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Elevated carbon dioxide in storage rooms prior to establishment of controlled atmosphere affects apple fruit quality

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ABSTRACT

The objective of this study was to investigate the effect of elevated carbon dioxide (CO_2) associated with storage room loading on fruit quality and external CO_2 injury in 'McIntosh' and 'Empire' apples. Effects of fruit cooling rate and the postharvest application of 1-methylcyclopropene (1-MCP) were also evaluated. Over two consecutive years, two strains of 'McIntosh' (Pioneer and Summerland) and 'Empire' apples were harvested from orchards during the commercial harvest period. In the first year, both strains of 'McIntosh' were cooled rapidly to 3 °C and treated with 1-MCP (1 $\mu L L^{-1}$) overnight. In the second year, half of the apples from each 'McIntosh' strain and 'Empire' were cooled rapidly to 3 °C, while the other half were cooled slowly from ~22 °C down to 14–16 °C. All 'McIntosh' and half of the 'Empire' fruit from each cooling regime were also treated with 1-MCP (1 $\mu L L^{-1}$) overnight. All apples from both years were then held in ~17 kPa O_2 + 4 kPa CO_2, to mimic typical CO_2 build-up during commercial storage room loading, at 3 °C for up to 6 weeks. Overall, Summerland was more susceptible to external CO_2 injury than Pioneer 'McIntosh', developing notable symptoms after 4 weeks of storage. 1-MCP treatment increased the incidence of external CO_2 injury in 'Empire', regardless of cooling rate. However, 1-MCP-treated 'Empire' cooled rapidly developed less external CO_2 injury than those cooled slowly. Other fruit quality attributes (internal ethylene concentration, firmness, soluble solids concentration, and malic acid content) were only marginally affected by short-term holding in elevated CO_2, in combination with cooling rate and/or 1-MCP treatment. These results suggest 'McIntosh' and 'Empire' apples are susceptible to developing external CO_2 injury without the establishment of low oxygen and typical controlled atmosphere (CA) storage. Furthermore, slow cooling after harvest may exacerbate the development of external CO_2 injury in apples during storage room loading.

1. Introduction

External CO_2 injury is a common postharvest physiological disorder in certain susceptible apple cultivars. It is characterized by rough bronze lesions that are often partly sunken with well-defined edges on the peel (Fawbush et al., 2008; Meheriuk et al., 1994; Watkins et al., 1997). Development of CO_2 injury generally occurs during the early stages of CA storage, and tends to be more prevalent with rapid establishment of elevated CO_2 partial pressures (Argenta et al., 2000; Elgar et al., 1999; Fawbush et al., 2008; Watkins et al., 1997), especially when fruit are not cooled sufficiently prior to CA (Burmeister and Dilley, 1995; Meheriuk et al., 1994; Watkins and Liu, 2010; Watkins et al., 1997). In most instances, external symptoms progress with minimal internal flesh browning or damage. However, fruit showing symptoms of external CO_2 injury are considered unmarketable and undesirable to consumers, regardless if the flesh tissue is healthy.

'McIntosh' and 'Empire' are two major apple cultivars produced in the northeastern United States of America and Canada. Both cultivars are chilling sensitive and therefore, require CA storage temperatures above 0 °C. 'McIntosh' and 'Empire' apples are also very susceptible to external CO_2 injury during CA storage (DeEll and Ehsani-Moghaddam, 2012; Fawbush et al., 2008; Watkins and Nock, 2012). Postharvest application of the ethylene antagonist 1-methylcyclopropene (1-MCP; SmartFresh™) maintains fruit firmness and quality characteristics in apples during storage (DeEll et al., 2007, 2008; Watkins, 2007; Watkins et al., 2008). Unfortunately however, 1-MCP can also exacerbate the incidence of CO_2 injury in 'McIntosh' and 'Empire' apples (DeEll and Ehsani-Moghaddam, 2012; DeEll et al., 2003; Fawbush et al., 2008). Treatment with the antioxidant diphenylamine (DPA) controls the development of external CO_2 injury (Argenta et al., 2002; Fawbush

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et al., 2008; Fernández-Trujillo et al., 2001; Watkins et al., 1997), but the use of DPA on apples may soon become restricted or banned due to regulatory issues.

Rapid establishment of CA storage has been encouraged for 'Empire' and 'McIntosh' apples, in order to retain maximum levels of fruit firmness and quality (DeEll, 2012; Watkins et al., 2008). Conversely, it has been reported that delaying CA storage can be beneficial in the reduction of CO_2-related disorders, such as external CO_2 injury (DeEll and Ehsani-Moghaddam, 2012). Watkins and Nock (2012) found that 1-MCP-treated 'McIntosh' apples developed a higher incidence of external CO_2 injury when CA was established within 7 days after harvest, compared to fruit stored in CA established 14 days after harvest. Moreover, holding 'Empire' apples in air storage for 1 or 2 months prior to CA has been shown to reduce the development of external CO_2 injury (DeEll and Ehsani-Moghaddam, 2012). Recent reports have also shown that the incidence of external CO_2 injury can vary from year-to-year (DeEll and Ehsani-Moghaddam, 2012; Gapper et al., 2013), as well as from orchard-to-orchard (Deyman et al., 2014; Watkins and Liu, 2010; Watkins et al., 1997). The underlying etiology and metabolic processes pertaining to the development of external CO_2 injury in apples remains to be fully understood.

'Empire' apples developed external CO_2 injury within the initial 3 weeks of CA storage with 2 kPa O_2 and elevated CO_2 of 2.5 or 5 kPa (Fawbush et al., 2008). However, the possibility remains that external CO_2 injury may develop in apples due to elevated CO_2 exposure prior to CA establishment, specifically during storage room loading. High fruit respiration rates prior to cooling and/or CA establishment can allow CO_2 to accumulate as high as 4–5% in commercial storage rooms during loading (DeEll, personal observation). Preliminary results from one season within a study by Fawbush et al. (2008) showed external CO_2 injury in 'Empire' apples within 8 days of elevated CO_2 exposure (5 kPa CO_2) prior to CA storage.

The objective of this study was to investigate the effects of elevated CO_2 associated with storage room loading (prior to CA establishment) on fruit quality and external CO_2 injury in 'McIntosh' and 'Empire' apples. The effect of cooling rate was also investigated, as well as postharvest 1-MCP treatment in 'Empire'. Apples were held in ~17 kPa O_2 + 4 kPa CO_2 at 3 °C for 1–6 weeks after harvest, to mimic the potential natural build-up of CO_2 during commercial storage room loading.

2. Material and methods

2.1. Plant material

'McIntosh' apples (Pioneer and Summerland strains) were harvested from the same orchards located near Simcoe, Ontario, Canada, within the commercial harvest periods of 2013 and 2014. There were 12 and 16 boxes (~90 fruit per box) of apples harvested in 2013 and 2014, respectively, with each strain from each harvest time having three treatment box replicates in 2013 and two treatment box replicates in 2014. 'Empire' apples were also harvested from an orchard located near Simcoe, Ontario, Canada, during the commercial harvest period of 2014. There were 24 boxes (~90 fruit per box) of 'Empire', with two box replicates per treatment. Each box from each cultivar and strain contained fruit from several trees and various locations within the trees. All apples were transported immediately to the nearby storage research facility after harvest.

2.2. Postharvest treatments

In the first year of study, all 'McIntosh' apples were cooled rapidly to 3 °C within 4 h of harvest and then treated with 1-MCP (1 $\mu L\,L^{-1}$) for 24 h (as postharvest treatment with 1-MCP has become standard industry practice for 'McIntosh'). 1-MCP application was made using SmartFresh™ tablets (AgroFresh Inc., Spring House, PA) within air-tight aluminum storage chambers (Storage Control Systems Inc., Sparta, MI). Subsequently, all fruit were held in ~17 kPa O_2 + 4 kPa CO_2 (within the same storage chambers) at 3 °C for 1, 2, 4 or 6 weeks.

In the second year of study, half of the apples from each 'McIntosh' strain and 'Empire' were cooled rapidly to 3 °C within 4 h of harvest and then treated with 1-MCP (1 $\mu L\,L^{-1}$) for 24 h, as in the first year of study. The remaining apples were cooled slowly from ~22 °C down to 14–16 °C overnight, during which time 1-MCP treatment occurred. Subsequently, all fruit were held in ~17 kPa O_2 + 4 kPa CO_2 at 3 °C. 'McIntosh' apples were held in these conditions for 1, 2, 4 or 6 weeks, while 'Empire' apples were held for 1, 2, or 4 weeks.

2.3. Fruit quality and external CO_2 injury evaluations

Initial fruit maturity at the time of harvest was evaluated on 10-apple samples for each cultivar and strain. Internal ethylene concentration (IEC) was determined by withdrawing a 3-mL gas sample from the core of each fruit using a syringe and injecting the gas sample into a Varian CP-3380 gas chromtograph (Varian Canada Inc., Mississauga, Ontario, Canada) equipped with a 0.5 mL sample loop, flame ionization detector, and 15 m × 0.32 mm Restek Rt-SPLOT™ capillary column (Chromatographic Specialties Inc., Brockville, Ontario, Canada). The injector, column and detector temperatures were 120, 35 and 225 °C, respectively. High-grade helium was used as the carrier gas at a flow rate of 0.37 mL s^{-1} with a typical run time of 1.5 min.

Fruit firmness was determined on opposite sides of each apple after peel removal, using an electronic texture analyzer fitted with an 11-mm tip (GÜSS, South Africa). Titratable acidity (expressed as mg equivalents of malic acid per 100 mL of juice) was determined by titrating a 2-mL juice sample with 0.1 N NaOH to an end point of pH 8.1 (as indicated by phenolphthalein). Soluble solids concentration (SSC) was determined on a juice sample using a digital refractometer (BRX-242; Erma Inc., Tokyo, Japan). Starch content was determined using the Generic Starch-Iodine Index Chart for Apples (Blanpied and Silsby, 1992). Apples were cut in half at the equator, dipped in potassium-iodine solution and rated on a scale of 1–8, where 1 represents 100% starch staining and 8 equals no starch staining.

Upon removal from elevated CO_2 conditions, fruit were warmed to room temperature (~20–22 °C) and then evaluated for fruit quality and external CO_2 injury on the same day. Ten fruit from each box replicate per treatment were measured for IEC, firmness, SSC, and malic acid content. Only five fruit per box replicate of each treatment were used for IEC measurements in 2014. The incidence of external CO_2 injury was determined using all apples (~90) in each box replicate of each treatment. Disorder incidence was calculated as a percentage of fruit displaying the disorder regardless of severity.

2.4. Statistical analyses

Data were analyzed using *Proc GLM* and *Proc GLIMMIX* of the SAS program (version 9.2; SAS Institute Inc., Cary, NC), incorporating a split-plot experimental design for each cultivar and strain. All data were subjected to testing of normality and assumptions for ANOVA, and transformed for analysis when appropriate. Mean separations were examined using Duncan's multiple range test and only differences significant at $P \leq 0.05$ are discussed.