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Evaluation of Short Postharvest Gaseous Treatments for Inhibition of Alternaria Black Spot of Persimmon cv. 'Rojo Brillante'

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Abstract

Persimmon (*Diospyros kaki* Thunb.) is an expanding crop in Spain. The most important production area is València (about 90% of total planted area) and astringent 'Rojo Brillante' is by far the most planted cultivar. An increasingly important factor limiting storability of persimmon is postharvest decay due to Alternaria black spot (ABS) caused by the pathogen *Alternaria alternata*. As in other EU countries, no chemical fungicides are currently approved in Spain for postharvest treatment of persimmon and alternative antifungal treatments are required. The availability of commercial facilities in persimmon packinghouses for fruit deastringency through CO₂ treatments opens the door for potential application of antifungal gaseous treatments. 'Rojo Brillante' persimmons were artificially wound-inoculated with *A. alternata* and exposed 24 h later for 48 h to air at 20°C (ambient atmosphere, control), air at 35°C, 95 kPa CO₂ at 20 or 35°C, or 30 kPa O₂ + 70 kPa CO₂ at 20 or 35°C, all at 90% RH. Incidence (% of infected wounds) and severity (lesion size) of ABS were evaluated after 3, 5, and 10 days of incubation at 20°C and 80% RH. Another lot of treated fruit was cold-stored at 1°C and 90% RH for up to 82 days and fruit quality attributes such as weight loss (WL), firmness, maturity index (MI), and peel color index (CI) were also determined on non-inoculated but treated fruit. After 10 days of incubation at 20°C, none of the gaseous treatments applied at 20 or 35°C significantly reduced ABS incidence and ABS severity was even higher on some gas-treated persimmons than on control fruit. After 40 days at 1°C, ABS incidence was reduced by 30 to 40% on fruit treated with 95 kPa CO₂ at both temperatures, but these reductions were not significant after 82 days. ABS severity on cold-stored fruit was also greater on gas-treated persimmons than on control fruit. In general, gas-treated and cold-stored persimmons, especially those treated with 95 kPa CO₂, showed lower CI, higher MI and greater firmness than control fruit.

Keywords: *Diospyros kaki* Thunb., *Alternaria alternata*, carbon dioxide, oxygen, controlled atmosphere exposure, postharvest decay control

INTRODUCTION

Cultivation of Asian persimmon (*Diospyros kaki* Thunb.) in Spain has greatly increased in the latest decade due to consumer demand in European markets for the cultivar 'Rojo Brillante', mainly produced in the Valencian region. This variety is astringent when harvested and since its astringency naturally diminishes as the fruit firmness plummets, it was traditionally consumed overripened as a soft persimmon. However, soft fruit is difficult to handle and transport without damage, limiting its commercialization.

The current application in packinghouses of a new postharvest deastringency technology based on fruit exposure to a controlled atmosphere of 95 kPa CO₂ at 20°C for 24 h (Arnal and del Río, 2003), which efficiently removes the astringency while maintaining firmness, allows Spanish persimmon to be commercialized with an appreciated crisp texture and extends its exportation to distant markets. The ripening season of 'Rojo Brillante' is short (October to December), making cold storage necessary to maintain fruit quality and prolong the commercial marketing season. Although this cultivar suffers chilling injury when stored at temperatures lower than 11°C, application of the ethylene inhibitor 1-methylcyclopropene (1-MCP) to 'Rojo Brillante' persimmon allows prolonged storage at 0 to 1°C while reducing chilling injury (Besada et al 2008). However, the extended fruit storage life has been limited in recent seasons by a high incidence of postharvest decay caused by fungal pathogens, leading to important economic losses. *Alternaria* black spot (ABS), caused by *Alternaria alternata* (Fr.:Fr.) Keissler is the main postharvest disease on persimmon in Spain (Palou et al, 2012). This fungus may infect flowers and young fruits in the field, remaining latent and developing rot after harvest. The fungus may also infect peel wounds inflicted before, during or even after harvest, causing decay on commercially-mature fruit (Palou et al., 2015). In Spain, as in other EU countries, no chemical fungicides are currently approved for postharvest treatment of persimmons and alternative antifungal treatments are required.

Cold storage in controlled atmospheres (CA) is a common commercial practice to extend shelf life of some commodities. CO₂-enriched atmospheres suppress the development of many fungal pathogens in fresh fruit (Kader, 1986; Sommer, 1989). The effect of disease suppression by CO₂ is attributed not only to direct inhibition of fungal growth, but also to delayed fruit ripening, which maintains fruit resistance to pathogenic attack. The CO₂ concentration required to inhibit mycelial growth and/or spore germination varies with the species of fungi, but generally concentrations above 10% are needed (El-Goorani and Sommer, 1981). However, long-term CA storage is an expensive technology that sometimes is difficult to apply and maintain during transportation. Short-time exposure to high O₂ or CO₂ can also reduce postharvest diseases on fresh fruit. For example, postharvest treatment of avocado with 30 kPa CO₂ for 24 h at 20 to 25°C delayed the development of anthracnose, caused by *Colletotrichum gloeosporioides* (Prusky et al., 1993). Similarly, 30 or 60 kPa CO₂ for 24 h reduced the growth of *A. alternata* both in vitro and on artificially-inoculated 'Triumph' persimmons (Prusky et al., 1997). Heat treatments are the most common physical means used to control postharvest diseases of fresh fruit (Palou, 2009). Curing is a heat treatment regimen that keeps the fruit at 30 to 46°C and 90 to 80% RH for 12 to 94 h, which controls decay of fresh fruit, especially in citrus (Schirra et al., 2000). Exposure of artificially inoculated citrus fruit to 15 kPa CO₂ for 24 h or 30 kPa O₂ for 48 h at 33°C (low curing temperature) significantly inhibited blue and green molds caused by *Penicillium* spp. (Montesinos-Herrero et al., 2012). Exposure to 95 kPa CO₂ or the mixture 30 kPa O₂ + 70 kPa CO₂ for 48 h at 20 or 35°C reduced gray mold incidence and severity on 'Mollar de Elche' pomegranates artificially inoculated with *Botrytis cinerea* and incubated at 20°C or cold-stored at 5°C (Palou et al., 2016). The availability of commercial facilities in persimmon packinghouses for fruit deastringency through CO₂ treatments opens the door for potential application of antifungal gaseous treatments.

This work evaluated short postharvest gaseous treatments, alone or in combination with curing temperatures, for inhibition of ABS on artificially-inoculated 'Rojo Brillante' persimmons. The effect of treatments on fruit quality was also assessed.

MATERIALS AND METHODS

Fruit

'Rojo Brillante' persimmons, provided by CANSO (Cooperativa Agrícola Ntra. Sra. de l'Oreto, L' Alcúdia, València, Spain), were harvested at commercial maturity and transported to the CTP-IVIA facilities, where sound fruit of uniform medium size were selected and randomized.

Fungal inoculation

Isolate QAV-6 of *A. alternata*, obtained from a decayed persimmon from the Valencia area, was selected for its pathogenicity on persimmon fruit and grown on potato dextrose agar (PDA) petri dishes at 25°C for 7 to 14 days. A conidial suspension of the pathogen was prepared in sterile water with 0.05% Tween 80 and adjusted to a concentration of 10^5 spores mL⁻¹ after counting spores with a haemocytometer. Fruit were wounded at four equidistant points along the cheek with a sterilized stainless steel probe, 1 mm wide and 2 mm long, previously immersed in the spore suspension. Inoculated fruit were kept at 20°C and 90% RH for 24 h to favor spore germination.

Gas exposure and storage conditions

Exposure chambers consisted of hermetically sealed, transparent polymethyl methacrylate cabinets (82 x 62 x 87 cm) fitted with inlet and outlet ports through which O₂ or CO₂ (Alphagaz; Air Liquide España S.A., Madrid, Spain) were injected until the desired concentrations were achieved. The cabinets were also fitted with internal basal water trays that maintained RH at 90±5%. Concentrations of O₂, CO₂, temperature and RH were continuously monitored by the computer-controlled Control-Tec® system (Tecnidex SA, Paterna, València, Spain). Cabinets were located in a 40 m³ standard storage/curing room, where the temperature was set to each experimental condition (20 or 35 ±2°C). Fruit were treated for 48 h at 20 or 35°C with air (control), 95 kPa CO₂, or the mixture 30 kPa O₂ + 70 kPa CO₂. Four replicates of five artificially inoculated persimmons each (20 fruits, 80 wounds) were used for each gaseous atmosphere, exposure temperature and storage condition. After treatment, fruit were arranged in plastic trays and divided into two lots. The first was incubated at 20°C and 90% RH for 10 days and the second was kept at 1°C and 90% RH for 82 days.

Effect on ABS development

The effect of gaseous treatments on the development of ABS disease caused by *A. alternata* on artificially inoculated persimmon fruit was assessed by measuring disease incidence (percentage of infected wounds) and severity (lesion diameter of infected wounds). Fruit incubated at 20°C were evaluated after 3, 5 or 10 days. Fruit stored at 1°C were evaluated every 14 days for an 82-day period.

Quality of cold-stored fruit

To assess the effect of gaseous shocks on the quality of treated and cold-stored fruit, in parallel with the experiment described above, a lot of uninoculated fruit was treated for 48 h at 20 or 35°C with air (control), 95 kPa CO₂, or the mixture 30 kPa O₂ + 70 kPa CO₂, then stored at 1°C and 90% RH for 12 weeks. External and internal quality was determined at harvest (initial quality of untreated fruit) and after 12 weeks of cold storage. The physico-chemical characteristics of 20 persimmon fruits were evaluated for weight loss, firmness, (Instron Universal Machine, Model 3343, Instron, Barcelona, Spain), external color (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Osaka, Japan), soluble solids content (SSC) (Atago Pocket refractometer, Atago company Ltd., Japan), and total acidity (TA) (Titration Excellence T50, Mettler Toledo, Barcelona, Spain). External color, expressed as color index (CI), was calculated using the Hunter L, *a*, *b* color space parameters, and fruit firmness was the maximum force in Newtons (N) required to penetrate fruit flesh after removing the skin on the equator. Maturity index was calculated as SSC/TA.

Statistical analysis

Statistical analysis was performed using Statgraphics v. 5.1 (Statpoint Technologies Inc., Warrenton, VA, USA). Specific differences between means were determined for each parameter and evaluation date by the least significant difference (LSD) test at $P \leq 0.05$ applied after a one-way analysis of variance (ANOVA). Disease incidence data were arcsine-transformed.

RESULTS AND DISCUSSION

Effect of gaseous exposure on ABS development

Exposure of fruit to the mixture 30 kPa O₂ + 70 kPa CO₂ for 48 h at 20°C reduced ABS incidence by 33% below that of fruit exposed to air at the same temperature (control) after 3 days of incubation (Fig. 1A). However, after 10 days of incubation at 20°C, no gaseous treatment reduced ABS incidence, independently of exposure temperature (Fig. 1A, 1C). ABS severity was greater on some gas-treated persimmons than on control fruit, especially fruit treated with 30 kPa O₂ + 70 kPa CO₂ for 48 h at 35°C (Fig. 1B, 1D). In contrast, exposure to 50 and 95 kPa CO₂ for 48 h at 20°C significantly reduced the incidence of gray mold by 82 and 92%, respectively, on pomegranates artificially inoculated with *B. cinerea* after 5 days of incubation at 20°C (Palou et al., 2016).

Regardless the exposure temperature, gaseous shocks with 95 kPa CO₂ for 48 h significantly reduced ABS incidence on fruit cold-stored for 54 days. At 20°C, ABS incidence was reduced by 100, 88, 32 and 10% in comparison to that of the control after 12, 26, 40 and 54 days at 1°C, respectively, and similar reductions were observed on fruit treated at 35°C. However, these treatments lacked persistence and did not control ABS incidence in subsequent evaluations (Fig. 2A, 2C). Therefore, the effect of CO₂ on the fungus was primarily fungistatic instead of fungicidal, since disease incidence increased with incubation time. Similar conclusion were reached regarding sweet cherries inoculated with *Monilinia fructicola* (De Vries-Paterson), mandarins inoculated with *Penicillium* spp. (Montesinos-Herrero et al., 2012), avocados inoculated with *C. gloesporioides* (Prusky et al., 1993), or pomegranates inoculated with *B. cinerea* (Palou et al., 2016) and treated with CO₂ gaseous shocks.

Exposure of inoculated persimmons to 30 kPa O₂ + 70 kPa CO₂ for 48 h was less effective than shocks with 95 kPa CO₂ in reducing ABS incidence on fruit stored at 1°C: when applied at 20°C, this treatment significantly reduced ABS incidence, with reductions of 85 and 44% after 12 and 26 days of cold storage, respectively, but without significant reduction thereafter (Fig. 2A). When applied at 35°C, ABS incidence was significantly reduced (by 54%) after 12 storage days only (Fig. 2C).

All treatments, regardless of the exposure temperature, significantly increased ABS severity on fruit stored at 1°C, especially on fruit treated with 30 kPa O₂ + 70 kPa CO₂ for 48 h (Fig. 2B, 2D). In contrast, gaseous shocks with 95 kPa CO₂ or 30 kPa O₂ + 70 kPa CO₂ at 20 or 35°C for 48 h significantly reduced gray mold severity on 'Mollar de Elche' pomegranates artificially inoculated with *B. cinerea* and either incubated at 20°C or cold-stored at 1°C (Palou et al., 2016).

Figure 1. Alternaria black spot (ABS) incidence and severity on 'Rojo Brillante' persimmons artificially inoculated with *Alternaria alternata*, then treated 24 h later with air (control), 95 kPa CO₂ or the mixture 30 kPa CO₂ + 70 kPa O₂ at 20 (A, B) or 35°C (C, D) for 48 h, and incubated at 20°C for up to 10 days. For gaseous treatments applied at 35°C (C and D), an additional control treatment of air at 20°C was included. For each evaluation, uneven letters and 'ns' indicate significant and not significant differences, respectively, according to Fisher's Protected LSD test ($P = 0.05$) applied after an ANOVA. Incidence data was arcsine transformed. Actual means are shown. For severity, only infected wounds were considered.

Figure 2. Alternaria black spot (ABS) incidence and severity on 'Rojo Brillante' persimmons artificially inoculated with *Alternaria alternata*, then treated 24 h later with air (control), 95 kPa CO₂ or the mixture 30 kPa O₂ + 70 kPa CO₂ at 20 (A, B) or 35°C (C, D) for 48 h, and stored at 1°C for 12 weeks. For gaseous treatments applied at 35°C (C and D), an additional control treatment of air at 20°C was included. For each evaluation day, uneven letters and 'ns' indicate significant and not significant differences, respectively, according to Fisher's Protected LSD test ($P = 0.05$) applied after an ANOVA. Incidence data was arcsine transformed. Actual means are shown. For severity, only infected wounds were considered.

Effect of gaseous exposure on fruit quality

Exposure of fruit to 95 kPa CO₂ for 48 h at 35°C or to the mixture 30 kPa O₂ + 70 kPa CO₂ for 48 h at either 20 or 35°C slightly increased weight loss (~ 5.3%) with respect to the control (~ 4.6%, Table 1).

In general, external color changed after cold storage by increasing CI, especially on control treatments. However, regardless of exposure temperature, fruit exposed to 95 kPa CO₂ had a lower CI than the rest of treatments, with similar values to those at harvest.

Similarly, fruit firmness decreased during storage, but fruit exposed to 95 kPa CO₂ at 20 or 35°C were firmer than control fruit (~10 N after 12 weeks at 1°C). The reduction in firmness of persimmon fruit during storage has been linked to changes in external color (Salvador et al., 2006), which agrees with our results. Firmness of persimmons treated with 30 kPa O₂ + 70 kPa CO₂ at 35°C sharply diminished after 82 days at 1°C.

SSC increased during storage, but there were no significant differences among treatments. Nevertheless, fruit treated with 95 kPa CO₂ at 20 or 35°C had significantly lower TA and higher MI than control fruit. 'Mollar de Elche' pomegranates exposed to 95 kPa CO₂ by 48 h had a similar response (Palou et al., 2016). Lower acidity has been linked to exposure to elevated CO₂ concentrations (Kader 1986).

Table 1. Effect of gaseous treatments on external and internal quality attributes of 'Rojo Brillante' persimmons after storage at 1°C for 12 weeks.

Temp (°C), gaseous treatment	WL (%)	Firmness (N)	SSC (%)	TA (%)	MI	CI
20						
At harvest		21.3±6	14.0±0.5	0.13±0.01	104.8±9.8	17.5±3
Air (control)	4.7a	4.9a	15.7a	0.11b	147.8a	27.4c
95 kPa CO ₂	4.5a	10.5b	15.5a	0.06a	264.2b	21.3a
30 kPa O ₂ + 70 kPa CO ₂	5.2b	4.6a	16.1a	0.06a	289.9b	23.7b
35						
At harvest		26.9±6	12.8±0.1	0.14±0.00	91.3±3.7	16.7±8
Air 20°C (control)	4.6ab	3.8b	14.5a	0.07b	200.5b	24.2c
Air 35°C	4.5a	3.1b	14.3a	0.10c	138.7a	21.7b
95 kPa CO ₂	5.3bc	9.3c	13.6a	0.05a	251.8c	15.4a
30 kPa O ₂ + 70 kPa CO ₂	5.4c	0.8a	14.0a	0.07b	204.3b	21.5b

^z Abbreviations: WL: weight loss; SSC: soluble solids content; TA: total acidity (g malic acid/100 g); MI: maturity index (SSC/TA); CI: color index (1000a/Lb; Hunter parameters). Shown values are means ± standard deviation. For each temperature and quality attribute, means in columns followed by the same letter are not statistically different according to Fischer's protected LSD test ($P < 0.05$).

CONCLUSION

Application of 95 kPa CO₂ or the mixture 30 kPa O₂ + 70 kPa CO₂ at 20 or 35°C for 48 h did not reduce ABS incidence on artificially inoculated 'Rojo Brillante' persimmons after 10 incubation days at 20°C. ABS severity was even greater on treated than on control fruit stored at either 20 or 1°C. However, fruit exposure to 95 kPa CO₂ at 20°C for 48 h significantly reduced ABS incidence for 54 days at 1°C. Nevertheless, the treatment lacked persistence and its effect was more fungistatic than fungicidal. The application of curing temperatures (35°C) combined with CO₂ exposure did not improve the efficacy of ABS control. Fruit treated with 95 kPa CO₂ had lower CI, higher MI, and was firmer than control fruit. The main shortcoming of a control strategy based on short-term treatments with CO₂ is the low persistence of their positive effects.

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