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Effect of biocontrol agent *Bacillus amyloliquefaciens* and 1-methyl cyclopropene on the control of postharvest diseases and maintenance of fruit quality

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ABSTRACT

Efficacy of biocontrol agent *Bacillus amyloliquefaciens* PPCB004 was evaluated on the control of anthracnose and phomopsis rot in 'Solo' papaya pre-treated with 1-methyl cyclopropene (100 μl) (1-MCP) during storage. This treatment was compared to the untreated control, commercial treatment (washing in chlorinated water), stand alone 1-MCP and PPCB004 treatment. Although fruit pre-treated with 1-MCP delayed the ripening (100% yellow) after cold storage by 9–10 d, it showed higher incidence and severity of anthracnose and phomopsis rot than the fruit subjected to commercial treatment. Application of PPCB004 after 1-MCP pre-treatment (1-MCP + PPCB004) reduced the anthracnose and phomopsis incidence and severity after cold storage (10 °C, 85% RH for 14 d) and ripening at 25 °C. The 1-MCP + PPCB004 treatment helped to retain the fruit firmness, overall quality and uniform yellow skin (100%) and flesh colour after ripening. The PPCB004 was effectively recovered from stand alone PPCB004 and 1-MCP + PPCB004 treated fruit after cold storage and ripening. The PPCB004 population showed an increase by 1 log units after ripening in 1-MCP + PPCB004 treated fruit. After ripening the recovery of PPCB004 population was higher (0.7 log units) in 1-MCP + PPCB004. The total recovery of fungal population on the fruit surface after ripening was lower in 1-MCP + PPCB004 and stand alone PPCB004 treated fruit. It can be concluded that application of *B. amyloliquefaciens* PPCB004 with 1-MCP pre-treated papaya (at 25–30% skin yellow stage) can significantly reduce disease incidence associated with 1-MCP treatment. This treatment has the potential for commercial application in the 'organic' papaya industry.

1. Introduction

Papaya (*Carica papaya* L.) is a popular desert fruit cultivated in the tropical and subtropical regions of the world. Although this exotic fruit is favoured on the local markets due to its excellent taste and well known health benefits and nutritional value, it has not emerged as a major traded fresh fruit. This is mainly due to rapid flesh softening after harvest resulting in reduced shelf life, thin skin that can easily be damaged during harvesting and handling often coupled with high incidence of postharvest decay. Postharvest diseases were identified as the major cause of quality loss in the supply chain. Anthracnose, caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., is identified as a major postharvest disease in the tropics affecting many fruit crops (Snowdon, 1990). Postharvest diseases caused by classic wound pathogens such as *Phomopsis* Petr. & Cif and *Rhizipous stolonifer* (Ehrenb.Fr.) Vuill. (=*R. nigricans* Ehrenb.) are also considered important postharvest pathogens particularly during poor field and facility sanitation and improper harvesting and handling practices.

Postharvest application of prochloraz or propiconaziole (Sepiah, 1993) or combination of fungicides and hot water treatments (Couey and Farias, 1979) were recommended to control postharvest diseases on papaya. However, hot water dip treatment was reported to affect fruit ripening (Paull, 1990) and is often difficult to manage properly in commercial settings. Due to global concern over the often indiscriminate use of pesticides and its hazardous side effects on nature and human health, more stringed product registration requirements have been developed. Due to perceived low profit margins by major agricultural chemical companies, re-registration of existing pesticides for small niche crops has not been considered a priority. The lack of strategic development of new chemical products for exotic fruits does not favor future growth in this market segment. On the other hand, due to the emergence of fungicide resistant strains, postharvest fungicide application is

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often not considered a long term solution for the industry. Therefore, the search for natural environmental friendly alternative products and processes becomes important for the exotic fruit industry.

Physiologically papaya is a climacteric fruit and during ripening it shows a climacteric rise in respiration and ethylene production. The application of ethylene inhibitor 1-methyl cyclopropene (1-MCP) has been adopted for some climacteric fruit including papaya (Hofman et al., 2001; Ergun and Huber, 2004) to prevent non-homogenous ripening due to exogenous ethylene exposure or rapid fruit softening because of poor postharvest handling practices. According to Manenoi et al. (2007), disease development was delayed and -severity reduced with 1-MCP treated fruit than in non-treated (control fruit) 'Solo' papaya. Fruit treated with 1-MCP at more than 25% ripe stage also showed a delay in fruit softening and may have commercial utility. However, according to Hofman et al. (2001), although the 1-MCP treatment delayed ripening in fruit harvested at commercial maturity, the incidence of post-harvest diseases, anthracnose, black rot (*Phoma caricae-papayae* (Tarr) Punith), black spot (*Asperisporium caricae* (Speg.) Maubl) and stem black rots (*Lasiodiplodia theobromae* Syn. *Botryodiplodia theobromae* Patouillard), were observed to increase after ripening. Therefore, to ensure even ripening, extend shelf life and reduce decay, 1-MCP could provide a commercial solution given it does not increase disease incidence. In order to assess the effect of 1-MCP treatment during prolonged cold storage (14 d at 10 °C) and after ripening at 25 °C under simulated marketing conditions, an additional protectant should be included to prevent 1-MCP disease-associated development.

The biocontrol agent, *Bacillus amyloliquefaciens* PPCB004 was selected as a potential antagonist to control *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* on peach fruit (Arrebola et al., 2010a). The HPLC data of PPCB004 indicated the lipopeptides iturin A, fengycin and surfactin as secondary metabolites (Arrebola et al., 2010a). The GC/MS analysis of PPCB004 showed 3-hydroxy-2-butanone as dominant compound (Arrebola et al., 2010b). The objective of this study was therefore, to determine the effect of *B. amyloliquefaciens* PPCB004 application on papaya pre-treated with 1-MCP on decay control and quality retention of 'Solo' papaya after cold storage (for 14 d, 10 °C and 80% RH) and after ripening at 25 °C market simulated conditions.

2. Materials and methods

2.1. Pathogen inoculum

Phomopsis caricae-papayae and *C. gloeosporioides* were isolated from symptomatic papaya fruit. Purified cultures were maintained on Potato Dextrose Agar (PDA, Merck, Johannesburg, South Africa) slants at 25 °C. Spore suspensions were prepared by removing the spores from the sporulating edges of the culture with a sterile glass rod by adding 5 ml of sterile deionised water with 0.02% of Tween 80 (Merck) for better spore separation. Spore suspensions were filtered through sterile double-layered cheesecloth and the spore concentration was determined using a haemocytometer and diluted to obtain a final concentration of 10^5 spores ml^{-1} for each fungus according to Arrebola et al. (2010b).

2.2. Biocontrol agent

B. amyloliquefaciens PPCB004 isolated from the surface of citrus 'Valencia' was used in this study. Molecular identification of *B. amyloliquefaciens* PPCB004 was confirmed according to Arrebola et al. (2010a). The biocontrol PPCB004 formulation of PPCB004 was prepared for this trial by Stimuplant cc., Pretoria, South Africa. The formulation was mixed with water (1:6 v/v) to obtain a final concentration of 10^9 cfu ml^{-1} according to the manufacture's instruction.

2.3. Effect of 1-MCP treatment and B. amyloliquefaciens PPCB004 on disease development in vivo

Freshly harvested 'Solo' Papaya fruits at 25–30% yellow (maturity stage) and uniform fruit size, free from decay and defects were collected from the Rodney Copper's packhouse, Tzaneen, South Africa. Prior to inoculation trials, the fruits were disinfected with 70% ethanol spray. The fruits for inoculation trial were subjected to following treatments; 1-MCP treatment, 1-MCP + PPCB004, PPCB004 alone, and commercial treatment (200 μl l^{-1} NaOCl) for each test pathogen (*C. gloeosporioides* or *Phomopsis*) separately. Each treatment with respect to specific pathogen had 10 replicate fruits. A set of 40 fruits was placed in an air tight chamber for 100 μl 1-MCP treatment (SmartFresh™ powder, active ingredient (0.14%), Rohm and Hass, South Africa) for 24 h at 20 °C. After 1-MCP treatment, fruits were air equilibrated for 6 h at 25 °C prior to inoculation or further treatments. 1-MCP treated (40) or non-treated fruit (40) was wounded with No 2 Cork borer (1.2 cm diameter) on the fruit surface. The wound was inoculated with 100 μl of spore suspension (10^5 spores ml^{-1}) of the test pathogen separately and incubated for 24 h at 25 °C to initiate infection. Inoculated wounds in 1-MCP treated fruits (40) and non-treated fruits (40) were treated with 1 ml PPCB004 formulation (10^9 cfu ml^{-1}). After treatment fruits were held at 25 °C. A set 10 wound inoculated fruits for each pathogen without any treatments served as control. Disease incidence was recorded and disease severity was evaluated by measuring the lesion diameter (mm) after 5 d at 25 °C and the experiment was repeated twice.

2.4. Effect of 1-MCP treatment and B. amyloliquefaciens PPCB004 on control of anthracnose and phomopsis rot in naturally infected fruit

A set of fruits at 25–30% yellow was subjected to the following four postharvest treatments; 1-MCP (100 μl) treatment, 1-MCP + PPCB004 [fruits were initially subjected to 1-MCP (100 μl) treatment for 24 h at 25 °C, equilibrated in air for 8 h and thereafter, subjected to dip treatment in PPCB004 (1:7 v/v, 10^9 cfu ml^{-1})]; PPCB004 alone; commercial treatment (fruits washed in chlorinated water sodium hypochlorite, 250 μl ml^{-1}) and untreated fruits (control). At completion of these treatments, fruits were packed and stored for 14 d at 10 °C and at 80% RH. After cold storage, fruits were allowed to ripen at 25 °C. Each treatment had five replicate boxes and each box had five fruits per box. The experiment was repeated twice in order to confirm the observations.

Incidence of anthracnose or phomopsis rot was recorded as the ratio of fruits showing disease development against the total number of fruits treated. Disease severity was determined by measuring the lesion diameter in mm. For each treatment number of days to reach 100% yellow skin and for initial occurrence of disease was assessed.

2.5. Recovery of B. amyloliquefaciens PPCB004 from naturally infected fruit after cold storage and ripening

Three fruits were randomly selected from each treatment after cold storage and after ripening at 25 °C, and washed in 500 ml quarter strength Ringer's solution (Merck) in an ultrasonic bath (Ultrasonic Manufacturing Company (Pty) Ltd., Johannesburg) at 25 °C for 30 s (Govender et al., 2005). The surface washing was filtered through a 0.22 μm filter in a vacuum assembly. The filters