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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 μL 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-methylcyclorpropene (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-therephtalate (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 N_2 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: $WL = 100 \times (W_i - W_f)/Wi$, 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 CO_2 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 CO_2 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

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