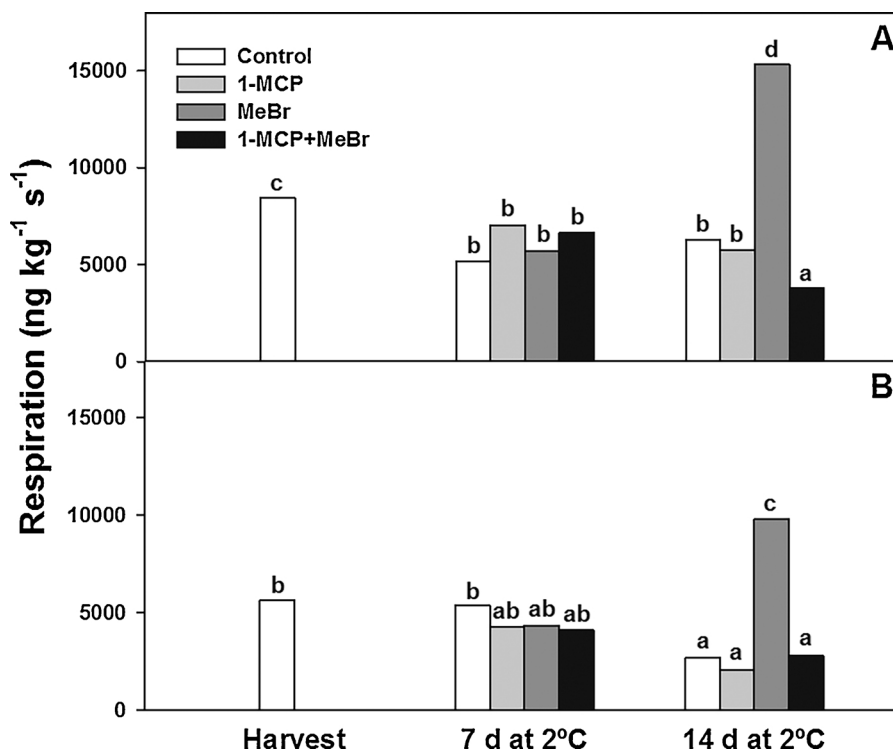


Fig. 2. Respiration rate ($\text{ng kg}^{-1} \text{s}^{-1}$) in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. ‘Jewel’ (A) and ‘Emerald’ (B) during storage at 2 °C for 0, 7 or 14 d. Different letters indicate significant differences based on a Tukey test at a significance level of $P < 0.05$.

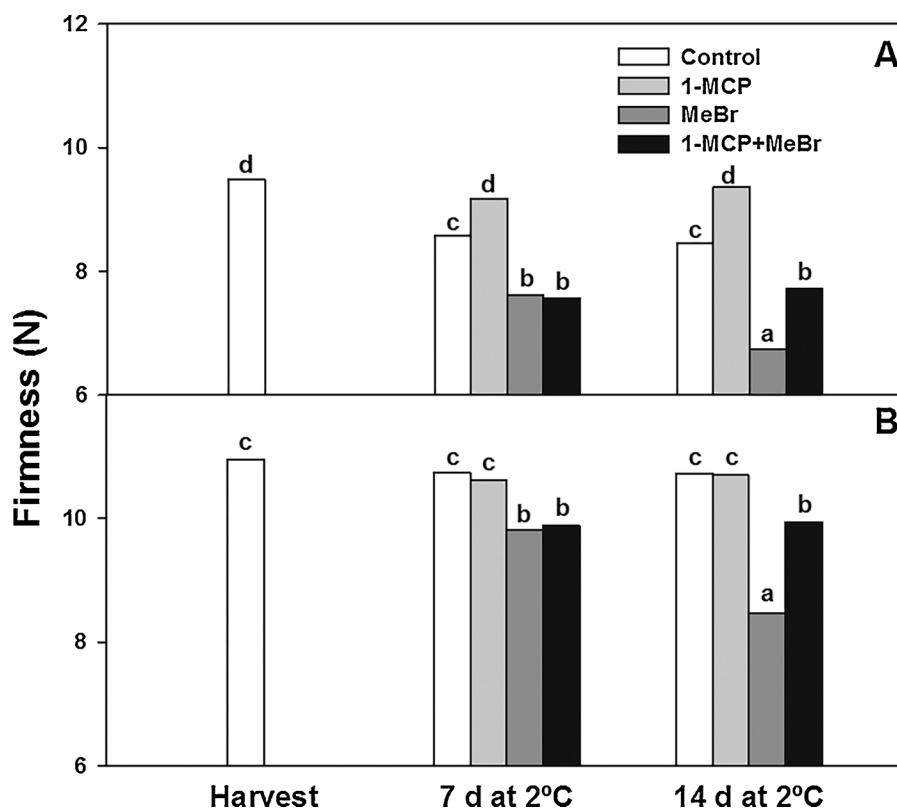


Fig. 3. Firmness (N) in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) during storage at 2 °C for 0, 7 or 14 d. Different letters indicate significant differences based on a Tukey test at a significance level of $P < 0.05$.

had a relatively rapid firmness loss, but 'Emerald' berries did not (Fig. 3). 1-MCP treated 'Jewel' fruit were firmer than control fruit. This differs from previous reports that 1-MCP treatment does not affect blueberry firmness (De Long et al., 2003; MacLean and NeSmith, 2011). Nevertheless, 1-MCP berries stored in CA remained firmer than fruit held only in CA (Chiabrando and Giacalone, 2011). Although post-climacteric fruit produce low concentrations of ethylene and are not highly responsive to the hormone (Deng et al., 2014), results here show that blueberry responses to 1-MCP are depend on the cultivar considered. MeBr-fumigated fruit showed the most rapid firmness loss during storage (Fig. 3). After just one week at 4 °C, MeBr-treated berries were softer than the control. At the last sampling date, the differences in firmness between control and MeBr-treated fruit were even greater. Fruit subjected to 1-MCP followed by MeBr were softer than the control, but remained firmer than MeBr-treated blueberries that received no 1-MCP priming. This suggests that ethylene accounts for part of the accelerated firmness loss observed in MeBr-fumigated berries.

Moisture loss has been reported as the major cause of firmness changes in stored blueberry (Paniagua et al., 2013). However, here differences in softening were observed even in the absence of variations in WL. This suggests that cell wall degradation may also contribute to blueberry postharvest firmness loss, especially under high relative humidity, so changes in cell wall components were tracked in berries affected by MeBr and 1-MCP. Xylose (*Xyl*) and arabinose (*Ara*) were the most abundant neutral sugars in both blueberry cultivars and comprised ~75% of the total neutral sugar moieties (Supplementary Table 3). *Xyl* was reported as the most abundant non-cellulosic neutral sugar blueberry cell walls, something uncommon in *Dicot* species (Vicente et al., 2007b). Glucose (*Glc*) and arabinose (*Ara*) accounted for ~13 and 10% of fruit wall non-cellulosic neutral sugars respectively, with *Rha*, *Fuc* and *Man* representing less than 2% of the total. No changes in the overall proportion of non-cellulosic neutral sugars was found in during storage or in response to 1-MCP or MeBr fumigation.

To evaluate whether the change in firmness was related to modifications in pectin and neutral sugar solubilization, we conducted a sequential extraction of cell wall polysaccharides in water (WSF), Na_2CO_3 (NSF) and 4M KOH (KSF) to determine the proportion of loosely- and tightly-bound pectin and the concentration of uronic acids associated with hemicellulose fractions. At harvest, less than 15% of total UA was in the WSF as opposed to tightly-bound NSF, which contained almost 80% of the extractable pectin (Fig. 4). As in other fruit (Brummell, 2006), blueberry postharvest softening was accompanied by increased loosely-bound pectin (Fig. 4). Although pectin solubilization increased slightly in blueberries ripening on the plant between the 75 and 100% blue stages (Vicente et al., 2007b), there is evidence that postharvest softening involves changes in pectin polysaccharides. Berry softening during storage correlated with increased pectin solubilization (Deng et al., 2014). Preharvest calcium application, delaying postharvest solubilization of pectin, reduced firmness loss of two blueberry cultivars (Angeletti et al., 2010). This also provides indirect support for a role of polyuronide integrity on blueberry firmness. Also of note is that MeBr treatments increased the UA content of the WSF and, as with internal breakdown and respiration; this effect was prevented if the fruit was exposed to 1-MCP prior to fumigation.

Pectin solubilization in berries, including blueberry, occurs in association with the removal of neutral sugar-side chains from branched rhamnogalacturonan type I pectins (Smith and Gross, 2000; Vicente et al., 2007a). Consequently, we evaluated the percentage of galactose in water-soluble polysaccharides. An increase in water-soluble galactose was found during berry storage (Fig. 5). The extent of the accumulation of water-soluble, galactose-rich polysaccharides was significantly greater in MeBr-treated 'Jewel' and 'Emerald' fruit than in the blueberries exposed to 1-MCP followed by MeBr fumigation. Previous work reported *Ara*, rather than *Gal*, as the primary non-cellulosic neutral sugar solubilizing in water during blueberry preharvest softening in *planta* (Vicente et al., 2007a), with *Ara* being the main sugar

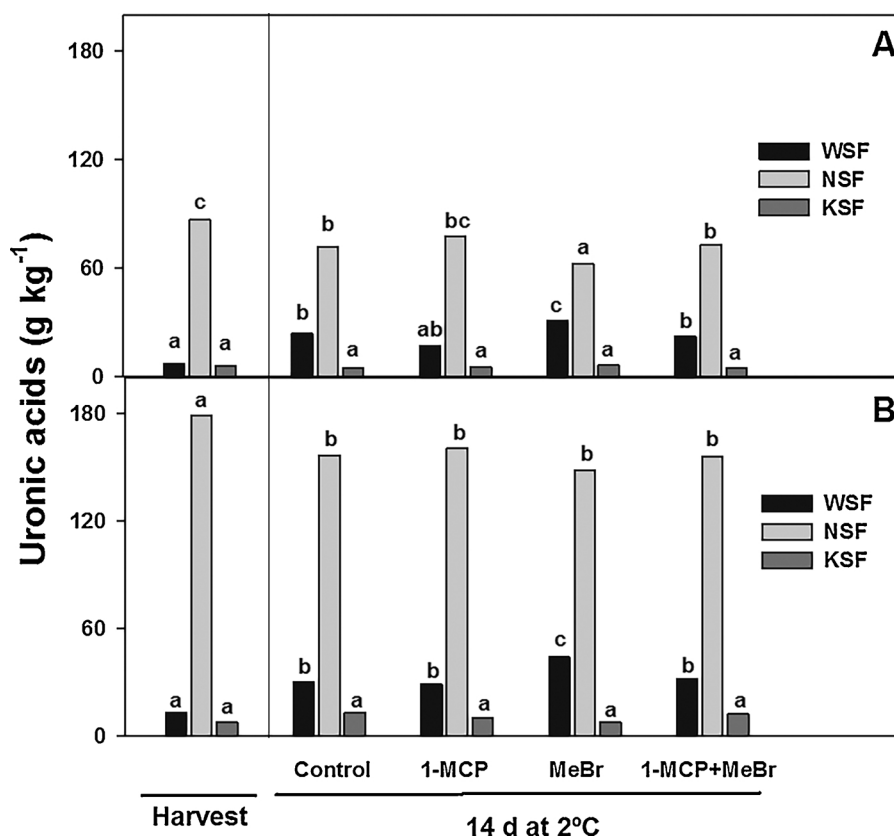


Fig. 4. Uronic acids (g per kg AIR) in the water (WSF), Na₂CO₃ (NSF) and 24% KOH (KSF) soluble fractions in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) at harvest and after 14 d at 2 °C.

after *Xyl*. In contrast, in the present work galactans were much more prominent based on wall neutral sugar GC-analysis (Supplementary Table 3). The accelerated softening caused by MeBr fumigation was related to increased galactose and uronic acid solubilization and both of these changes were retarded by 1-MCP.

3.4. Ascorbate and glutathione

The mechanism of MeBr toxicity in plants has not been studied in detail. By analogy with mammalian systems, MeBr damage could result from alkylation of macromolecules, accumulation of toxic metabolites such as methanethiol and formaldehyde and/or overproduction of reactive oxygen species (ROS) beyond the ability of the tissue to detoxify (Hallier et al., 1990). Ascorbate (AsA) and glutathione (GSH) are two of the most important cell quenchers of ROS, including both free radical (superoxide, hydroxyl, perhydroxy and alkoxy radicals) and non-radical (hydrogen peroxide and singlet oxygen) forms. Together with phenolic compounds, non-protein amino acids and α -tocopherols, they control cascades of uncontrolled oxidation and protect stored commodities from oxidative damage (Gergoff Grozeff et al., 2013). After seven days storage, concentrations of GSH was reduced in MeBr-fumigated berries compared to control and 1-MCP treated fruit, supporting a role for this compound in fruit responses to the xenobiotic molecule (Figs. 6 and 7). The GSH concentration was 50–60% lower in MeBr fumigated berries than in the control. A similar trend was found for AsA in 'Jewel' but not in 'Emerald'. GSH may participate in the detoxification of methyl halides through direct conjugation by both enzymatic (*via* glutathione transferase, EC 2.5.1.18) and non-enzymatic mechanisms (Hallier et al., 1990). After two weeks of storage, MeBr-treated fruit recovered the GSH levels found in non-fumigated berries after one-week cold storage. A similar trend was reported in lemons, although three weeks were necessary for complete GSH recovery (Ryan et al., 2007). In methyl

iodide-treated lemons, the duration of aeration after fumigation was a major factor determining the time required to recover GSH and the degree of fruit injury. It would be interesting to evaluate whether this was due to *de novo* synthesis of GSH, enzyme hydrolysis of the S-methylglutathione presumably formed upon exposure to methyl halides, or both. In blueberry, 1-MCP did not prevent MeBr-induced reductions in total AsA or GSH after one week of storage (Figs. 6 and 7). Although further work is needed to characterize the different forms of antioxidant within the tissue and the redox state of the system, this suggests that 1-MCP protective effect against MeBr was not due to an increase in the fruit total antioxidant pool.

4. Conclusions

In this work, we evaluated whether the ethylene action inhibitor 1-methylcyclopropene (1-MCP) could counter-act the detrimental effects caused by MeBr in stored blueberries. MeBr-treated fruit showed increased internal breakdown and a higher respiration rate after long-term storage and all three effects were alleviated by 1-MCP pre-treatment. The combined 1-MCP followed by MeBr treatment prevented polyuronide and galactose solubilization and softening without affecting fruit color, anthocyanins, weight loss, soluble solids content or acidity. MeBr fumigation reduced GSH concentration regardless of the use of 1-MCP, indicating that the quality retention benefits observed could not be attributed to changes in the total pool of GSH, and were more likely caused by inhibition of ethylene-dependent over-ripening and senescence symptoms. Pre-treatment with 1-MCP alleviated MeBr-induced internal breakdown, firmness loss and wall degradation in blueberry.

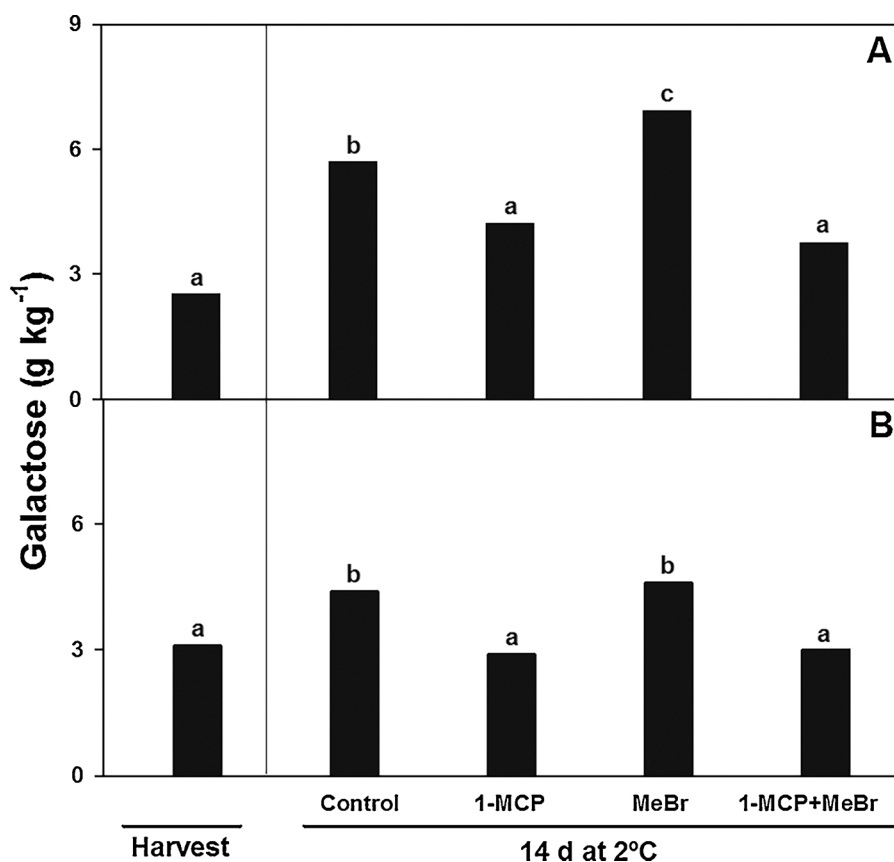


Fig. 5. Galactose (g per kg AIR) in the water-soluble fraction of control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) at harvest and after 14 d at 2°C. Different letters indicate significant differences based on a Tukey test at a significance level of P < 0.05.

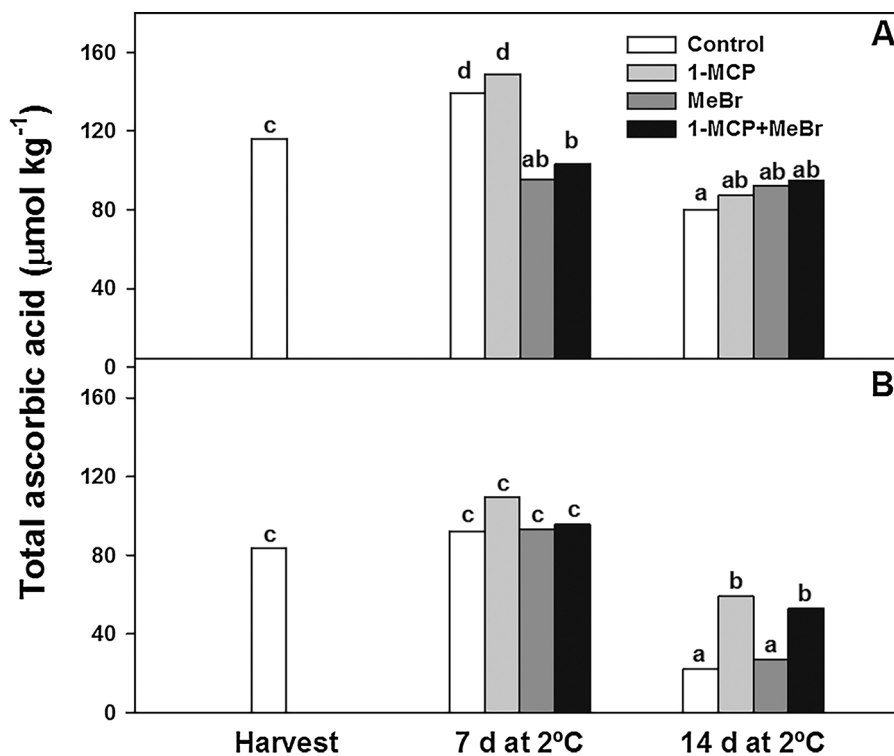


Fig. 6. Ascorbic acid (μmol per kg on fresh weight basis) in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) during storage at 2°C for 0, 7 or 14 d. Different letters indicate significant differences based on a Tukey test at a significance level of P < 0.05.

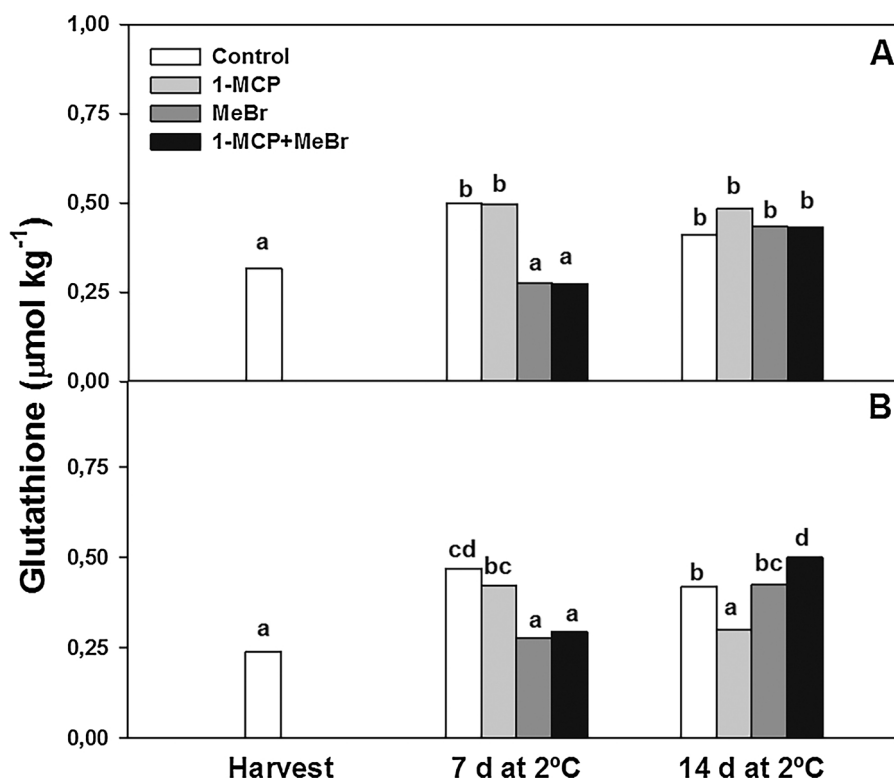


Fig. 7. Glutathione content (μmol per kg on fresh weight basis) in control, 1-MCP, MeBr and MeBr + 1-MCP treated blueberry fruit cvs. 'Jewel' (A) and 'Emerald' (B) during storage at 2°C for 0, 7 or 14 d. Different letters indicate significant differences based on a Tukey test at a significance level of $P < 0.05$.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.postharvbio.2018.08.018>.

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