Food Chemistry 234 (2017) 163–167

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Control of anthracnose disease via increased activity of defence related enzymes in 'Hass' avocado fruit treated with methyl jasmonate and methyl salicylate

Marcin Glowacz [a,b,*], Nico Roets [c], Dharini Sivakumar [b]

[a] Natural Resources Institute, University of Greenwich, Chatham ME4 4TB, UK
[b] Postharvest Technology Group, Department of Crop Sciences, Tshwane University of Technology, Private Bag X680, Pretoria West 0001, South Africa
[c] Agricultural Research Council: Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200, South Africa

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 6 April 2017
Accepted 11 April 2017
Available online 12 April 2017

Keywords:
Persea americana Mill.
Anthracnose
Epicatechin
Chitinase
β-1,3-Glucanase
Supply chain

ABSTRACT

Development of anthracnose disease caused by *Colletotrichum gloeosporioides* Penz. is one of the major issues within the avocado supply chain. Exposure to methyl jasmonate (MeJA) and methyl salicylate (MeSA) vapours at 10 and 100 μmol l^{-1} was investigated as an alternative solution to commercial fungicide – prochloraz® that is currently being used by the industry. The incidence of anthracnose disease was found to be significantly reduced in 'Hass' avocado fruit treated with MeJA or MeSA vapours, especially at 100 μmol l^{-1}. The mechanism involved enhanced activity of defence related enzymes, i.e. chitinase, *β*-1,3-glucanase and PAL, and higher content of epicatechin.

1. Introduction

Avocado (*Persea americana* Mill.) is becoming a popular fruit mainly due to its nutritional content (Dreher & Davenport, 2013), in particular being rich in monounsaturated fatty acids (Lu et al., 2009; Ozdemir & Topuz, 2004). The production of avocados in South Africa is mainly export driven, and according to the latest (2015) food trade and supply chain directory (www.foodtradesa.co.za), the European Union, and United Kingdom in particular, is the biggest export market.

Development of postharvest disease, such as anthracnose (caused by *Colletotrichum gloeosporioides* Penz.), is one of the major issues within the avocado supply chain, affecting marketability of the produce. At the moment prochloraz®, a synthetic fungicide, is being used in the packhouses to control anthracnose disease. However, since there is an increasing demand to reduce the use of fungicides (Bill, Sivakumar, Thompson, & Korsten, 2014), there is clearly a need for new techniques that could reduce undesired fungal decay.

One of the possible options to reduce the disease development is via inducing the defence mechanisms in the fruit (Romanazzi et al., 2016). There are numerous methods used to reduce microbial contamination of fresh produce and to extend its storage life (Ramos, Miller, Brandao, & Silva, 2013), however the postharvest use of jasmonates and salicylates seems to be overlooked. These are natural plant signalling compounds that play a role in stimulating natural defence mechanisms against both biotic and abiotic stress, and the information on their use to reduce losses within the fruit supply chain has recently been reviewed (Glowacz & Rees, 2016).

It has been reported that dipping 'Hass' avocado fruit in 2.5 μmol l^{-1} methyl jasmonate solution for 30 s reduced the development of chilling injury in fruit subsequently stored for 2 weeks at 1 °C (Sivankalyani et al., 2015) and 4 weeks at 2 °C (Meir et al., 1996), respectively. It was further confirmed in our trials with methyl jasmonate and methyl salicylate that these compounds have the ability to maintain the postharvest quality of cold stored 'Hass' avocado fruit by altering their fatty acids content and composition (Glowacz, Bill, Tinyane, & Sivakumar, 2017). However, to the best of our knowledge there is no information in the literature on the effects of methyl jasmonate and methyl salicylate on the anthracnose disease susceptibility in 'Hass' avocado fruit, while the ability of these compounds to reduce fungal decay has already

* Corresponding author at: Natural Resources Institute, University of Greenwich, Chatham ME4 4TB, UK.
E-mail addresses: M.M.Glowacz@greenwich.ac.uk (M. Glowacz), SivakumarD@tut.ac.za (D. Sivakumar).

http://dx.doi.org/10.1016/j.foodchem.2017.04.063
0308-8146/

been reported for numerous products, e.g. loquat (Cao, Zheng, Yang, Tang, & Jin, 2008; Cao, Zheng, Yang, Tang, Jin, Wang, et al., 2008) and mango (Zeng, Cao, & Jiang, 2006).

It is well known that the activity of defence related enzymes, i.e. chitinase and β-1,3-glucanase is enhanced when the produce is challenged by the fungal pathogen (Mauch, Mauch-Mani, & Boller, 1988). These enzymes, acting synergistically, are capable of hydrolysing polymers of fungal cell walls – chitin and β-1,3-glucan respectively, leading to weakened cell wall and cell lysis (Stintzi et al., 1993). Chitinase and β-1,3-glucanase are thus involved in the plant defence mechanisms preventing/delaying the fungal growth and in this way reducing the decay (Theis & Stahl, 2004).

The activity of PAL is often induced by both abiotic and biotic stress (Dixon & Paiva, 1995), e.g. in response to wounding or pathogen attack, where synthesised phenolics could either act directly as defence compounds or indirectly, due to being precursors of lignin and suberin, producing a barrier and strengthening cell walls (Passardi, Penel, & Dunand, 2004), which would prevent the infection and limit pathogen expansion in infected fruit.

Finally, epicatechin, an antioxidant present in the peel, is also involved in delaying/preventing the fungal decay via lowering the activity of lipoxygenase during the activation of quiescent infection (Karni, Prusky, Kobiler, & Kobiler, 1989) and slowing the rate of decline of antifungal 1-acetoxy-2-hydroxy-4-oxo-henei cosa-12,15 diene compound (Ardi, Kobiler, Keen, & Prusky, 1998).

The concentrations of 10 and 100 μmol $l^{-1}$of MeJA and MeSA were used in majority of the studies reviewed by Glowacz and Rees (2016), e.g. loquat treated with MeJA at 10 μmol l^{-1} (Cao, Zheng, Wang, Jin, & Rui, 2009; Cao, Zheng, Yang, Tang, Jin, Wang, et al., 2008), mangos treated with MeJA at 10 μmol l^{-1} and 100 μmol l^{-1} (Gonzalez-Aguilar, Buta, & Wang, 2001) or MeSA at 100 μmol l^{-1} (Han, Tian, Meng, & Ding, 2006), papaya treated with MeJA at 10 and 100 μmol l^{-1} (Gonzalez-Aguilar, Buta, & Wang, 2003), peaches treated with MeJA at 100 μmol l^{-1} (Meng, Han, Wang, & Tian, 2009), pears treated with MeJA at 100 μmol l^{-1} (Zhang et al., 2009), pomegranates treated with MeJA or MeSA at 10 and 100 μmol l^{-1} (Sayyari et al., 2011), and tomatoes treated with MeJA or MeSA at 10 and 100 μmol l^{-1} (Ding, Wang, Gross, & Smith, 2002).

Thus, the objective of this study was to investigate the effect of methyl jasmonate (MeJA) and methyl salicylate (MeSA) vapours exposure at two concentrations of 10 and 100 μmol l^{-1} on i) disease incidence ii) epicatechin content, and iii) activity of defence related enzymes (chitinase, β-1,3-glucanase, PAL) in naturally and artificially infected 'Hass' avocado fruit kept at 2 °C for 14 d, followed by 6–7 d shelf-life at 20 °C.

2. Materials and methods

2.1. Plant material and handling

Freshly harvested, unblemished late season 'Hass' avocado fruit were obtained from Koeltehof Packers (Nelspruit, Mpumalanga province, South Africa) at commercial maturity (28–30% DM). Fruit were organised into the following treatment: i) untreated control – fruit that were transported to the laboratory and then left untreated; ii) dipped for 5 min in 0.05% prochloraz® – fruit were treated at the pack house, i.e. the commercial treatment, prior to being transported to the lab; iii) fruit that were transported to the laboratory and then exposed to methyl jasmonate (MeJA) or methyl salicylate (MeSA) vapours at two concentrations of 10 and 100 μmol l^{-1} for 24 h at 20.0 ± 0.5 °C.

After placing the fruit in a 10 l air-tight container, the appropriate volume of MeJA or MeSA to reach the desired concentration of 10 and 100 μmol l^{-1}, respectively was deposited on the Petri dish at the bottom of the container (Gimenez et al., 2016), using the system set up previously designed for the thyme oil fumigation (Bill, Sivakumar, Beukes, & Korsten, 2016). The container was immediately hermetically-sealed and solutions were left to evaporate over the 24 h period. Control and prochloraz® treated fruit were also kept in similar sealed containers. Thereafter half of the fruit were wounded and inoculated at the equatorial region with 20 μl of *C. gloeosporioides* spore suspension (10^5 spores ml^{-1}) as previously described (Bill et al., 2016). Both naturally and artificially infected 'Hass' avocado fruit were subsequently kept at 2.0 ± 0.2 °C for 14 d followed by 6–7 d shelf-life at 20.0 ± 0.5 °C, RH 70%.

2.2. Pathogen

Colletotrichum gloeosporioides was obtained from the Fruit and Vegetables Technology Laboratories, Tshwane University of Technology, South Africa. The *C. gloeosporioides* isolate was cultured and maintained on potato dextrose agar (PDA) (Merck, Johannesburg, South Africa) and incubated at 25 °C for 12–13 d. Spore suspension was prepared following a method of Bill et al. (2016). The mycelia fragments were removed from the suspension by filtering through three layers of muslin cloth. Spores count was determined using a haemocytometer and adjusted to 10^5 spores ml^{-1}. Fruit were prepared for artificial infection by disinfecting the place of inoculation with 70% ethanol (left to dry for ~30 min). The inoculation was then performed by uniformly wounding the fruit with a sterile needle (1 mm × 1 mm) and transferring 20 μl of spore suspension (10^5 spores ml^{-1}).

2.3. Disease incidence

At the 'ripe and ready to eat' stage (firmness near to 6.7 N, which has been defined by Arpaia, Collin, Sievert, and Obenland (2015) as the optimal eating firmness) fruit were assessed for signs of rotting (anthracnose), by giving them a score of 0 or 1 – no/signs of rotting, respectively. In case of stem-end rot being noticed in naturally infected fruit, the note of it was taken. Disease incidence was expressed as the proportion (%) of fruit showing signs of rotting out of the total number of fruit in each treatment.

2.4. Physical properties of the fruit

Firmness was determined along the equator of the fruit using a Chatillon Penetrometer, Model DFM50 (Ametek, Largo, Florida, USA) with an 8 mm diameter flat-head stainless steel cylindrical probe (Mpho, Sivakumar, Sellamuthu, & Bautista-Banos, 2013) to ensure that only ripe fruit are being assessed.

2.5. Biochemical analysis

2.5.1. Epicatechin content

Epicatechin was determined following the method used by Guetsky et al. (2005), with some modifications. Freeze-dried samples (20 mg) were homogenised in methanol: water (1:1; v/v) solution. Thereafter, the Eppendorf tubes were centrifuged for 10 min at 14,000×*g*. The supernatant was transferred to the new tubes, and subsequently filtered through a 0.45 μm membrane (Nylon syringe filter, PerkinElmer™, China) prior to injection. The analyses were carried out using a Flexar™ HPLC system (PerkinElmer, USA) consisting of a Flexar Isocratic LC Pump Platform and a variable wavelength Flexar UV/ViS LC detector. Separation was done on an Analytical C18 column (100 × 4.6 mm; 5 μm) at 25 °C, using water/methanol (25:75 v/v) as a mobile phase, with a flow rate of 1 ml min^{-1}. Chromatographic peak of the epicatechin