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Postharvest Biology and Technology 37 (2005) 111–121
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Postharvest 1-methylcyclopropene application in ripening control of 'Stark Red Gold' nectarines: Temperature-dependent effects on ethylene production and biosynthetic gene expression, fruit quality, and polyamine levels

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Received 18 January 2005; accepted 5 April 2005

Abstract

"Stark Red Gold" nectarines were harvested at 30 N of flesh firmness (FF) and 1 nl g^{-1} h^{-1} of ethylene production, and treated in sealed plastic containers with 1 μl l^{-1} (1 ppm) 1-methylcyclopropene (1-MCP) for 12 h at 25 °C. Treated and control fruit were then transferred either to a growth chamber at 25 °C or to a cold room at 4 °C for 3 days. At the end of treatment with 1-MCP; ethylene production in control fruit had increased relative to production at harvest, and this rise was abolished by the presence of the chemical. Moreover, treated fruit showed lower soluble solids content (SSC) and higher FF and titratable acidity (TA) compared to control ones, and putrescine and spermine levels were moderately enhanced in the mesocarp at the end of treatment. In contrast with the inhibition of ethylene production, 1-aminocyclopropane-1-carboxylate synthase (ACS) and especially 1-aminocyclopropane-1-carboxylate oxidase (ACO1 and ACO2) transcript levels were enhanced relative to controls. During storage, 1-MCP affected ethylene production and biosynthetic gene expression, fruit softening and other quality parameters in a temperature-dependent manner: in fruit held at 25 °C a strong decrease in ethylene production, a delay in ripening and lower ACS and ACO1/ACO2 levels were recorded, while in fruit held at 4 °C an opposite trend was observed. Results suggest that 1-MCP application followed by storage at 25 °C appears effective in controlling postharvest ripening. The lack of efficacy of the chemical in cold-stored fruit is discussed in relation to changes in SAMDC gene expression and putrescine accumulation in treated fruit relative to controls, which may be part of a stress response.

Keywords: 1-Aminocyclopropane-1-carboxylate oxidase (ACO); 1-Aminocyclopropane-1-carboxylate synthase (ACS); Ethylene biosynthesis; Polyamines; 1-Methylcyclopropene (1-MCP); *Prunus persica*; *S*-Adenosylmethionine decarboxylase (SAMDC)

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doi:10.1016/j.postharvbio.2005.04.006

1. Introduction

Since the discovery of 1-methylcyclopropene (1-MCP) as an ethylene antagonist, a great number of studies have examined its mechanism of action (Sisler and Serek, 1997), and its effect on extending the shelf-life and quality of edible crops (fruit and vegetables) and ornamentals (Blankenship and Dole, 2003; Sisler and Serek, 2003). It also represents a useful tool in studies aimed at clarifying the role of ethylene in plant development, including fruit ripening.

Peaches and nectarines are typical climacteric fruit which exhibit a sharp rise in ethylene synthesis at the onset of ripening, associated with changes in sensitivity to the hormone itself (Rasori et al., 2002), and changes in colour, texture, aroma and other biochemical features (Mathooko et al., 2001; Ruperti et al., 2001). Therefore, treatment with inhibitors of ethylene biosynthesis (Garner et al., 2001) or with ethylene antagonists, such as 1-MCP, has been performed with the intent of extending postharvest storage and shelf-life (Dong et al., 2001; Fan et al., 2002; Liguori et al., 2004). Melting flesh peaches and nectarines undergo rapid ripening and soften quickly after harvest, leading to losses during marketing. Therefore, the fruit are often picked at an early stage of ripening to better withstand handling. However, although after harvest they soften, they never reach full flavour and aroma. Thus, treatment to inhibit rapid softening after harvest would allow fruit to be picked at a tree-ripe stage.

Fruit ripening is partially controlled by ethylene as evidenced by application of exogenous ethylene or propylene which enhances it. Conversely, the ethylene action inhibitor 1-MCP delays ripening, but may adversely affect flesh quality (internal browning, mealiness) and increase storage disorders (Dong et al., 2001, 2002; Fan et al., 2002).

Another class of plant growth regulators known to play a role in plant (Bagni and Torrigiani, 1992), including fruit, development are the aliphatic polyamines (PAs) putrescine, spermidine and spermine which share with ethylene the precursor *S*-adenosylmethionine (SAM) which is decarboxylated by SAM decarboxylase (SAMDC) in the PA (spermidine and spermine) biosynthetic pathway, or is, alternatively, converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS), and then to ethylene via ACC oxidase (ACO). During peach fruit development, endogenous PA titres undergo dramatic changes with maximum levels during the early stages (cell division phase and S1), and minimum levels at ripening (S4) when climacteric ethylene production occurs (Tonutti et al., 1997; Kushad, 1998; Ziosi et al., 2003). In earlier studies on peach and nectarine, we have also demonstrated that pre-harvest applications of PAs strongly interfere with fruit ethylene production resulting in delayed on-tree ripening as assessed by evaluating several quality parameters (Bregoli et al., 2002), and that these effects correlated with altered gene expression of ethylene and polyamine biosynthetic enzymes (Torrigiani et al., 2004).

In the present work, 1 $\mu l\,l^{-1}$ 1-MCP was applied at room temperature (25 °C) for 12 h to detached tree-ripe nectarines. After treatment, fruit were held at 25 °C or stored at 4 °C for up to 3 days. During this time, ethylene production and biosynthetic enzyme (ACS, ACO) gene expression, fruit quality parameters, PA titres, and SAMDC mRNA levels were monitored to further analyse the metabolic relationship between ethylene and PAs, also during postharvest ripening.

2. Materials and methods

2.1. Plant material

Nectarines (*Prunus persica* L. Batsch cv Stark Red Gold) were harvested from 6-year-old trees grown at the experimental farm of the University of Bologna, Italy. During ripening, ethylene and the main fruit quality parameters were monitored daily.

Nectarines, harvested on 4 August 2001 (flesh firmness 30 N and ethylene production 1 $nl\,g^{-1}\,h^{-1}$) were placed, on the day of harvest, in sealed 30-l plastic jars. SmartFresh™ (AgroFresh Inc., Philadelphia, PA, USA), a commercial powder containing 0.14% (w/w) 1-MCP a.i., was prepared as a 10-fold concentrated stock solution following the technical bulletin of the company, and injected as 10 ml of air (final concentration 1 $\mu l\,l^{-1}$ equivalent to 1 ppm). Ninety fruit (two jars with 45 fruit each) were incubated with 1-MCP for 12 h at 25 °C (conditions which were previously shown to be effective in peaches; Rasori et al., 2002). The same total number of fruit were kept in two sealed jars for 12 h at 25 °C without 1-MCP (controls). In one experiment fruit were incubated for only 6 h. Treated and control