ON-LINE UV-C PROTOTYPE FOR POSTHARVEST ANTIFUNGAL TREATMENT OF CITRUS FRUITS

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Abstract

Synthetic fungicides used for postharvest treatment of citrus fruits are considered by consumers as a risk to human health and environment and alternatives to these chemicals that leave no residue on fruit are increasingly demanded. UV-C light irradiation is a clean and safe technology that when applied at the right doses may induce fruit resistance to postharvest diseases, reducing the need for chemical treatments.

The objective of present study was to evaluate the preventive effect of UV-C light treatments against citrus postharvest green mould caused by the pathogen *Penicillium digitatum*. For this purpose, 'Lanelate' oranges and 'Ortanique' mandarins were puncture-injured in one side of the equatorial zone, treated with UV-C light at doses of 2.5 or 5 kJ/m^2 and held at 20ºC and 90% RH for 6 days before inoculation with *P. digitatum* at 10^3 or 10^4 spores/ml. Control fruit was inoculated but not treated. Four replicates of 10 fruit each were used for each treatment.

A new UV-C reactor prototype was used for postharvest treatment of citrus fruits. This prototype was able to treat the fruit while rolling, therefore exposing the surface of the fruit more homogeneously to UV-C light. The reactor, assembled on a commercial 3-m long roller conveyor, consisted of three low pressure 55-W mercury-vapour lamps, mounted on an aluminium frame placed 16 cm above the conveyor belt. UV-C average power supplied by the lamps at 6 cm above the conveyor belt was 5 mW/cm^2. In order to regulate the flow rate and thus the UV-C exposure dose, the speed of the conveyor belt was set to provide a treatment time from 25 s to 2 min, which yielded a UV-C light dose from 2.25 to 5 kJ/m^2, respectively.

The number of infected fruit (disease incidence) as well as the diameter of the lesion (disease severity) and the percentage of fruit showing sporulated lesions (pathogen sporulation) were assessed after 3 and 7 days of incubation at 20ºC. Green mould incidence on 'Lanelate' oranges treated with a UV-C dose of 5 kJ/m^2 was 64 and 42% lower than on control fruit after 3 and 7 days of incubation, respectively. Pathogen sporulation was 70 and 45% lower than on control fruit after these incubation periods. Disease severity was not significantly affected by UV-C treatments. On 'Ortanique' mandarins, treatments with UV-C light at 2.5 kJ/m^2 reduced green mould incidence by 56% compared to non-treated fruit after 7 days of incubation. However, disease incidence and pathogen sporulation on 'Ortanique' mandarins treated with UV-C light at 5 kJ/m^2 were similar to those on control fruit. In this case, although no rind damage was observed at the naked eye, UV-C light application probably resulted on slight phytotoxicity that counteracted the resistance induction. It can be concluded from these experiments that the election of the right UV-C dose was essential for the suitability of the treatments.

Key words: UV-C radiation. Prototype. Postharvest Citrus. *Penicillium digitatum*. Green mould

1. Introduction

1.1 Background

Currently, consumers are increasingly demanding food free from chemical residues. Alternatives to the use of chemical fungicides for postharvest treatment of fruits and vegetables are needed (Palou et al., 2008). Far-ultraviolet radiation (UV-C; λ = 254 nm) is a physical method potentially useful for the control of diseases caused by *Penicillium* spp. in citrus fruit (Ben-Yehoshua et al., 1992). UV-C light may show a direct effect by the destruction of fungal structures and an indirect effect by the induction of fruit resistance to disease (D'hallewin et al., 1999). At the right doses, UV-C is a clean, safe, and residue-free technology for the postharvest antifungal treatment of fresh produce that may substitute or reduce the use of chemicals. However, overdose of UV-C may result in phytotoxicity and therefore proper doses must be assessed experimentally.

The IVIA and the UPV have been working for the last few years on the development of a UV-C reactor prototype suitable for the postharvest treatment of citrus fruits. Our previous results (Adrados et al., 2009) showed that high UV-C radiation doses of up to 20 kJ/m^2 caused damages in fruit skin which, although invisible to the naked eye, increased the susceptibility of fruit to postharvest green mould, caused by *Penicillium digitatum*.

1.2. Objectives

The objective of the present work was to establish the most appropriate UV-C doses to control green mould, the most important citrus postharvest disease in our Spanish environmental conditions, on 'Lanelate' oranges and 'Ortanique' mandarins.

2. Materials and methods

2.1 Prototype

The prototype used in these experiments (Figure 1) was designed to be integrated into packinglines in citrus packinghouses. This prototype was able to treat the fruit while rolling, therefore exposing the surface of the fruit more homogeneously to UV-C light. The reactor, assembled on a commercial 3-m long roller conveyor, consisted of three low pressure 55-W mercury-vapour lamps, mounted on an aluminium frame placed 16 cm above the conveyor belt. Lamps were 5-cm overlapping each other to overcome the loss of power at their ends. UV-C average power supplied by the lamps at 6 cm above the conveyor belt was 5 mW/cm^2. In order to regulate the flow rate and thus the UV-C exposure dose, the speed of the conveyor belt was set to provide a treatment time from 25 s to 2 min, which yielded a UV-C light dose from 1.25 to 6.0 kJ/m^2, respectively. Before every experiment, doses were calibrated using a photodiode-type sensor, and the speed was set to yield the right dose by measuring the exposure time with a chronometer.

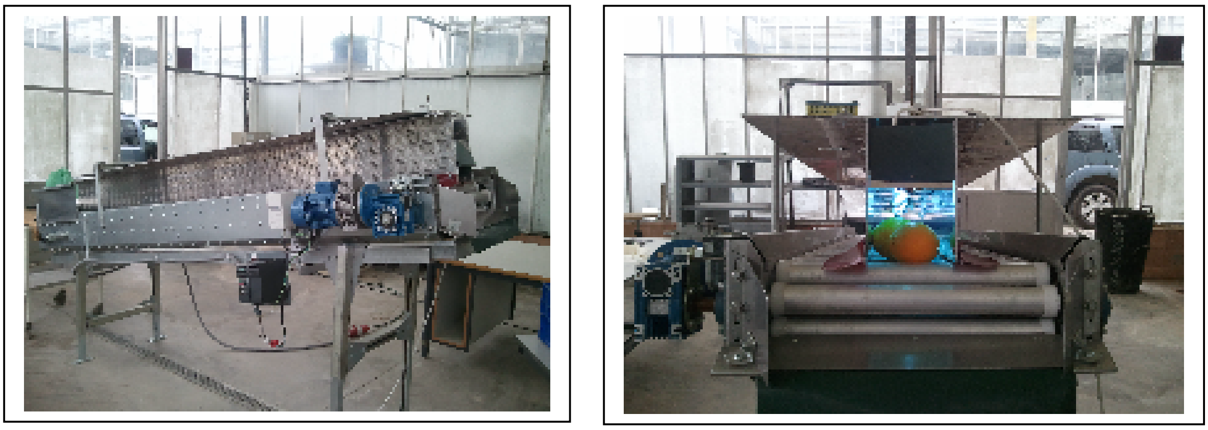

FIGURE 1: UV-C prototype images showing the gear motor, speed setting device and position of the lamps.

2.2 Fruit and Treatments

Fruit used in this study were oranges cv. Lanelate (*Citrus sinensis* L. Osbeck) and hybrid mandarins cv. Ortanique [*C. reticulata* Blanco × (*C. sinensis* × *C. reticulata*); synonym: 'Topaz']. Fruit were collected from commercial orchards in Valencia (Spain) and received no fungicide treatments or coatings. Prior to each assay, fruit were selected, randomised, rinsed with fresh water, and allowed to air dry at room temperature.

In order to test the preventive effect of UV-C irradiation against green mould, fruit were wounded at two opposite sites on the equatorial zone with a sterilised stainless steel rod with a probe tip 1-mm wide and 2-mm long. Thereafter, 'Lanelate' oranges were treated with 5 kJ/m^2 UV-C and 'Ortanique' mandarins with 2.5 or 5 kJ/m^2 and then stored at 20ºC and 80% RH for 6 days. According to previous preliminary tests and other workers (Kuniga et al, 2006), this is the time when the maximum induction of antifungal scoparone occurs in citrus fruit after being UV-C stimulated. *P. digitatum* isolate NAV-7, obtained from a decayed orange from the Valencia area, was grown on PDA in petri dishes at 25ºC for 7 to 14 days. A conidial suspension was prepared in Tween 80 (0.05% w/v) in sterile distilled water, the concentration determined with a hemacytometer and diluted to a concentration of 10^3 and 10^4 spores/ml for 'Lanelate' oranges and 'Ortanique' mandarins, respectively. Fruit were inoculated by immersing the above described rod in the spore suspension and then introducing the tip in the same wound. Fruit were then stored at 20ºC and 80% RH, and after 3 and 7 days the number of decayed fruit (incidence, %), sporulated fruit (sporulation, %), and disease lesion diameter (severity, mm) were assessed. In every experiment, four replicates of ten fruit each were used for each treatment.

2.3 Statistical Analysis

Data on incidence and sporulation was arcsine transformed. Only symptomatic infected fruit were considered for disease severity assessment. One-way analyses of variance (ANOVA) were performed using Statgraphics Plus 4.1 (Manugistics, Inc., Rockville, MD, USA) with disease incidence, sporulation, or severity data as dependent variables and treatment as factor. Where appropriate, means were separated by Fisher's Protected LSD test (P=0.05).

3. Results

Results of the experiments with 'Lanelate' oranges and 'Ortanique' mandarins are shown on Table 1 and Figure 2, respectively.

TABLE 1: Incidence, sporulation and severity of green mould on 'Lanelate' oranges treated with 5 kJ/m^2 UV-C irradiation, artificially inoculated 6 days later with *Penicillium digitatum* (10^3 spores/ml), and incubated for 3 and 7 days at 20ºC after inoculation. Different letters denote significant differences among means within each column according to LSD test (*P*<0.05).

Treatment	Incidence (%)		Sporulation (%)		Severity (mm)	
	3 days	7 days	3 days	7 days	3 days	7 days
Control	82,5 a	95 a	67,5 a	82,5 a	94,9 a	115,8 a
UV-C 5 kJ/m^2	30 b	55 b	20 b	45 b	96,6 a	110,2 a

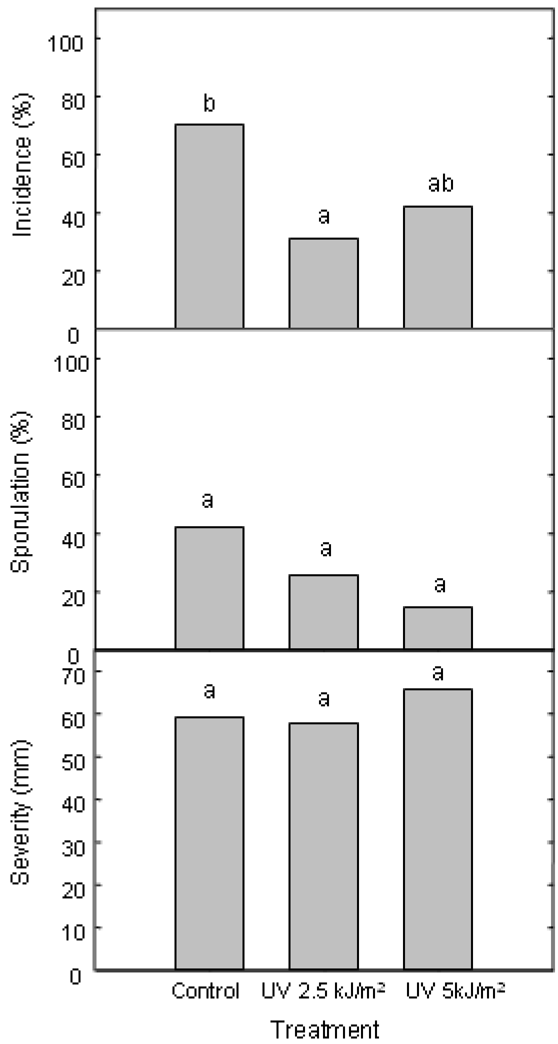

FIGURE 2: Incidence, sporulation and severity of green mould on 'Ortanique' hybrid mandarins treated with 2.5 or 5 kJ/m^2 UV-C irradiation, artificially inoculated 6 days later with *Penicillium digitatum* (10^4 spores/ml), and incubated for 7 days at 20ºC after inoculation. Different letters denote significant differences among means within each group of columns according to LSD test (*P*<0.05).

4. Discussion

The experimental design in this study was based on results from previous unpublished and published research (Adrados et al, 2009). It was observed that for ‘Lanelate’ oranges and ‘Ortanique’ mandarins, UV-C irradiation doses over 5 kJ/m^2 were harmful and increased fruit susceptibility to green mould. On ‘Lanelate’ oranges, UV-C irradiation at 5 kJ/m^2 applied 6 days before inoculation was effective to prevent green mould, since this treatment significantly reduced the incidence and sporulation rates on treated fruit compared to control fruit. Nevertheless, disease severity was not significantly affected by the treatments. On the other hand, green mould prevention was higher on ‘Ortanique’ mandarins treated with 2.5 kJ/m^2 than on mandarins treated with 5 kJ/m^2. In this case, although no significant differences were found between doses, only the lower irradiation dose significantly reduced the incidence of green mould compared to control fruit. UV-C irradiation at 5 kJ/m^2 might cause some unnoticeable damage to fruit skin that can counteract the potential protective effects of UV-C treatment against green mould.

As described by other authors (Ben-Yehoshua et al., 1992; Droby et al., 1993), the synthesis of phytoalexins such as scoparone induced by UV-C light increases up to certain threshold and then decreases. The time elapsed until a maximum induction level is achieved, the amount of phytoalexins, and whether this amount is high enough to prevent disease development depend on treatment conditions (dose, time between treatment and fungal infection), pathogen (species, strain, inoculum density) and host fruit (species, cultivar, fruit physical and physiological condition). This variability explains the importance of testing the performance of UV-C treatments for every particular case in order to establish the optimum commercial treatment conditions.

In this study, UV-C light at 2.5 and 5 kJ/m^2 applied to ‘Ortanique’ and ‘Lanelate’ citrus fruit, respectively, prevented green mould sufficiently to be considered as part of a strategy to substitute or reduce the use of conventional synthetic fungicides for the control of postharvest diseases.

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