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Effect of ethylene degreening on the development of postharvest penicillium molds and fruit quality of early season citrus fruit

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ABSTRACT

The effect of commercial degreening with ethylene gas on fruit susceptibility and quality and development of postharvest green (GM) and blue (BM) molds on early season citrus fruit was investigated. Each cultivar was harvested with different peel color indexes (CI). Fruit were exposed for 3 d to 2 $\mu L L^{-1}$ ethylene at 21 °C and 95–100% RH before or after artificial inoculation with *Penicillium digitatum* or *Penicillium italicum*. Control fruit were kept at the same environmental conditions without ethylene. Fruit were stored at either 20 °C for 7 d or 5 °C for 14 d and disease incidence (%) and severity (lesion diameter) were assessed. No significant effect of commercial degreening was observed on fruit susceptibility to both GM and BM on citrus cultivars inoculated after degreening. Likewise, no significant effect was observed on disease incidence on citrus cultivars inoculated before degreening and stored at either 20 °C for 7 d or 5 °C for 14 d. In contrast, in cultivars like 'Clemenules' mandarins and 'Navelina' oranges, degreening significantly increased the severity on fruit with higher initial CI (−3.6 and 1.7, respectively). GM and BM severity on degreened and control 'Clemenules' mandarins incubated at 20 °C for 7 d was 146 and 118 mm and 56 and 46 mm, respectively. In general, commercial degreening did not significantly affect external and internal quality attributes of citrus cultivars. Commercial degreening after inoculation of less green (more mature) fruit showed a trend to increase mold severity, presumably through an aging effect (acceleration of peel senescence).

1. Introduction

Postharvest green mold (GM), caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and postharvest blue mold (BM), caused by *Penicillium italicum* Wehmer, are the most economically important postharvest diseases of citrus in Spain and all production areas characterized by low summer rainfall (Eckert and Eaks, 1989). Blue mold is especially important on citrus fruit kept under cold storage (Palou et al., 2001).

External color of citrus fruit is more related to climatic conditions than to internal maturity. Degreening with ethylene (C_2H_4) is a common commercial practice employed in many parts of the world to accelerate artificially the natural color break in which fruit turn from green to orange/yellow (Porat, 2008; Peng et al., 2013). Particularly, it is extensively implemented in Spain from September to December to market early season mandarins and oranges. Since the 1970s, several studies have been conducted to study the relationship between ethylene applications and postharvest decay by phytopathogenic fungi on citrus fruit, the results showing inconclusive trends. Discrepancies observed in research results seem to be related to different environmental conditions, the amount of ethylene applied and/or different host–pathogen interactions. On the one hand, fruit degreening has been reported to induce resistance to penicillium molds or anthracnose caused by *Colletotrichum gloeosporioides*, especially when applied at 30 °C and relative humidity (RH) higher than 90% (Brown, 1973, 1975; El-Kazzaz et al., 1983a; Porat et al., 1999). On the other hand, other results have suggested an increase in the incidence of citrus postharvest diseases such as stem-end rots or penicillium molds following ethylene exposure (McCornack, 1971; Barmore and Brown, 1985; Brown, 1986; Zhang, 2004). It has also been reported that degreening in standard conditions at 20 °C after fungal inoculation had no effect on the incidence of both GM and BM on 'Clemenules' mandarin fruit (Plaza et al., 2004). Given these different degreening effects depending on specific handling and environmental conditions, it is important to establish them for each particular situation. Standard commercial degreening practices for early season mandarins and oranges in Spain, California and other Mediterranean-climate areas consist of fruit exposure for 2–4 d to 2–5 $\mu L L^{-1}$ ethylene at 20–22 °C and RH > 90% (Sdiri et al., 2012a),

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while in other citrus producing areas like Florida or Brazil, commercial degreening is typically performed at temperatures around 30 °C (Brown, 1973, 1975).

The responses of the effects of ethylene exposure on the quality of harvested horticultural produce are numerous and varied, and can be beneficial or detrimental depending on each case (Saltveit, 1999; Palou et al., 2003). In the case of citrus fruit, it is important to examine the effects of ethylene degreening on fruit quality attributes other than peel color, especially on fruit destined to prolonged storage or long-distance markets. Thus, the effects of ethylene or 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, on fruit weight loss, firmness, total soluble solids content (SSC), titratable acidity (TA), or consumer acceptance for different citrus cultivars have been assessed with different results (Porat et al., 1999; Plaza et al., 2004; Tietel et al., 2010; Mayuoni et al., 2011a). Furthermore, the knowledge of the effects on nutritional quality and content of bioactive compounds is increasingly gaining importance (Mayuoni et al., 2011b; Sdiri et al., 2012b).

Before the Spanish citrus industry can optimize degreening treatments and overall fruit handling in packinghouses for the most representative commercial cultivars, it is important to determine the influence of ethylene degreening in our particular conditions on the development of GM and BM, the most economically important cause of postharvest decay, and also on fruit susceptibility to these diseases. Therefore, the aims of this research were to: (i) determine the effect of commercial degreening with ethylene gas on the susceptibility to GM and BM of intact early season mandarins and oranges, (ii) assess the effect of degreening on the incidence and development of GM and BM on fruit previously inoculated with *P. digitatum* or *P. italicum* and incubated at 20 °C or stored at 5 °C, and (iii) study the effect of degreening under standard commercial conditions on internal and external fruit quality attributes. Preliminary results from this research have been recently published (Moscoso-Ramírez and Palou, 2013).

2. Materials and methods

2.1. Fruit

The trials were conducted from 2008 to 2011 with 'Clemenpons' and 'Clemenules' clementine mandarins (*Citrus reticulata* Blanco), 'Navelina' oranges (*Citrus sinensis* (L.) Osbeck), and 'Nova' [*C. reticulata* × (*Citrus reticulata* × (*Citrus reticulata* × *Citrus paradisi*)), synonym: 'Clemenvilla'] hybrid mandarins. Fruit were collected from commercial orchards in the Valencia area (Spain) and used the same day or stored up to 1 week at 5 °C and 90% RH before use. Before each experiment, fruit were selected, randomized, washed, disinfected superficially by immersion for 2 min in a 0.5% sodium hypochlorite solution, rinsed with tap water to eliminate residual chorine, and allowed to air-dry at room temperature. Depending on the experiment, fruit of each cultivar were harvested at different rind color indexes (CI = −0.07, 0.9 for 'Clemenpons' mandarins; CI = −6.5, −3.6, 2.2 for 'Clemenules' mandarins; CI = −5.3, 1.1, 1.7 for 'Navelina' oranges; CI = 12.3 for 'Nova' hybrid mandarins), in some cases during the same season and in other cases in different seasons.

2.2. Commercial degreening procedure

Fruit were transported to a 1000 t commercial degreening room in a local citrus packinghouse (Fontestad S.A., Montcada, Valencia). Fruit were exposed to 2 μL L^{-1} ethylene (±0.5 μL L^{-1}) at a constant temperature of 21 °C (±0.5 °C) and RH > 95% for 72 h, with the exception of fruit from one of the experiments with 'Clemenules' mandarins that needed 24 h of additional exposure to develop acceptable commercial color. At the same time, non-degreened (control) fruit were exposed to the same environmental conditions but without exposure to exogenous ethylene in a storage room located in the IVIA CTP pilot plant.

2.3. Fungal inoculation

P. digitatum and *P. italicum*, isolates NAV-7 and MAV-1, respectively, from the fungal culture collection of the IVIA CTP, were cultured on potato dextrose agar (PDA, Sigma–Aldrich Chemical Co., St. Louis, MA, USA) plates at 25 °C. Conidia of each 7- to 14-day-old fungus were taken from the plate surface with a sterile glass rod and transferred to a sterile aqueous solution of 0.05% Tween 80® (Panreac, S.A.U., Barcelona, Spain). Conidial suspensions were filtered through two layers of cheesecloth to separate hyphal fragments and adjusted to a concentration of 1×10^5 spores mL^{-1} using a haemocytometer. Fruit were wounded and inoculated with the pathogens at the same time by immersing the tip of a stainless steel rod (2 mm length and 1 mm diameter) into the conidial suspension and making a puncture on the peel in the equatorial region of the fruit. Different lots of fruit were inoculated with each fungus.

2.4. Effect on fruit susceptibility to disease

To evaluate the effect of degreening on the fruit susceptibility to GM and BM, fruit were artificially inoculated with *P. digitatum* or *P. italicum* about 2 h after ethylene degreening. This set of experiments was performed with fruit of each cultivar harvested at the lowest CI (CI of −0.07, −6.5, −5.3 and 12.3 for 'Clemenpons', 'Clemenules', 'Navelina' and 'Nova' cultivars). Each treatment consisted of 4 replications with 10 fruit each. Degreened and non-degreened inoculated fruit were incubated at 20 °C and 90% RH for 7 d. Fruit were examined after 3 and 7 d to determine the incidence (% of infected wounds) and severity (diameter of lesion in mm measured only in infected fruit) of the molds.

2.5. Effect on disease development

To evaluate the effect of degreening on fungal development, fruit were inoculated with the pathogens as previously described 2 h before ethylene degreening. For each cultivar, these experiments were performed with fruit harvested at all different CI previously mentioned. After commercial degreening, degreened and control fruit were stored under two different conditions: (i) incubation at 20 °C and 90% RH for 7 d, and (ii) cold storage at 5 °C and 90% RH for 2 weeks. Stored fruit were periodically examined to determine disease incidence and severity. For each pathogen, treatment and storage condition, 4 replications of 10 fruit each were used.

2.6. Effect on citrus fruit quality

Non-inoculated oranges and mandarins were used for quality assessment. Fruit external and internal quality was determined just after harvest (initial quality) and 2–3 d after degreening on degreened and non-degreened (control) fruit (final quality).

2.6.1. External quality

Peel color was measured using Hunter parameters (*L*, *a*, *b*) with a colorimeter (Model Minolta CR-300, Konica Minolta Business Technologies, Inc., Tokio, Japan). A color index (CI) was calculated: CI = 1000.*a*/*L*.*b* (Jiménez-Cuesta et al., 1981). For each treatment, three measurements on the equatorial area of 25 fruit were performed.

Firmness of 20 fruit per treatment was determined using an Instron Universal Testing Machine (Model 4301, Instron Corp., Norwood, MA, USA). Each fruit was compressed between two flat