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Pre-harvest spray of oligochitosan induced the resistance of harvested navel oranges to anthracnose during ambient temperature storage

Lili Deng [a, b], Yahan Zhou [a], Kaifang Zeng [a, b, *]

[a] College of Food Science, Southwest University, Chongqing 400715, PR China
[b] Laboratory of Quality & Safety Risk Assessment for Agro-products on Storage and Preservation (Chongqing), Ministry of Agriculture, Chongqing 400715, PR China

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ABSTRACT

In this study, the ability of pre-harvest oligochitosan spray to control *Colletotrichum gloeosporioides* of navel orange during ambient temperature storage was examined. Navel orange trees were sprayed thrice with oligochitosan (15 g L^{-1} water) after physiological fruit drop. Results indicated that disease incidence and lesion diameter were lower in oligochitosan-treated fruit compared with the respective control. The inhibitory effects of volatiles in navel orange rind on the spore germination of *C. gloeosporioides* were significantly enhanced in the treated fruit compared with the control. Biochemical evaluations revealed that the contents of hydroxyproline-rich glycoprotein were increased. Protopectin degradation was delayed during storage. In addition, the activities of defense-related enzymes, including pectin methylesterase, peroxidase, chitinase, and phenylalanine ammonia-lyase, were increased in oligochitosan-treated navel orange fruit rinds. Our results suggested that pre-harvest oligochitosan spray can be a potential alternative to conventional control methods to prevent post-harvest anthracnose in navel orange.

1. Introduction

Navel orange (*Citrus sinensis* L. Osbeck) is cultivated worldwide to produce fresh and processed products. However, anthracnose, a post-harvest disease caused by *Colletotrichum gloeosporioides* Penz., can cause post-bloom fruit drop, key lime anthracnose and considerable economic losses in navel orange production, particularly with prolonged storage (Ballester et al., 2006). Other early season fruits are also susceptible to this pathogen (Peres et al., 2008). Synthetic fungicides, such as benomyl and thiabendazole, were used to control this disease (Timmer et al., 1998), but pathogens develop fungicide resistance, hence, public concerns on fungicide residues in food have compelled scientists to search for new alternatives (Tripathi and Dubey, 2004).

Biogenic and non-biogenic elicitors can be used to induce resistance of fruits to pathogenic infection; this method has been considered as a promising approach to control post-harvest diseases. For example, salicylic acid, oligosaccharides, and other elicitors can induce fruit resistance to infections (Zeng et al., 2006a; Mazaro et al., 2008). Moreover, pre-harvest treatment with elicitors is an effective method to enhance the post-harvest disease resistance of fruits (Silimeta and Korsten, 2008; Elmer et al., 2007; Meng et al., 2010a).

Oligochitosan, prepared by the enzymatic hydrolysis of deacetylated chitosan polymers, is currently viewed as a plant disease vaccine (Yin et al., 2010). Oligochitosan and chitosan can effectively control the development of anthracnose in banana (Meng et al., 2012) and papaya (Eryani et al., 2009), due to the combination of a direct antifungal effect on the pathogen and an indirect effect. In addition, oligochitosan can elicit multiple plant defensive reactions against various kinds of biotic and abiotic stresses in several plants (Yin et al., 2010). Oligochitosan can promote the accumulation of several components, such as volatile components exhibiting antibacterial activity, hydrogen peroxide (Li et al., 2009), and defense-related enzymes (Yan et al., 2012; Ma et al., 2013). Oligochitosan can also enhance the upregulated expression of β-1,3-glucanase (GLU) and chitinase (CHI) (Lin et al., 2005; Meng et al., 2012) as well as the

Abbreviations: HRGP, hydroxyproline-rich glycoprotein; PME, pectin methylesterase; PDA, potato dextrose agar; POD, peroxidase; VOCs, volatile organic compounds; PG, polygalacturonase; PAL, phenylalanine ammonia-lyase; CHI, chitinase; GLU, β-1,3-glucanase.

* Corresponding author. College of Food Science, Southwest University, Chongqing 400715, PR China.
E-mail address: zengkaifang@hotmail.com (K. Zeng).

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contents of structural-related compounds (Orlita et al., 2008; Meng et al., 2010b). In addition, the direct and broad-spectrum antimicrobial activity of oligochitosan should be considered (Yang et al., 2012). These findings suggest that oligochitosan is a promising alternative to synthetic fungicides to control post-harvest diseases in horticultural products. It was reported that pre-harvest oligochitosan sprays can induce post-harvest disease resistance in jujube (Yan et al., 2012). However, limited information is available regarding the regulatory effects of pre-harvest oligochitosan spray on anthracnose and related mechanisms in navel orange. This study investigated whether or not the pre-harvest spray of oligochitosan influences anthracnose development in post-harvest navel orange (*C. sinensis* L. Osbeck). This study also elucidated the mechanisms underlying this process.

2. Materials and methods

2.1. Plant growth, oligochitosan treatments, and sampling

Oligochitosan (molecular mass = 1500 Da–2000 Da) was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd. (Shandong, China).

Fruit treatment. Experiments were conducted in a commercial orchard in Nanchong, Sichuan Province from 2007 to 2008. A randomized block design was used with three replications consisting of nine trees. Each tree was numbered and one planting line was used for each treatment, and the treated lines were separated by untreated lines. The trees were equidistanced from each other by 4 m in each planting line, and the distance between each line was 5 m. Considering the results of our preliminary experiment (Deng et al., 2009a, 2009b), we sprayed oligochitosan (15 g L^{-1} water) on the whole trees thrice for 30, 60, and 90 d after physiological fruit drop, with a 16-L easy carry 'knapsack' sprayer equipped with a pressure release nozzle valve and a trigger lock (Model, AM-S18, Zhejiang Allied Agro Materials Company, China, the pressure of the nozzle is about 1.6 MPa), and about 1 L for one tree. Water was used as a control treatment. All sprays were carried out in the morning when the fog disappeared, and the last spray was performed about 10 days before harvest. Other cultural practices of orchard management were carried out according to regular commercial production, but without using of any fungicides. Fruit from the oligochitosan spray treatment and the water treatment in the field were then harvested when the control group reached commercial maturity (approximately 190 d after full flowering). For biochemical analysis and disease evaluation, oranges with uniform size and free from physical damage and infections in these two treatments were selected and stored in air at 20 °C and 85%–90% RH. Rind tissue from the fruit equator was cut into small pieces, frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis (Cao and Jiang, 2006).

2.2. Inoculation and measurement of disease progress

C. gloeosporioides was isolated from an infected orange fruit showing typical anthracnose symptoms and then incubated in potato dextrose agar (PDA) medium at 28 °C. We observed that the orange fruit was infected with this microorganism and typical anthracnose symptoms reappeared. This observation is consistent with Koch's postulates. The isolates were initially identified morphologically, physiologically, and pathogenically as *C. gloeosporioides* Penz. strains (McGovern et al., 2012). Fungal spores were then obtained by flooding the 12-day-old cultures with sterile distilled water containing 0.05% (v/v) Tween-80. Spore suspensions were filtered using four layers of sterilized cheesecloth to remove adhering mycelia. The spore concentration was adjusted with the aid of a hemocytometer before use (Zeng et al., 2006a).

Navel orange fruit from the oligochitosan spray treatment and the water treatment with uniform size and free of physical damage and infection were selected, and disinfected with 2% (v/v) sodium hypochlorite solution for 2 min. Afterward, these oranges were rinsed with tap water and dried in air. The orange fruit were then wounded using a sterile syringe at three points [4 mm (depth) × 4 mm (width)] at the equator. Approximately 15 μL of the conidial suspension of *C. gloeosporioides* Penz. containing 1×10^5 spores mL^{-1} to 10^6 spores mL^{-1} was pipetted to each wound. The fruit were individually packed in plastic bags (polyethylene; area = 150 mm × 150 mm; thickness = 0.015 mm) 4 h after these fruit were inoculated with fungal spores and incubated at 20 °C and 85%–90% RH for 32 d. A fruit was considered decayed when the visible rot zone outside the wounded area on the fruit measured >1 mm in width. Each treatment was performed in three replicates with 10 fruit per replicate, and the experiment was conducted twice.

The disease incidence and lesion diameter (symptoms were visible and countable) of each fruit were recorded every second day after inoculation. Disease incidence was expressed as the percentage of fruit showing disease symptoms, and lesion size was calculated as 3.14 × (lesion diameter/2)^2 (Cao and Jiang, 2006). Each treatment contained 10 fruit with three replicates arranged in block randomization. This experiment was also conducted twice.

2.3. Inhibitory effects of volatile organic compounds (VOCs) in navel orange rind on the spore germination of C. gloeosporioides

The inhibitory effects of VOCs in navel orange rind on the spore germination of *C. gloeosporioides* were evaluated as described by

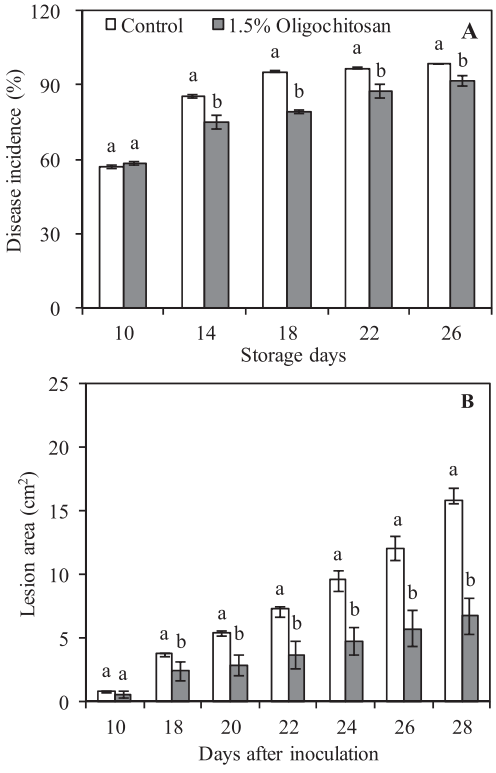

Fig. 1. Effects of oligochitosan spray (15 g L^{-1} water) on disease incidence (A) and lesion size (B) in navel oranges inoculated with *C. gloeosporioides*. Fruits were treated thrice and water was used as a control treatment. Data represent the means ± SD, n = 3. Bars represent standard deviations of the means. Values followed by different letters at the same time are significantly different according to Student's t-test (*P = 0.05).