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Environmentally friendly approach to postharvest quality maintenance of mango (*Mangifera indica* L.) cv. 'Tommy Atkins' & 'Kent'

By

Francois Johannes van Deventer

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DECLARATION

I, Francois Johannes van Deventer, declare that the dissertation, which I hereby submit for the degree Magister Scientiae Agriculturae (Plant Pathology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Postharvest decay control and fruit quality maintenance of mango, cv's 'Tommy Atkins' and 'Kent'.

Supervisor: **Prof. L Korsten**
Co-supervisor: **Prof. D Sivakumar**

The mango (*Mangifera indica* L.) is an appealing subtropical fresh fruit with a pleasant flavor and taste, high nutritional value, beneficial medicinal properties and various processing options. However, as is the case with most subtropical fruit, it is a sensitive commodity, prone to losses postharvestly. The South African mango industry is highly dependent on a hot water and cold prochloraz dip treatment, to control postharvest anthracnose and soft brown rot on fruit destined for export. However, negatve public perceptions of synthetic fungicides and its use on fresh produce for disease control has been increasing in major export markets such as the European Union. This growing concern from a public point of view is forcing industry to consider more environmentally acceptable methods to maintain quality of mangoes during extended export periods. 'Tommy Atkins' and 'Kent' mangoes either uninoculated or artificially inoculated with *Colletotrichum gloesporioiedes*, *Botryosphaeria parva* or sterile agar, were used to evaluate softer, greener alternatives, in this study. Fruit were subjected to either a hot, *Bacillus amyloliquefaciens* (PPCB004) containing dip treatment for two minutes or a 24 hour 1-methylcyclopropene (1-MCP) gas treatment at 16 ºC or no treatment. Fruit were then stored at 10 ºC under either 5% O_2 and 5% CO_2 (CA-1) or 3% O_2 and 8% CO_2 (CA-2) controlled atmospheres (CA) for 18 days and allowed to ripen for five days at 25 ºC. Similarly, uninoculated or artificially inoculated fruit subjected to *B. amyloliquefaciens*, 1-MCP or a combination of the two treatments was stored at 10 ºC for 18 days under conventional storage. 'Tommy Atkins' fruit were packed into bags made from four different film types, untreated or after being subjected to a cold *B. amyloliquefaciens* dip treatment and stored for 23 days at 10 ºC. Overall, 'Kent' fruit were more susceptible to anthracnose and SBR after artificial inoculation. *In vivo* inoculated 'Tommy Atkins' fruit, storage under CA-1 gave the best control of soft brown rot whilst CA-2 storage gave the best control of anthracnose. For quality retention no definite conclusion could be made for both cultivars after CA storage or the combination of 1-MCP pre-treatment and CA storage. The combination of 1-MCP pre-treatment and *B.*

amyloliquefaciens maintained the quality of 'Kent' mangoes under conventional storage the best. Anthracnose severity on both cultivars was reduced with 1-MCP treated fruit combined with the biocontrol pre-treatment. Modified atmosphere packaging in this study was found to be ineffective in maintaining quality of mangoes.

CHAPTER 1

General Introduction

The mango, *Mangifera indica* L. is sometimes referred to as the 'king of fruit'. It's popularity can be attributed to its excellent exotic flavour, attractive fragrance, beautiful colour, taste and nutritional properties (Arauz, 2000). According to Lebrun *et al.* (2008), there are 49 species and thousands of mango cultivars. Some cultivars, such as those from the Indian and the Sri Lankan regions, show strong aroma, intense peel colouration, delicious taste, and have a high nutritional value (Thanaraj *et al.*, 2009). In addition, mangoes are a good source of ascorbic acid, carotenoids and phenolic compounds, and other dietary antioxidants (bioactive compounds) (Talcott *et al.*, 2005). Consumption of mangoes can provide significant amounts of bioactive compounds with antioxidant activity.

Mango belongs to the order Sapindales in the family *Anacardiaceae.* According to Mukherjee (1971), mangoes originate from the Indo-Burmese region from where it was introduced to the rest of the world. The fruit is popular and sometimes considered exotic and of economic importance. It is widely cultivated in the tropical and subtropical regions of the world (Subramanyam *et al.*, 1975). Today, the fruit is mostly eaten fresh and also used in several other by-products, including juices, nectars and purees (Ploetz *et al.*, 1994).

Commercial mango production is reported in more than 87 countries. The prominent mango producing countries are India, China, Thailand, Indonesia, Philippines, Pakistan and Mexico (Tharanathan *et al.*, 2006). India is currently the worlds leading country in terms of production volumes (Food and Agriculture Oragnization statistics, 2008). However, plantings of mangoes outside the traditional geographical regions are increasing. This is mostly due to the increasing global importance of the fruit (Tharanathan *et al.*, 2006). In the Republic of South Africa, the fibreless Florida cultivars (Tommy Atkins, Kent and Keitt) are mainly grown for export purposes and are harvested from December to April. Although the initial focus of the industry was on export (up to 15% of fresh fruit), industry challenges forced a shift. Only 3% of fruit was exported in 2008. These changes in export volumes can be mainly attributed to fluctuating national and international market trends, a stronger Rand, land reform challenges, good quality mangoes from competitor countries, poor quality fruit, socio-economic challenges and climate.

However, postharvest losses due to *Botryosphaeria* spp. and *Colletotrichum gloeosporioides* ((Penz.) Penz. And Sacc. In Penz.) (causing soft brown rot and anthracnose respectively) remain one of the biggest concerns for producers (Fivaz, 2009). Mango postharvest losses are primarily due to harvesting fruit at improper maturity, mechanical damage caused during harvesting or improper field handling, sap burn, spongy tissue, lenticels discolouration, fruit softening, decay, chilling injury, and pest damage, among others (Yahia, 1998). During export fruit losses can vary dramatically depending on initial fruit quality and export conditions, in terms of temperature control, handling and hygiene, which can affect rates of decay and/or physiological breakdown.

Generally, control of postharvest disease in mango is achieved by adopting proper pre- and postharvest management practices such as strict orchard hygiene management, application of fungicides and temperature management during storage and shipping. In South Africa, only prochloraz is registered as a postharvest fungicide treatment on mangoes that can be applied in the packhouse. This treatment is done in combination with a hot water dip on fruit (Nel *et al.*, 2003). The maximum residue limit (MRL) for prochloraz on fruit exported to the European Union (EU) is currently set at 5 ppm (Fivaz, 2009). It is suggested by Swart *et al.* (2009) that prochloraz in combination with fludioxonil and hot water can be a viable alternative to the control of postharvest diseases on 'Kent' and 'Keitt' mangoes. Given this, more options are needed to control postharvest diseases and retain quality, especially technologies that are less dependant on 'hard chemicals' to ensure market access.

In the 1980's the use of agricultural chemicals were the major strategies developed to control postharvest diseases as well as using cold chain management systems. The use of biocontrol agents to control postharvest diseases of fruit and vegetables provided a suitable alternative and has since been extensively reviewed (Janisiewich and Korsten, 2002; Wisniewski *et al.*, 2007). Due to global concern over the often indiscriminate use of pesticides and its hazardous side effects on nature and human health, regulatory changes and more stringent product registration requirements were introduced. 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. A further lack in strategic development of new chemical products for exotic fruits threatens future growth in this market segment. Resistance to prochloraz in *Pseudocercosporella herpotrichoides* isolates has been reported by Cavelier *et al.* (1994). Considerable variability in sensitivity among the *C. gloeosporioides* isolates from mango was also reported by Arauz (2000). Prochloraz is the only fungicide used for postharvest disease control in mangoes. The search for natural

environmental friendly alternative products and processes therefore becomes important for small industries.

Necrotrophic wound-invading pathogens are susceptible to biocontrol and the application of an antagonist directly to wounds on fruit can reduce decay significantly. This allowed biocontrol to become an attractive alternative way to control postharvest decay of fruit (Janisiewicz and Korsten, 2002). *Bacillus licheniformis* (Govender *et al.* 2005), *Brevundimonas diminuta*, *Stenotrophomonas maltophilia* and *Candida membranifaciens* (Kefialew and Ayalew, 2008) are all antagonists that has previously shown to have potential to control one or both of the major postharvest pathogens of mango. *Bacillus amyloliquefaciens* PPCB004 showed potential as a biocontrol agent to control *Botrytis cinerea*, *Penicillium expansum* and *Rhizopus stolonifer* on peach fruit (Arrebola *et al*., 2010a). The HPLC data of this isolate (PPCB004) indicated the lipopeptides iturin A, fengycin and surfactin as secondary metabolites (Arrebola *et al.*, 2010b). The biocontrol agent *B. amyloliquefaciens* was isolated from the surface of citrus cv. Valencia and showed multiple modes of action in decay control. *Bacillus amyloliquefaciens* and other members of the *B. subtilis* group are considered safe and have "generally recognized as safe" (GRAS) status (Food and Drug Administration 1999). However, metabolites emitted by *B. amyloliquefaciens* that are toxic to human or other mammals have so far only been reported in connection with the commercial production of L-tryptophan. Therefore, a critical assessment of the safety of *B. amyloliquefaciens* strain used in this study on fresh fruit surface needs to be investigated prior to commercialization.

Postharvest disease control through maintenance of host resistance can be achieved by shipping or storing fruit after harvest in controlled atmosphere (CA) storage. Short storage life of mango fruit limits export distances. Shipping mangoes under modified atmosphere / controlled atmosphere (MA/CA) allows extending the storage life of fruit and therefore contributing towards increased shipping distances to reach further markets. The MA/CA with higher CO_2 and lower O_2 (than normal air atmosphere) can delay ripening by inhibiting the production of ethylene, slowing down the changes in skin and flesh colour, flavour, aroma and texture (fruit softening), retaining fruit firmness and thus promoting resistance to the attack of postharvest pathogens. According to Prusky and Keen (1993), the concentration of antifungal compounds present in immature mango fruit declines during ripening due to the oxidative process. A reduced O_2 concentration of around 3 - 5% and an elevated CO_2 concentration of 5 – 10% are the suggested atmosphere regimes for a successful MA/CA system for mango fruit (Yahia, 1998, Kader, 1994). Higher concentrations of CO_2 (above

10%) can prevent the incidence of postharvest diseases due to its fungistatic or toxic activity. However, CO_2 concentrations must not result in any quality defects in terms of off-flavour development. Further, certain CO_2 levels may cause injury such as skin discolouration or greyish flesh colour or fruit softening (Lalel *et al.*, 2003). Controlled atmosphere (3% O_2 and 10% CO_2) resulted in lower anthracnose incidence in 'Tommy Atkins' and after removal from cold storage to room temperature conditions, the residual effect of CA controlled anthracnose (Kim *et al.*, 2007).

The application of 1-methylcyclopropene (1-MCP) inhibits fruit ripening by irreversibly binding to the ethylene binding sites. Fruit softening in mangoes occurs as a result of degradation of protopectins and increased soluble pectins levels in the flesh. Application of 1-MCP was reported to inhibit fruit softening, delaying the climacteric peak, rate of respiration, weight loss and increased ascorbic acid content during mango fruit storage (Jiang and Joyce, 2000; Alves *et al.*, 2004). Further, 1- MCP concentrations between 1 and 100 µl l^{-1} were reported to be effective in extending the storage life of mangoes (Jiang and Joyce, 2000). These concentrations may however increase the incidence of stem-end rot during storage (Hofman *et al.*, 2001).

Postharvest treatments that extend storage life are important in developing countries where the cold chain infrastructure is not well established. Under those circumstances, application of 1-MCP may provide a suitable alternative to extend the postharvest life of mangoes at ambient temperature (25º C) for the domestic markets. According to Watkins and Miller (2005), cultivars that have lower ethylene production rates after harvest respond better than the cultivars that produce higher levels of ethylene, and the delays between harvest and 1-MCP application also affect the effectiveness of the treatment. Application of *B. amyloliquefaciens* PPCB004 in 1-MCP pre-treated 'Solo' papaya at 30 % yellowing (colour stage) significantly reduced the anthracnose severity (Osman *et al.*, 2010). Furthermore, according to Watkins and Nock (2003), the two technologies of 1-MCP application and CA storage are effective when used in combination. This shows that combinations of different alternative treatments can complement one another and could be integrated to enhance control and retain quality.

On this basis, the objective of this research is to retain the overall mango fruit quality and reduce postharvest losses due to decay and fruit softening of the South African export cultivars 'Tommy Atkins' and 'Kent' during export by evalauting suitable postharvest novel technologies such as:

- To determine the effect of *B. amyloliquefaciens* PPCB004 or 1-MCP under two different CA storage conditions.
- To determine the effect of *B. amyloliquefaciens* PPCB004 or 1-MCP with standard hot water treatment and wax application.
- To determine the effect of *B. amyloliquefaciens* PPCB004 and different modified atmosphere conditions.

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CHAPTER 2

Review of Literature

1. INTRODUCTION

Mango is a delicate and tasty subtropical fruit, popular on the international markets. It is mainly grown in the subtropical regions of the world and exported to developed countries. Postharvest diseases result in major losses on these markets and effective control measures are required to retain product quality, directly ensuring profitable production and trade. This literature review will primarily focus on postharvest disease and quality management, with trade, production and quality parameters as supportive background information.

1.1 Origen and description

The mango (*Mangifera indica* L.) is a dicotyledonous fruit belonging to the family Anacardiaceae that originated from the Indo-Burmese region (eastern India, Burma and Andaman Island) (Mukherjee, 1971). The fruit is a simple, large resinous drupe, that vary in shape (round, oval, ovoid-oblong) and colour (red, green, yellow, purple), depending on the variety. The fruit has a smooth leathery skin (exocarp) that surrounds the soft edible mesocarp and the stony endocarp. Harvest maturity indices used vary from physical (colour, shoulder growth, size, shape, specific gravity), to chemical (total soluble solids, titratable acidity, starch, phenolic compounds, carotenoids) parameters (Tharanathan *et al.*, 2006). Fruit harvested at an unripe stage have a lower total-soluble-solid (TSS) to acid ratio, and do not further develop in terms of colour and flavour. Fruit harvested at an over mature stage have a shorter storage life and often display poor quality attributes.

1.2 Global estimations for mango

Most of the world production of tropical fruit is done in developing countries, with the developed countries being the major importers. India is the biggest producer of mangoes

in the world contributing to around 50% of the global production (13.5 million tonnes produced) and also the biggest exporter. Other major producers include China, Thailand, Phillipines, Indonesia, Mexico, Pakistan, Nigeria, Bangladesh and Brazil, with Mexico, Brazil, Pakistan, Thailand and the Philippines recognised as major exporters as well (FAOstat 2010; Tharanathan *et al.,* 2006).

1.3 South African market

In South Africa, mainly fibreless Florida originating cultivars ('Tommy Atkins', 'Keitt', 'Kent' and 'Sensation') are grown in the subtropical regions of the Limpopo (Hoedspruit, Levubu and Tzaneen) and Mpumalanga (Kiepersol, Malelane and Nelspruit) provinces. The European markets have shifted preferences for cultivars 'Kent' and 'Keitt' and currently the volumes exported of cultivars 'Tommy Atkins' and 'Sensation' are decreasing (Fivaz, 2009).

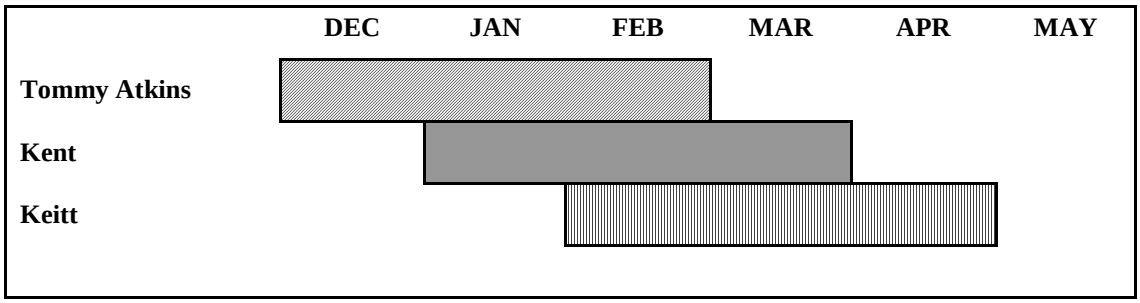

Fig 1: *The harvesting periods of the three major mango cultivars, grown in South Africa.*

South Africa contributes only 0.22% towards the world mango production (Fivaz, 2009). Over the past 20 years South African production figures increased from 25 000t to 80 000t but the ratio of local vs. export market changed drastically over the past six years. During the 2007/08 season, only 3% (1.3 million cartons) of the total production was exported, and 23% was distributed on the local market. This compared to the 2001 - 2003 seasons where approxamitely 4.25 million boxes (20% of the total production) were exported. The majority of fruit produced in the 2007/08 season were juiced (40%). During 1993 to 2002 seasom, on average, more than 15% of the total production was exported, with the 2003 season having 26% of the total produce exported (Theo Bekker, Subtrop; Perishable Products Export Control Board (PPECB) Export Directory 2007-2009).

The major factors contributing to the drastic changes in export volumes, can be ascribed to: national and international market trends, a stronger Rand making it not so profitable to export to the European countries (where price per kg is strongly correlated with size of fruit), good quality mangoes from Brazil, Peru and Ecuador that compete with South African fruit, land reform constraints, socio-economic situations and climate (Fivaz, 2009). The major destinations to which South African mangoes are exported include the Mid-East / Mediterranean (United Arab Emirates and Saudi Arabia), Central Europe (Belgium, Switzerland and the Netherlands), United Kingdom, Africa (Ghana), the Far – East and Asia (PPECB Export Directory 2007-2009; Mango market chain profile by the DAFF, 2009). During the early season (January) South African fruit competes with fruit from Peru, Brazil, Israel and Thailand and during the late season (March), with fruit from Puerto Rico, Venezuela, Costa Rica and other West African countries on these markets (Mango market chain profile by the DAFF, 2009).

1.4 Maturity indices for harvesting mangoes

Mango fruit attain physiological ripeness around 90 days after fruit set or 4-5 months after flowering. Traditionally, mangoes are harvested based on assessment of external and internal appearances of the fruit. When mangoes are harvested for shipping to distant markets or long-term storage, it is done at a mature green stage while still firm. Ripening will continue after harvest but the maturity level at harvest is very important for acceptable flavour development during the ripening process (Kader and Mitcham 2008). The selection of suitable maturity indices for optimum harvesting is very important windows, since the quality and the postharvest life of mango fruit depends on the maturity stage at harvest (Yahia, 1998). When fruit are harvested at an immature stage they tend to be more sensitive to chilling injury and may also fail to ripen adequately. On the other hand, if fruit are harvested at an over mature stage, they are more susceptible to mechanical damage (bruising, decay, water loss) resulting in quality deterioration (Yahia, 1998). Other disorders like jelly seed or jelly pulp are also more prone to develop postharvestly in fruit harvested at an over mature stage (Yahia, 1998).

Physical, physiological and chemical parameters are used to define the maturity stage. Physical methods to determine maturity in mango include specific gravity, external and internal colour, shoulder development and softness of cheeks (Kosiyachinda *et al.,* 1984). Soluble solids content (SSC) are generally not used as a commercial parameter for mango

maturity, but it complements other indicators such as flesh colour and shoulder formation, and for most markets, mangoes need to be harvested at about 9-10% SCC (Tharanathan *et al.*, 2006).

2. POSTHARVEST DECAY

Anthracnose (caused by *Colletotrichum gloeosporioides* ((Penz.) Penz. And Sacc. In Penz.)) and soft brown rot/stem-end rot (*Botryosphaeria* spp. and its anamorphs) are the predominant postharvest diseases that affect mango fruit quality postharvest. Anthracnose symptoms are expressed as dark, sunken lesions on ripe fruit with pink, slimy spore masses (Jeffries *et al.*, 1990). Stem-end rot develops as a dark rot from the stem-end as fruit ripens with soft brown rot exhibiting similar symptoms except from starting at the stem-end.

Incidence of postharvest decay occurs as a result of inoculum coming in contact with the fruit. The spores are dispersed in the air or by contact and when suitable conditions are available, germination takes place and infection occurs (Arauz, 2000). Suitable conditions include the presence of high relative humidity (RH), warm temperatures, and nutrients (mainly sugars) present on the fruit surface or in and around wounding sites.

According to Johnson (1994), stem-end rot are reported to cause significant losses in mango during transit and storage, especially in situations where anthracnose is effectively controlled. Anthracnose and stem–end rot pathogens infect during fruit development, harvesting or de-sapping procedures and continue to advance after harvest, causing significant losses in storage (Johnson, 1994). Postharvest disease control begins in the field and involves cultural and chemical practices as well as cultivar selection. Cultivars such as `Tommy Atkins` and `Keitt` are known to be less susceptible to anthracnose than `Irwin`, `Kent`, and `Edward` and `Haden` (Campbell, 1992; Arauz, 2000; Nelson, 2008).

Generally, control of postharvest disease in mango is achieved by adopting proper preharvest and postharvest management practices. These include strict orchard hygiene management, application of fungicides and temperature management during storage and shipping.

2.1 Anthracnose

2.1.1 Taxonomy

Fungi that produce conidia within black acervuli cause anthracnose. *Glomerella* species are true fungi under the phylum Ascomycota and classed under the Pyrenomycetes and placed in the order Phyllachorales. *Colletotrichum* species are in most cases the anamorphic stage of *Glomerella* and these are placed in the class Deuteromycetes or mitosporic fungi (imperfect or asexual fungi) (Agrios, 2005). Ploetz (2004) and Swart (2007) reviewed anthracnose in avocado caused by *C. gloeosporioides* (Penz.) Penz. and Sacc. in Penz.) with its teleomorph *Glomerella cingulata* (Stoneman) Spauld. et H. Schrenk. Variants of this fungus also cause anthracnose in mango. For example, in India and Australia, *C. acutatum* Simmonds (teleomorph*: G. acutatum*) and in Australia *C. gloeosporioides* var. *minor* also causes anthracnose, but none of these variants have been reported in South Africa on either mango or avocado.

2.1.2 Symptoms

Several *Colletotrichum* species cause anthracnose in a wide variety of crops. On annual plants it causes amongst others anthracnose of beans, cucurbits, tomato, onion, strawberry, cashew as well as cereals and grasses. It also causes rots on fruit such as mango pre- and postharvest anthracnose, citrus postbloom fruit drop (anthracnose), bitter rot of apple, and ripe rot of grape (Agrios, 2005).

2.1.3 Description

Anthracnose may cause problems postharvest and pre-harvestly (may cause blossom blight or foliage damage). The postharvest infection in most cases remains latent and lesions only become visible after harvest in storage, usually in the radiating tearstain patterns, originating from the stem-end. *Colletotrichum gloeosporioides* mainly infect through conidia that gets easily dispersed by rain (Fig 1) (Arauz, 2000).

The wax on the surface of the fruit induces appresorium formation of *C. gloeosporioides*. Mainly the longer fatty alcohol chains induce appresoria formation and a certain balance between these long chains and the absence of inhibitory factors will allow the

pathogen to germinate and infect the fruit. *Colletotrichum gloeosporioides* is unable to infect non-host plants that do not exhibit this balance between long chain fatty alcohols and inhibitory factors (Podila *et al.*, 1993).

It was found in a South African study by Sanders and Korsten (2003) that *C. gloeosporioides* isolates from mango (*Mangifera indica* L.) and avocado were able to cross infect and cause lesions on both commodities. It was also observed that the pathogen isolated from mango has the ability to cause lesions on strawberries, guavas, peppers and papayas, but the pathogen was unable to cause lesions in oranges. From the study it seemed that *C. gloeosporioides* isolates from avocado were more virulent than those isolated from mango. The method used for infection was plug inoculations where the pathogen was inoculated in the flesh of the respective fruit (Sanders and Korsten, 2003). This allowed the pathogen to infect fruit without having to breach the cuticle thus excluding pathogen/host recognition.

Freeman and Shabi (1996) had conducted similar experiments with cross inoculation of different fruit with *C. gloeosporioides* isolated from almond, apple, avocado, mango and pecan, and *C. acutatum* isolated from apple, peach and pecan. Both pathogens were able to cause lesions in all the fruit (almond, apple, avocado, mango and nectarine) inoculated, although different sizes of lesions formed with respect to isolates and host. These pathogens are more virulent on the crop originally isolated from. It remains unclear if cross infection potential exists under natural conditions in the field.

Isolates of *C. gloeosporioides* was found, are heterogenetic different from the different crops it was isolated from, implicating that the pathogen for each crop originated from a different population. The heterogeneity can also be observed in the different sizes of lesions produced after artificial inoculation (Freeman and Shabi, 1996). Sanders and Korsten (2003) observed the same trend between *C. gloeosporioides* isolates from mango and avocado respectively.

A key virulence factor for disease development by *C. gloeosporioides* is a pectate lyase it secretes. This enzyme only functions at a higher pH than 5.8. *Colletotrichum gloeosporioides, C. acutatum* and *C. coccodes*, when inoculated into a medium with a low pH will secrete ammonia and thus increase the pH of the medium. This then allows pectate lyase to function optimally. It was found that when apples that is normally attacked by *C. acutatum*, were inoculated with *C. gloeosporioides* in conjunction with ammonia releasing compounds, the virulence of *C. gloeosporioides* was increased to levels similar to that of *C. acutatum*, indicating the importance of ammonia to increase the environmental pH as a virulence factor (Prusky *et al.*, 2001). When the pelB gene is artificially disrupted, via

homologous recombination, in *C. gloeosporioides* and fruit artificially inoculated, a decrease in decay diameter can be observed in comparison to fruit inoculated with a wild type or an undisrupted sample (Yakoby *et al.*, 2001).

2.2 Soft brown rot / Stem-end rot

The most economically important postharvest mango pathogen in South Africa that is associated with fruit stem-end rots and soft brown rot was originally described as *Dothiorella dominicana* (Petrak and Cif.) (see taxonomic reclassification as described under *2.2.1*. It was frequently isolated from stem-end rot lesions in association with *C. gloeosporioides* (Penz.) from anthracnose lesions (Darvas, 1993). Four different *Botryosphaeria* spp. were identified from mango fruit in South Africa that were pathogenic to the fruit, tree or blossoms and all were able to cause soft brown rot in mango fruit. *Botryosphaeria parva* is the most common of these (Jacobs, 2002).

2.2.1 Taxonomy

Soft brown rot / Stem-end rot in mango is caused by and associated with anamorphs of *Botryosphaeria* including *Dothiorella dominicana* (Petrak and Cif.), *D. mangifera* H. et P. Syd et But (also classified as *Nattrassia mangiferae* (Nattrass) Sutton et Dyko*)* and *Lasiodiplodia theobromae* Pat et Griff. et Maubl (Peterson *et al.*, 1991). It was found by Jacobs (2002), that all four *Botryosphaeria* spp. occurring on mango in South Africa were pathogenic, with *B. parva* (previously known as *B. ribis*) (anamorph: *F. parvum* Pennycook and Samuels) being the most common, followed by *B. rhodina* (Pat.) Griff. Et Maubl (anamorph: *L. theobromae*), *F. indigoticum* Jacobs, Slippers and Whingf. and *F. bacilliforme* Jacobs, Slippers and Whingf.

Soft brown rot on mango traditionally known as caused by a complex of fungal pathogens, among others and collectively known as a *Dothiorella spp.* or *Botryosphaeria / Dothiorella* complex, where the *Botryosphaeria spp.* usually is the teleomorph of the *Dothiorella spp* (Johnson *et al.*, 1993). *Dothiorella aromatica* has been found along with anthracnose (*Glomerella cingulata* var. *minor*) also to be some of the most economically important postharvest diseases of avocados (Darvas and Kotze, 1979; Fitzell and Muirhead, 1983; Muirhead *et al.*, 1982; Sanders and Korsten, 1997). The taxonomical classification of some

of the *Botryosphaeria* anamorphs has been re-evaluated and the classification is as follows: *D. dominicana* as *F. parvum* Pennycook and Samuels (teleomorph: *B. parva*), *D. mangiferae* as *F. mangiferum* and *D. aromatica* as *F. aesculi* (teleomorph: *B. dothidea*) (Slippers *et al.*, 2005).

2.2.2 Description

Stem-end rot in mango symptoms develop from fungi that endophytically colonise the peduncle of the fruit. These endophytic myceliums are not easily eradicated by applying fungicides externally. This explains why it is more difficult to control stem-end rot than anthracnose postharvest. In this particular study it was also found that the source of inoculum was not airborne (Johnson *et al.*, 1993). A study conducted in the Letsitele valley (Limpopo province, South Africa) indicated that the amount of endophytic infection accounts only for a minor percentage of the postharvest infection. Most of the postharvest infection is caused by inoculum that is washed, primarily by rain, from other affected areas on the tree. This implicates that well managed orchard sanitation and effective pre-harvest fungicide treatments can significantly control the disease at a postharvest stage in combination with postharvest treatments (Saaiman, 1997).

3. CONTROL OF POSTHARVEST DISEASE

Various postharvest treatments to control postharvest diseases, especially soft brown rot and anthracnose have been researched and evaluated. However, those that are successful are mainly based on some form of hot water or vapour treatment technology, usually in combination with synthetic fungicides. Good pre-harvest practices remain a crucial factor to consider when controlling postharvest diseases.

3.1 Prochloraz

Prochloraz is a non-systematic, imidazole-type compound that has a very high activity against fungi from the Ascomycetes and the Fungi Imperfecti. The mode of action of prochloraz has been described as the inhibition of fatty acid ergesterol synthesis, which is

vital to the structural formation of fungal cell walls, thus preventing mycelial growth of fungi (Danderson, 1986).

The potential of prochloraz as a postharvest disease control treatment were evident and today prochloraz is commonly used as a postharvest treatment to control diseases postharvestly on a wide variety of fruits. It was found that prochloraz applied as an ultra-low volume spray control postharvest rots the best in combination with wax (Everett and Korsten, 1996). Arauz (2000) reported that for sensitivity towards prochloraz, there exist a considerable variability among the *C. gloeosporioides* isolates from mango. However, Kuo (2000) reported that none of the *C. gloeosporioides* isolates they isolated, from 43 different orchards in Taiwan where prochloraz was frequently used as a pre-harvest to control anthracnose, exhibited any resistance towards the fungicide. Sanders *et al.* (2000), reported that only one highly resistant strain of *C. gloeosporioides* were found in their three year survey of isolates collected from the South African production areas. Although no other resistant strains were found, it was suggested that prochloraz resitance among *C. gloeosporioides* strains are closely monitored. Widespread resistance is however, unlikely, since prochloraz is mostly only used as a postharvest application in South Africa. Since prochloraz is the only fungicide registered to be used in South African packhouses to control postharvest diseases of mangoes, resistance of pathogens against this fungicide have potential to result in a problem for producers and exporters alike. Research by Grünfeld and Bonefeld-Jorgensen (2004), suggested that prochloraz might pose a health risk to mammals and this might have an impact on future use of prochloraz as a safe fungicide used on fresh produce.

3.2 Heat treatments to control postharvest mango diseases

Hot water treatment (HWT) as a decay control measure is practiced commercially in various countries due to its efficacy (Jacobi *et al.*, 1995; Anwar and Malik, 2007). Bagged mangoes before harvest and a 10 min HWT (52-55°C) was reported to reduce the anthracnose infection by 83% and stem-end rot by 100% (Aveno and Orden, 2004). Stage of maturity, size and weight of fruit, cultivar and growing conditions are all factors that influence the efficacy of HWT and should therefore be considered when determining the temperature and duration of the treatment (Jacobi *et al.*, 2001; Anwar and Malik, 2007). It is further advised to subject mangoes to a HWT within 24 hours after picking (Aveno and Orden, 2004).

The advantages of HWT technology, except for being an environmentally friendly method, include that it can easily be implemented into the supply chain and can be practiced by any size farming enterprise with success (Aveno and Orden, 2004). *Colletotrichum gloeosporioides* can be controlled by dipping fruit in hot water (55 °C for 5 min) or by vapor heat treatment (VHT) (46.5 °C for 10min) or combining the treatments. The effect of the heat treatment becomes evident on mango as soon as the fruit begin to ripens, when compared to untreated fruit (Sopee and Sangchote, 2005). Mansour *et al.* (2006), showed that a combination of a hot air treatment (40 °C for 4 hours) followed by a hot water (50° for 5min) dip treatment, controlled *Alternaria alternate* (Fr.) Keissl., *Botryodiplodia theobromae* Pat. and *Botrytis cinerea* (De Bary) Whetzel in 'Tommy Atkins', 'Kent' and 'Keitt' mangoes without injury caused to fruit after the prolonged heat treatment.

It has been reported that stem-end rot is not readily controlled by hot treatments but Coates *et al.*, (1996) suggested that certain species of the different pathogens that cause stem-end rot are more resistant to vapor heat treatments than others. They showed that *D. dominicana* are more sensitive to a 47.5 °C (core temperature of fruit kept at 46.5 °C for ten minutes) vapor heat treatment than *L. theobromae.* Hot water technology was reported to maintain fruit quality and therefore the product can be sold at higher prices especially at the export markets. This technology requires minimal labour and it is cost effective to implement, can be implemented on the farm, or in packing sheds and it is cost effective if the water used is recycled.

3.3 Heat treatments in combination with synthetic fungicides to control postharvest mango diseases

The combination of imazalil and hot water (53 ºC) has been shown to be effective in controlling anthracnose at a postharvest level. However, this treatment is not effective in controlling stem-end rot in cultivars 'Kent', 'Tommy Atkins' and 'Palmer' (McGuire and Campbell, 1993). Although in 'Kensington Pride' mangoes the stand –alone hot water (49 ºC for 30-40 min to obtain a core temperature of 47 ºC for 10 min) treatment was effective in controlling anthracnose. However, this treatment could not control stem-end rot and caused heat damage. A hot benomyl dip (52 ºC) for five minutes showed effective control of both stem-end rot and anthracnose (Ploetz, 2004, Fivaz, 2009). However, this treatment is not allowed in many production regions, including South Africa. A hot water dip (49 ºC for 30-40 min) followed by an ambient prochloraz treatment showed near total control of

anthracnose (Rappel *et al.*, 1991). A combination treatment of hot benomyl (52 ºC for five min) followed by an ambient prochloraz dip (30 s) gave total control of anthracnose and reduced stem-end rot incidence and severity (Rappel *et al.*, 1991).

Similarly stem-end rot and anthracnose control was achieved with 'Kesar' mangoes after a hot Bavistan/Captan dip (52ºC for 10 min), but stand-alone hot water treatment, failed to show complete control of both *Botryosphaeria* and *Colletotrichum* associated diseases. This treatment also increased the shelf life of the fruit (Waskar, 2005). Treatment with hot water brushing and prochloraz at 55 ºC and waxing with 2, 4-dichlorophenoxyacetic acid (2, 4-D) incorporated into the polyethylene emulsion wax, showed a reduction in stem-end rot incidence (Kobiler *et al.*, 2001). Dipping 'Zill' and 'Kent' mangoes in a 50 ºC, prochloraz containing (180ml Omega / 100l water) water bath for 5 min also significantly reduced the incidence of anthracnose.

Esguerra *et al.* (2004), found contradictory results on 'Carabao' mangoes treated with hot water. They used a hot water dip (53 ºC) for 10 min or a 20-35 s. dip in 60 ºC hot water or hot water brushing (60 ºC for 20-35 s) on fruit and afterwards exposing the mangoes to a vapour heat treatment (46 ºC for 10 min). This controlled the incidence of stem-end rot and anthracnose on fruit to an acceptable level (Esguerra *et al.*, 2004).

3.4 Biological control

Originally Plant Pathologists adopted the entomological definition of biocontrol in the sense that "*one organism is controlled by another organism*" (Wisniewski *et al*., 2007; Droby *et al*., 2009). That definition has since been adapted to the "*control of a plant disease by a biological process or the product of a biological process*", because a plant disease is not an organism but rather a process (Wisniewski *et al*., 2007; Droby *et al*., 2009). With controlled atmosphere, refrigeration, heat, chemicals and irradiation as the major strategies developed to control postharvest diseases of commodities, the use of biocontrol agents to control postharvest diseases of fruits and vegetables began to gain attention from researchers in the early 1980's. Chemical control, with the exception of cooling, was the only method widely used to control postharvest diseases. However, resistance of pathogens against these chemicals fuelled the need to develop an alternative and successful control method. These lead researchers to focus on the field of biological control and the development of second generation biocontrol products (Wilson and Pusey, 1985; Wisniewski *et al*., 2007).

3.4.1 Biocontrol agents

Janisiewicz and Korsten (2002) reported that necrotrophic wound-invading pathogens are sensitive to biocontrol and that application of the antagonist directly to wounds on fruit reduces decay significantly. This allowed biocontrol to become an attractive alternative way to control postharvest decays in fruit. Biocontrol has limiting factors and these can be changed by amongst others postharvest environmental manipulation, integration with other control measures and enhancement of the biological control organism. Biological control using microbial antagonist could be used on its own or in combination with a reduced concentration of synthetic fungicides (El Ghaouth *et al.*, 2002).

The use of reduced-pathogenicity mutants of *C. gloeosporioides* as biocontrol agents to control the same pathogen are able to reduce symptom development, primarily because it induces the defence systems by increasing antifungal diene levels in fruit (Yakoby *et al.*, 2000). A wide range of *B. subtilis* strains have been identified that have potential antimicrobial properties. These can be exploited to develop biocontrol products or were developed into commercial biocontrol products (Pruvost and Luisetti, 1991; Kilian *et al.*, 2000; Marrone, 2002; Schisler *et al.*, 2004; Demoz and Korsten, 2006).

The potential of *Bacillus amyloliquefaciens* GA1 as a potentially good biocontrol agent was noted after it was found that it synthesise a wide range of antibiotic compounds like surfactin, iturin A, fengycin A and fengycin B (Arguelles-Arias *et al.*, 2009). The use of *B. amyloliquefaciens* PPCB004 in combination with lemon grass oil (applied through a pad delivery system) and modified atmosphere packaging on peaches, showed absence of disease after cold storage as well as retaining overall quality of fruit (Arrebola *et al.*, 2009). It was found that the iturin lipopeptides family, specifically iturin A, are the major metabolites responsible for the antagonistic properties of *B. amyloliquefaciens* PPCB004 (Arrebola *et al.*, 2010). The antagonistic effect against specific pathogenic *Penicillium* spp. was shown by inhibition of mycelial growth *(in vitro)* and reduced *in vivo* incidence and severity on citrus (Arrebola *et al.*, 2010).

In vitro studies with the endophytic fungus, *Muscodor albus*, showed that it exhibits a wide spectrum of antimicrobial activity when applied collectively to various pathogenic microbes (human and plant). It produces a wide range of volatile organic compounds (esters, ketones, alcohols, acids, lipids and naphtalene) that act synergistically in killing various bacteria and fungi (Strobel *et al.*, 2001; Ezra *et al.*, 2004). Another fungal genus,

Trichoderma, contains various strains showing potential as biocontrol agents (Wilson and Pusey, 1985; Howell, 2003).

Various biocontrol agents have been registered for commercial use on a variety of crops (Table 1.). A strain of *Pantoea agglomerans* was found to effectively control the bacterial disease fire-blight in apples and pears caused by *Erwinia amylovora* (Elmer *et al.*, 2005). This antagonist is commercially registered in New Zealand as BlossomBless® and PomaVita®. *Ulocladium oudemansii* (Simmons.) is a saprophytic fungi also registered in New Zealand as BOTRY-Zen®, to control *Botrytis cinerea* (Pers.:Fr.), the causal agent of botrytis or grey mould, on grapes (Elmer *et al.*, 2005). Another antagonist that has been registered in 1995 for commercial use in the United States of America and Israel is the yeast *Candida oleophila* Montrocher (strain I-182) (Ecogen Corporation, Langhorne, PA), commercially available as Aspire®. Aspire® is used postharvestly against green and blue mould, as well as sour rot of citrus (Droby *et al.*, 1998).

Other commercially available biocontrol products are the yeast *Cryptococcus albidus* (available as YieldPlus®) to control postharvest diseases of apples and pears (Janisiewicz and Korsten, 2002) as well as *Pseudomonas syringae* (available as BioSave 100™ and 110™) to control sour rot on pome and citrus fruit (Tripathi and Shukla, 2007; Janisiewicz and Korsten, 2002).

3.4.2 Postharvest biocontrol in mango

In South Africa, the first publication on biological control for mango was seen in 1987, with a biological control agent *Bacillus licheniformis* against *Xanthamonas campestris* pv. *mangiferaeindica* (Burger and Korsten, 1988). The effectiveness of this biocontrol agent when applied at (10^7 cfu ml^{-1}) was shown to improve when it was integrated with a hot water dip (5min at 45º C) followed by quarter strength of recommended rate prochloraz treatment for 20 s and waxing with Citrashine $^{(Tm)}$ (Citrashine Pvt Ltd., Johannesburgh, South Africa) (Govender *et al.*, 2005). *Bacillus licheniformis*, available as Mangogreen (Stimuplant, Pretoria, South Africa) tested under semi-commercial conditions and applied in hot water to 'Keitt' mangoes, followed by a quarter strength prochloraz dip, gave good control of anthracnose and stem-end rot (Govender *et al.*, 2005). The yeast *Candida tropicalis* was found to have potential in controlling *L. theobromae*, while another yeast, *Torulpsis glabrata*, have shown potential to control stem-end rot and anthracnose (Sangchote and Saoha, 1998).

Other organisms that might prove beneficial to control anthracnose on mango after *in vitro* and *in vivo* trials include *Brevundimonas diminuta*, a yeast isolate B-65-23 (Kefialew and Ayalew, 2008) and *Trichoderma harzianum* (Noiaium and Soytong, 2000).

Table 1: *Summary of some biocontrol agents commercially available to control postharvest diseases on certain crops.*

PRODUCT	**ANTAGONIST**	**CROP**	**REFERENCE**
Avogreen®	*Bacillus subtilis*	Avocado	Korsten *et al.*, 1997
YieldPlus®	*Cryptococcus albidus*	Apples and Pears	Janisiewicz and Korsten, 2002
BioSave 100™ /110™	*Pseudomonas syringae*	Pome and Citrus	Tripathi and Shukla, 2007
BlossomBless®	*Pantoea agglomerans*	Apples and Pears	Elmer *et al.*, 2005
PomaVita®	*Pantoea agglomerans*	Apples and Pears	Elmer *et al.*, 2005
BOTRY-Zen®	*Ulocladium oudemansii*	Grapes	Elmer *et al.*, 2005
Aspire®	*Candida oleophila*	Citrus	Droby *et al.*, 1998

3.4.3 Commercialisation and the future of biological control

Screening processes for potentially effective antagonists of postharvest diseases particularly favour organisms that have a mode of action based on nutrient competition. Although numerous other mechanisms for antagonism have been identified over the years they are not always used in the screening process. This led to a wide selection of potential individual antagonistic organisms, all from a fairly narrow range of species (Droby *et al.*, 2009).

From a commercial perspective, the major factor contributing to the success of a new biocontrol agent is that it gives consistent and acceptable control of a disease and that it performs effective under commercial conditions. This requires that a good strain of an antagonist is intensively tested. Evalaution will include all factors that might influence its performance in the field as well as during the formulation process of the product. All of this

must preferably be finished before registration for commercialising is attempted (Woodhead *et al.*, 1990; Mathre *et al.*, 1999; Droby *et al.*, 2009).

The future of biocontrol products (post – and preharvest) holds promise as alternatives to synthetic fungicides, but new innovative ideas for research and development of biocontrol products are needed. Examples of areas where innovation are required include genetic modification of antagonists, understanding of interactions between antagonists, targets, hosts and the environment and the combination of potential antagonistic microbes and/or its products with other environmentally friendlier technologies (Droby, 2006; Droby *et al.*, 2009).

3.5 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is the packaging of a product in a non-permeable bag or film (usually made of a type of plastic) in such a way that air (78% N_2, 21% O_2 and 0.03% CO_2 (Kader, 2002), water vapour and other gasses are replaced by a specific or combination of specific gasses. No control over the gas composition is kept after packaging and this will change because of metabolic processes by both microbes and the product in a non-perforated bag (Mir and Beaudry, 2002; Irtwange, 2006). In a bag that allows gas exchange, the gas composition will change gradually so that equilibrium and a modified atmosphere will be obtained. This technology is called "equilibrium modified atmosphere" or also "passive atmosphere modification". The change in gas composition is a result of respiration and gas permeability of the film. Prior to sealing, the gas in the bag can be replaced, as is the case with MAP (Mir and Beaudry, 2002).

3.5.1 Modified atmosphere packaging in mango

Modified atmosphere packaging of mango in different types of film show potential to prolong shelf life and control certain postharvest diseases and disorders. It was found that mangoes treated with thiabendazole and wax prior to being stored in ventilated polyethylene bags, reduce weight loss and disease incidence, compared to untreated fruit (Reddy and Raju, 1988). Similarly, 'Keitt' mangoes stored in high and low density polyethylene bags at a high temperature (20ºC) for a month, resulted in delayed ripening, reduced weight loss and no off-flavours (Gonzalez *et al.*, 1990). Fruit stored (12 – 13 ºC) in low density polyethylene bags with potassium permanganate as ethylene scavenger (Castro *et al.*, 2005) and granular

charcoal as carbon dioxide scavenger (Illeperuma and Jayasuriya, 2002), showed less physiological disorders and also a reduction in weight loss. Both treatments delayed ripening and ripening did proceed normally to consumer preferences after removal from MAP. The fruit stored in the presence of the ethylene scavenger also showed no decay symptoms after three weeks of refrigerated storage. Castro *et al.* (2009) reported that that the use of chitosan as a coating, in combination with a hot water dip (52 °C for 5 min) and packed in low density polyethylene packaging (containing potassium permanganate (5g / 3.2kg fruit) as ethylene absorber), prolonged the shelf life of 'Espada Vemelha' mangoes.

3.6 Controlled atmosphere

Controlled atmosphere (CA) is a system where the composition of the gasses in the containers is kept constant for the entire storage period, irrespective of temperature or environmental changes. This method requires that the system be constantly monitored to maintain the desired atmosphere (Irtwange, 2006; Beaudry *et al.*, 2006). The MAP or CA has an influence on postharvest pathology and the modification of the storage atmosphere may influence the development of postharvest diseases directly by suppressing the pathogen or by maintaining the resistance of the host to infection (Irtwange, 2006). The potential benefits of MAP and CA have been compard by Itwange (2006) for different commodities. The CA is successful because it is based on the principle that it reduces physiological postharvest processes like ethylene production (ripening) and respiration, keeping in mind that the lower O_2 and/or increased CO_2 must be within the tolerance range of the specific crop. Whether fruit are stored in a dynamic controlled atmosphere or a static controlled atmosphere, both will result in better ripe fruit quality (pathological or physiological orders), compared to fruit stored in air. Also, the time after harvest (1-15 days) when the CA is established, doesn't seem to influence the incidence of pathological disorders. It was found that fruit stored in a dynamic controlled atmosphere, ripen more rapidly and has a lower incidence of rots, compared to fruit stored in a static controlled atmosphere (Burdon *et al.*, 2008).

3.6.1 Use of controlled atmosphere to extend mango fruit quality

Kader (1994) suggested the optimum CA conditions for the storage of mango fruit are at an optimum temperature of 13 ºC and that care should be taken to avoid chilling injury. A

reduced O_2 concentration of around 3 - 5% and an elevated CO_2 concentration of 5 – 10%, are the suggested atmosphere regimes for a successful CA system. The reduced O_2 levels are expected to delay ripening and the elevated CO_2 levels to retain firmness of fruit, with a expected moderate efficacy. Injury symptoms on mango for low O_2 levels are skin discolouration, greyish flesh colour and off flavour, while too high CO_2 concentrations might lead to softening and off flavours. It has been shown that pre-climateric 'Tommy Atkins' and 'Haden' mangoes can tolerate 3 kPa O_2 for up to three weeks at temperatures ranging from 12 to 15 ºC without any visible injury, but this tolerance decreases as fruit ripens (Bender *et al.*, 2000).

This technology has also been adopted to control insects.'Manila' mangoes can tolerate and also be disinfected from quarantine pests by placing fruit for 160 min under CA (0kPA O_2 and 50kPa CO_2; RH 50%) at a high temperature (<44 ºC) and then be stored at 10 ºC (RH 80%) for up to 20 days without any severe quality damage (Ortega-Zaleta and Yahia, 2000).

3.7 Ethylene scavengers

Since the discovery of 1-MCP, the focus on ethylene control research has moved away from ethylene scavengers. However, a new Palladium (Pd) promoted material shows good potential as an ethylene scavenger. It can be an alternative to the commercially used potassium permangenate ($KmnO_4$) based mechanisms to remove ethylene from storage environments (Terry *et al.*, 2006).

3.8 1-Methylcyclopropene

3.8.1 History of 1-methylcyclopropene

Edward Sisler and Sylvia Blankenship (1991 – 1996) discovered 1-MCP as result from trying to identify the ethylene-binding site protein. Sisler synthesised a cyclic diolefin with an attached diazo group – diazocyclopentadiene (DACP), but this was a highly reactive gaseous molecule that was only active when exposed to fluorescent light (Reid and Staby, 2008). This lead to the argument that the gas was broken down into smaller products and that one of these products were the active component that inhibited ethylene binding and with the

help of Blankenship lead to the discovery of 1-MCP (Chemical structure: C_4H_6). Jim Daly made the major breakthrough in commercializing 1-MCP by developing a powdered cyclodextrin – bound formulation that made it possible to conveniently sell, store, transport and use 1-MCP (Reid and Staby, 2008). Commercially 1-MCP is available under the trade names EthylBloc®, SmartFresh™, SmartTabs™ and EthylBloc™ Sachet from AgroFresh, Inc (Rohm and Haas Company) (US Environmental Protection Agency, 2008). Currently, this technology is registered in various countries for use on food poducts, including South Africa. I is used to prolong shelf life of amongst others: tomatoes, avocado, papaya, banana, apple, apricot, peach, plum and mango (Watkins and Miller, 2005).

3.8.2 Mode of action of 1-methylcyclopropene

Sisler and Serek (1997), proposed a model of how 1-MCP reacts with the ethylene receptor. In this model, a hypothesis was that ethylene can leave the receptor, and that this departure is necessary for the formation of the active complex. Ethylene then would not be a part of the active complex, but the initiator of its formation. Steps in the proposed model are: 1) ethylene approaches the metal and electrons are withdrawn; 2) another ligand in a trans position to it moves away from the metal; 3) yet another ligand moves toward the metal and as it does, ethylene is lost, and an active complex is formed; and 4) 1-MCP acts in a similar manner to ethylene, but it is not lost from the complex, and an active complex is therefore not formed.

Another model where 1-MCP competes with ethylene for receptors is proposed by Stepanova and Ecker (2000). The ETR-1 protein is suggested to form an active dimer with six trans-membrane domains (three per polypeptide), a Cu I ion complexed with each polypeptide, and protein kinase domain on the C-terminus, presumably functioning in transmission of the ethylene signal. This ETR-1 protein is thought to act as a negative regulator whose normal function, presumably mediated by the C-terminal kinase, is to maintain an intermediary regulatory protein (CTR2) in an active state. This prevents the activity of a further step in the signal cascade catalyzed by EIN-1. When ethylene binds, it inhibits the kinase activity and thereby unleashes the ethylene cascade.

Reid and Celikel (2008), proposed another model for 1-MCP where the molecule competes with ethylene for ethylene receptor sites according to their new data. In this model, it is hypothesized that 1-MCP, rather than binding directly to the binding site, binds to a site instead that is exposed during the histidine kinase reaction that occurs when the binding site

is free of ethylene. The irreversible binding of 1-MCP, they postulate, would maintain the site in its 'on' state, modifying the molecule so that ethylene no longer is bound to it. The present model for ethylene action suggests that when ethylene is bound to the binding site, kinase activity is inhibited.

This hypothesis explains the inability of 1-MCP to attach to the binding site in the presence of ethylene and the slow acquisition of the inhibitory effect over a period of hours. This is because the proposed 1-MCP-binding site would not be exposed until ethylene is released from the ethylene-binding site and kinase activity resumes. It also explains the absence of any effects of CO_2 on 1-MCP inhibition. The presence of this molecule, which is a competitive inhibitor of ethylene action, would not be expected to interfere with the effects of 1-MCP. Certainly this hypothesis will require verification using advanced biochemical chemical and molecular tools, but it does provide an explanation of certain aspects on the behaviour of 1-MCP that previously couldn't be explained (Reid and Celikel, 2008).

3.8.3 1-Methylcyclopropene's use on mango fruit

The potential benefit that 1-MCP has for the mango industry, when used on its own, would include less postharvest management steps and reduced costs (Cocozza *et al.*, 2004a). Chaiprasart and Hansawasdi (2009) investigated extension of the ripening period with 1-MCP. They concluded that a 1000 ppb treatment for 6 or 12h can significantly lengthen the ripening period for cv. Namh-dawg-mai-sri-tong mangoes. Work done on 'Kent' mangoes shows that the time for these fruit to ripen can be extended by up to four days after removal from cold storage. This was achieved when fruit were treated with concentrations greater or equal to 300 nl L^{-1} 1-MCP for 20 hours after a hot water anthracnose preventing treatment (Osuna-Garcia *et al.*, 2009). On cv. Tommy Atkins mangoes, harvested at a specific maturity, a two day delay in ripening could be observed after treatment with two different concentrations 1-MCP compared to untreated fruit (Alves *et al.*, 2004).

Mangoes, harvested at different stages of maturity, treated with 1-MCP for 12 or 24hrs and stored at room temperature showed delayed ripening. A reduction in weight loss loss could also be observed, while maintaining total titratable acidity and total soluble solids (de Melo Silva *et al.*, 2004; Alves *et al.*, 2004). However, fruit harvested at a more advanced maturity proved to be less susceptible to 1-MCP (Alves *et al.*, 2004). The use of 1-methylcyclopropene (1-MCP) (also available as Smartfresh™) at a concentration of 100 nL/L, controlled the ripening of 'Tommy Atkins' mangoes best. In combination with

Xtend™ bags a reduction in weight loss could also be observed (Cocozza *et al.*, 2004b). Jiang and Joyce (2000) reported that if 'Zihua' mangoes were treated with 100 µL/L 1-MCP for 12 hrs and then held in polyethylene MAP bags for 10 days, a delay in the onset of ripening could be observed compared to untreated fruit held in MAP. It is suggested that fruit treated with 1-MCP and MAP should be treated with ethylene after removal from MAP to promote ripening before postharvest decay can cause losses.

3.8.4 1-Methylcyclopropene's uses on other crops

Avocado fruit treated with 1-MCP, prior to ripening with ethylene, shows a considerable delay in ripening when compared to fruit not treated with 1-MCP. This response is shown in a wide variety of cultivars (Jeong and Huber, 2004; Feng *et al.*, 2000). One-methylcyclopropene, applied at 30 – 70 nl l^{-1}, delays ethylene production and action, skin colour change and softening by nearly two weeks but is an non-competitive inhibitor when applied prior to ethylene. One-methylcyclopropene also suppresses polygalacturanose activity as well as cellulase activity in avocado (Feng *et al.*, 2000). Similar results have been obtained when fruit were waxed after 1-MCP treatment with the added effect that wax in combination with 1-MCP reduces weight loss more than 1-MCP treatment alone (Jeong *et al.*, 2002). Also 1-MCP reduces polyphenol oxidase and peroxidase activity, which is correlated with mesocarp browning at high activity, in avocado (Hershkovitz *et al.*, 2005). Woolf *et al.* (2004), also found that 1-MCP reduced diffuse flesh discolouration (flesh greying, grey pulp or chilling injury) as well as some of the other physiological disorders like vascular browning, vascular leaching, stringy vascular tissue and outer flesh blackening in 'Hass' avocado. It however failed to reduce external chilling injury or skin blackening when fruit were stored at 0 ºC.

In combination with controlled atmosphere (commercially available as Transfresh atmospheres), 1-MCP treated fruit showed a significantly higher incidence of vascular browning, internal anthracnose and stem-end rot in 'Hass avocados, compared to 1-MCP treated fruit alone (Maré *et al.*, 2002). On papaya it was found that pre-treatment with 1-MCP in combination with *B. amyloliquefaciens* PPCB004 retained quality of fruit and also controlled anthracnose and *Phomopsis* rot (Osman *et al.*, 2010).

It was found that high concentration 1-MCP treatment on strawberry maintained quality (firmness and colour) but disease formation (leak rot caused by *Rhizopus stolonifer* [Ehrenb.:Fr.] Vuill.) was accelerated. Lower concentrations of 1-MCP on the other hand

delayed the development of the disease (Jiang *et al.,* 2001). Other crops in which 1-MCP delay the ripening process includes: plums and apricots (Dong *et al.,* 2002), persimmon (Harima *et al.,* 2003), apple (Watkins *et al.,* 2000), kiwifruit (Kim *et al.,* 2001), tomatoes (Wills and Ku, 2002) and banana (Jiang *et al.,* 1999).

3.8.5 Effect of 1-methylcyclopropene on disease formation

Jiang *et al.* (2001) reported that phenylalanine ammonia lyase (PAL) activity was inhibited in strawberry fruit treated with 1-MCP at various concentrations (10, 10, 250, 500 or 1000 nl/l). Decay of fruit (leak rot caused by *Rhizopus stolonifer* [Ehrenb.: Fr.] Vuill.) treated with higher concentrations of 1-MCP (500, 1000nl/l), progressed faster than untreated fruit indicating that the use of 1-MCP might lower natural plant resistance. Dong *et al.* (2002), reported that treatment with 1-MCP at 100 nl/l decreased decay and at 1000nl/l completely inhibited decay on 'Canino' apricots. Jiang and Joyce (2000) reported that mango fruit treated with 1-MCP and stored in polyethylene bags remained decay free for 18 days longer than untreated fruit stored in similar bags. No mention is however made about decay incidence on fruit treated with 1-MCP and stored under conventional storage.

Watkins (2006) reviewed the effect of 1-MCP on fruit and vegetables and amongst others summarised the effect 1-MCP has on pathological decay. 1-MCP increased disease susceptibility of citrus (mold and stem rots), strawberry (Jiang *et al.,* 2001) avocado, custard apple, mango, papaya (Hofman *et al.,* 2001), apples (bitter rot and blue mold) (Janisiewicz *et al.,* 2003), grapes (Bellincontro *et al.,* 2006) but increased disease resistance of pears, plums (brown rot) (Watkins, 2006), peaches (Liu *et al.,* 2005) and guavas (Phebe and Ong, 2010).

In avocado fruit treated with 1-MCP, reduced activities of polyphenol oxidase (PPO) and peroxidase (POD) were observed, reducing browning of fruit, but increased susceptibility to decay (Watkins, 2006). Similary, lowered PAL enzyme activity and an increased rate of decay was observed in strawberry treated with 1-MCP (Jiang *et al.,* 2001). The opposite effect was observed for peaches where decay incidence was lower and PAL, PPO and POD activities higher (Watkins, 2006). Hofman *et al.,* (2001) found that on mango (cv. 'Kensington Pride') fruit treated with 1-MCP, the severity of stem-end rot almost doubled compared to untreated fruit.

Little is known about the effect of 1-MCP on disease incidence, but this will become more important as the commercial use of 1-MCP increase. Specific interaction between

plant, pathogen and environment are the important factors that will influence 1-MCP's effect on disease development in various crops.

4. CONCLUSION

Mango is economically one of the most important sub-tropical crops cultivated and traded around the world. The fruit has a high consumer preference mainly because of its appealing shape, colour and taste and, because of its excellent nutritional value. Over the past few years, mango exports from South Africa has declined to such an extent that the country is no longer recognised as one of the major global exporters. This can mainly be ascribed to poor prices received on the European markets, the weak Rand, land reform and high production costs. This placed more pressure on farmers to focus on top quality fruit fit for export, and complying with international voluntary standards.

The postharvest diseases soft brown rot and to a lesser extend anthracnose, causes severe losses for producers at export destinations. Control strategies for both these pathogens are limited and in South Africa, only prochloraz is registered as a postharvest treatment that can be applied in the packhouse. However, variable success has been reported for soft brown rot. Pressure from European markets to reduce the use of synthetic chemicals and the continuous lowering of minimum residue levels on fruit, make it even more difficult to deliver good quality mangoes on these markets. Good competitive quality fruit from other mango producing countries also puts pressure on the South African industry.

All these obstacles have forced many exporters to either deliver most of their produce to the processing industries for juice, achar or drying or, replaced orchards and planted other commodities, like citrus. The mango fresh cut industry can also be more beneficial for the small and medium scale industries and future research will have to focus on developing this technology for the industry. This raised the need to develop alternative disease control options for export mangoes that is less costly, provide more effective control of soft brown rot and anthracnose while, simultaneously maintaining quality of fruit. With the increasing international pressure to reduce the use of synthetic chemicals that might pose a risk to the environment and human health, many researchers have changed their focus to more environmentally alternative decay control and quality maintenance strategies.

For fresh fruit and vegetables, pre-harvest practices remain one of the most important factors that can ensure good quality produce that will continue to be acceptable to the retailers for a reasonable period. Effective cold chain management will also, besides pre-

harvest practices, remain very important. Controlled atmosphere storage of commodities is a system where the gas composition surrounding the fruit are artificially maintained in such a way that it is fungistatic or fungicidal to pathogens, but also delays or suppresses the metabolic processes responsible for ripening and deterioration of the fruit. This technology can also be applied to extend shelf life.

Modified atmosphere packaging is another technology that can be used for storing fresh commodities in permeable or semi-permeable packaging that maintain or result in a changed gas composition. This reduces moisture loss, depending on the permeability of the film. Basic packhouse activities like waxing of mango fruit is also represent a type of modified atmosphere environment, since it affects the respiration and regulates moisture loss of fruit.

Biological control has shown potential to control postharvest pathogens and various products are commercially available to control specific pathogens on crops. Many new or different antagonists show potential under experimental conditions to control pathogens on a wide variety of crops.

Recently, it has been shown that 1-MCP can be used with success to extend the storage life of, especially, climacteric fruit. Further, 1-MCP is an ethylene inhibitor that binds to the ethylene binding sites of fruit and thus delays the ripening process. Commercially, 1-MCP is available to the industry as Smartfresh® and has been successfully implemented in several fruit industries to prolong storage life and retain quality.

This literature review therefore provides an overview of some technologies that are used to maintain quality and control disease formation postharvestly in a variety of crops, including mango. The review focused primarily on more “environmentally acceptable” methods. The aim was to develop a critical understanding of these practices in order to conduct a relevant study on postharvest disease and quality maintenance of mangoes in the following chapters.

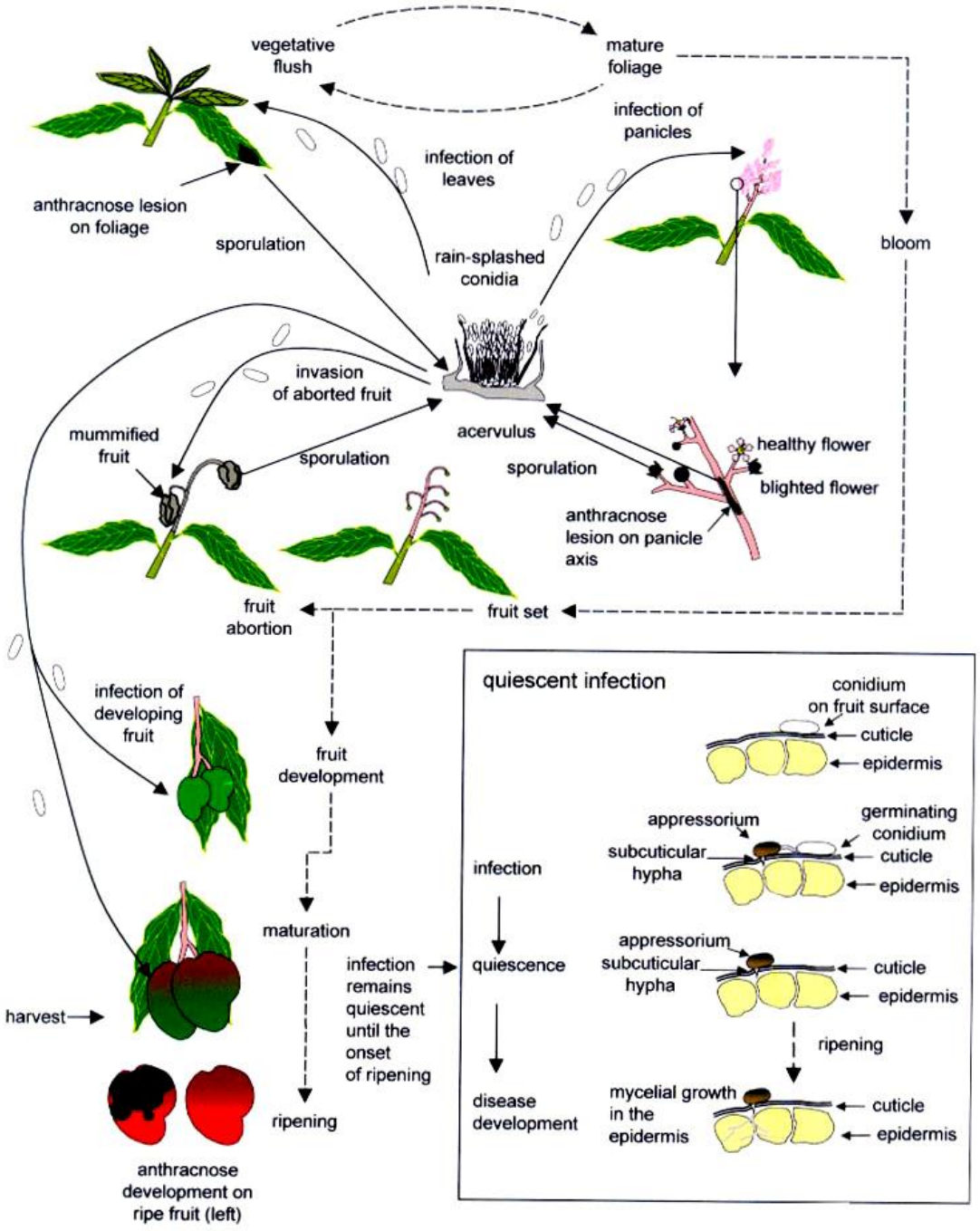

Fig 2. *The life cycle of anthracnose in mango, caused by Colletotrichum gloeosporioides ((Penz.) Penz. And Sacc. In Penz.) (Arauz, 2000).*

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CHAPTER 3

Responses of 1-methyl cyclopropene or biocontrol agent *Bacillus amyloliquefaciens* application in mango cultivars 'Tommy Atkins' and 'Kent', stored under controlled atmosphere storage

ABSTRACT

Mangoes are climacteric fruit and highly perishable, susceptible to bruising and wounding during postharvest handling. Poor handling contributes to increased postharvest decay as well as flesh softening. The effect of controlled atmosphere (CA) in combination with either 1-methylcyclopropene (1-MCP) or *Bacillus amyloliquefacies* (PPCB004) were evaluated to sustain quality of 'Tommy Atkins' and 'Kent' mangoes during cold storage. The respiration supressing and, fungicidal effect of CA and ethylene inhibition by 1-MCP on various fruit and vegetables have been shown, but not the combination effect on mangoes. *Bacillus amyloliquefaciens* might further provide an added antagonistic effect against anthracnose and soft brown rot. Fruit were pre-treated with either 1-MCP or *B. amyloliquefaciens*, prior to being stored under either 5% O_2 + 5% CO_2 or 3% O_2 + 8% CO_2 CA for 18 days at 10 ºC. Weight loss was overall reduced by CA storage and the combination of 1-MCP and 3% O_2 + 8% CO_2 were able to control anthracnose in both cultivars. Further work should focus on optimizing the CA conditions / 1-MCP treatment for mango storage.

1. INTRODUCTION

Rapid flesh softening, wounding due to poor picking and -handling practices as well as unhygienic conditions during packaging, storage and transportation were identified as major factors that affect mango fruit after harvest (Yahia, 1998). Anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz.) and soft brown rot (*Botryosphaeria* spp.) are the dominant postharvest diseases of mangoes. The cultivar 'Tommy Atkins' is known to be less susceptible than 'Kent' to anthracnose (Campbell, 1992; Arauz, 2000). Generally, storage temperatures of around 10º C can delay decay development

in mangoes (Abou Aziz *et al.*, 1976). However, a residual protection against postharvest pathogens is important once the fruit move through the cold chain and after removal from cold storage at the distribution centre. Control of postharvest decay is achieved commercially in South Africa through a combination of thermal and fungicide prochloraz treatments. Considerable variability in sensitivity to prochloraz among the *C. gloeosporioides* isolates from mango was reported by Arauz (2000) and Sanders *et al.* (2000).

Controlled atmosphere storage (CA) influence the development of postharvest pathogens and the modification of storage atmospheric conditions may affect disease expression directly by preventing pathogen growth or by maintaining host resistance (Irtwange, 2006). The CA treatment is reported to reduce postharvest decay development, retain fruit quality and to extend storage life (Arauz, 2000; Bender *et al.*, 2000; Lalel *et al.*, 2003).

The application of the ethylene inhibitor, 1-methyl cyclopropene (1-MCP) has been adopted for some climacteric fruit including mango (Hofman *et al.*, 2001). However, according to Hofman *et al*. (2001), although the 1-MCP treatment delayed ripening in mango fruit harvested at commercial maturity, increase stem-end rot was observed in 'Kensigton Pride' mangoes. Plotto *et al*. (2003), reported that 1-MCP delayed the ripening related changes such as fruit softening and colour development in 'Tommy Atkins' while ripening related events were not affected in 'Kent' mangoes. On the other hand according to Osuna-Garcia *et al.* (2009), ripening in Kent fruit was delayed after 1-MCP pre-treatment. On peach, the biocontrol agent, *Bacillus amyloliquefaciens* PPCB004 had shown potential as an antagonist to control *Botrytis cinerea* Pers.:Fr., *Penicillium expansum* Link and *Rhizopus stolonifer* (Ehrenb.:Fr.) (Arrebola *et al.*, 2010).

Different postharvest treatments, such as application of edible coatings, heat treatments, 1-MCP application, biocontrol agents, modified atmosphere storage and CA storage have already been researched. The objective of this study, however, is to determine the combined effect of *B. amyloliquefaciens* PPCB004 or 1-MCP separately under two different CA storage conditions on mango 'Tommy Atkins' and 'Kent'. The effect these treatments had on decay control and maintenance of fruit quality after cold storage (for 18 d, 10 ºC and 80% RH) and ripening at 25 ºC was investigated.

2. MATERIALS AND METHODS

2.1. Fungal pathogens

During the 2008-2009 season, isolations were made from anthracnose and soft brown rot lesions on symptomatic 'Tommy Atkins' and 'Kent' fruit. Isolations were made by surface sterilising fruit by spraying with a 70 % ethanol mixture and allowing it to air dry. Isolations were made from the edge of lesions by removing (2 x 2mm) blocks, using a sterile scalpel. The blocks were placed on malt extract agar (MEA) (Merck Biolab, Midrand) and incubated for 14 days at 25 ºC. Six isolates considered as *C. gloeosporioides* and *Botryosphaeria* spp., were selected for identification and confirmation.

To ensure genetic uniformity, single spore isolations of fungal cultures were made by plating out 100 µl of spore suspension of the isolates on bacteriological agar (Merck) plates. Plates were incubated for 12 h at 25 ºC and viewed under a stereo microscope for the presence of single germinated spores. Thereafter a single germinated spore was removed and inoculated onto MEA (Merck).

Selected cultures were identified as described in section 2.2. Pathogenicity was confirmed via artificial inoculation as described in section 2.4. From these, one positively identified culture of *C. gloeosporioides* and *Botryosphaeria parva* (Pennycook & Samuels) were selected for further *in vivo* studies.

2.2. Confirmtion of postharvest pathogens using the polymerase chain reaction

Single conidial isolates were produced and DNA was extracted using the DNeasy Plant Mini DNA Extraction kit (Qiagen, USA). During the extraction process, the fungal mycelium was macerated in a bead beater (Thermo, IEPSA) using micro silicone beads. Amplification was performed using the 16S rRNA internally transcribed spacer (ITS) gene region with primers ITS1 and ITS4 (White et al., 1990). PCR reactions were performed in 50 µl reaction mixtures containing 0.6 µl DNA (10 ng/ml), 5 µl reaction buffer, 37.4 µl sterile double distilled water (ddH2O), 1 µl dNTPs (10mM), 0.5 µl of each primer (100pmol/ µl) and 0.5 µl Taq DNA polymerase (2.5 U/ µl) (Bioline, Scotland). Amplification was performed as follows: 30s at 95 ºC, then 35 cycles of 94 ºC for 1 min, followed by primer annealing at 58 ºC for 90 s and extension at 72 ºC for 2 min. A final extension of 7 min followed after which the samples were kept at 4 ºC and later stored at -20 ºC. Sequence identification was performed in an ABI

Prism 3700 Genetic Analyser (AB Applied Biosystems) after pre- and post-cleaning methodology of the PCR amplicons. The cycle sequencing PCR reaction constituted a final volume of 10 µl and contained 1 µl of template DNA, 4 µl Dye Terminator Ready Reaction Mix, 4 µl ddH2O and 1 µl primer (4 pmol/ µl). Contigs were assembled and isolate identity was confirmed using sequence homology with the program NCBI Blast.

2.3. Fruit

Mango 'Tommy Atkins' and 'Kent' fruit (360 fruit of each cultivar) were hand harvested at commercial maturity by the orchard workers during the early morning from a commercial orchard (Farm Moria in the Hoedspruit area (Limpopo Province)). After harvest, fruit were de-sapped and transported to the on farm laboratory at the Lushof farm in Tzaneen (Limpopo Province), within an hour. Fruit were surface disinfected with a 0.05 µl 1^{-1} NaOCl solution dip for two min and rinsed three times with tap water. Thereafter, fruit were sorted for uniform size and absence of mechanical damage or disease.

2.4. In vivo inoculation

Prior to inoculation, fruit were surface sterilised by spraying with 70% ethanol and allowing it to air dry. Artificial inoculation of fruit was done by using a sterile scalpel (size 2) making a cross cut (10 x 10 mm) on the fruit surface penetrating only the skin, on opposing sides of the fruit.

An agar square (5 x 5 mm) with overgrown five-day old mycelial hyphae of *C.gloeoesporioides* or *B. parva* was placed separately, using a sterile inoculation needle, in the wound site. Fruit that served as control were inoculated with a plain agar disc. Batches of 72 fruit (12 per treatment) were inoculated with either *C. gloeosporioides, B. parva* or control. After inoculation, fruit were kept at 16 ºC for 6 h to allow pathogen establishment. Fruit were then subjected to different postharvest treatments prior to storage.

2.5. Postharvest treatments for naturally infected fruit

All the fruit were disinfected with 0.5 µl l^{-1} NaOCl for 2 min and sorted for uniform size and absence of mechanical damage or disease. Thereafter fruit were subjected to standard hot water treatment adopted by the South African mango industry (50° C for 2 min

in 20dm^3 tank equipped with RKCF4 temperature controller (Vizier Systems (Pty) Ltd, Cape Town), 2.5 kW immersion heating elements and a 0.37 kW pump to maintain the turbulence of the dip preparation.

2.5.1. Biocontrol treatment

The biocontrol agent, *Bacillus amyloliquefaciens* PPCB004 (8.3 x 10^9 cells/ml) was incorporated into the hot water treatment. The final concentration of the biocontrol agent in the 20 dm^3 bath was 8.3 x 10^6 cells/ml. The *B. amyloliquefaciens* PPCB004 formulation for this application was prepared by Stimuplant (Pty) Ltd (Boschkop). After *B. amyloliquefaciens* PPCB004 application with hot water treatment, fruit were randomised to provide seven sets of four replicates. Each replicate box had six fruit per treatment.

2.5.2. One- methylcyclopropene treatment

Fruit were subjected to the hot water treatment and there after treated for 24h in a sealed chamber with a volume of 1.5 m^3 with 1-MCP (2 mg/kg of fruit) SmartFresh ™ powder (0.14% active ingredient; Rohm and Haas Company, Philadelphia, Pensylvania) (Singh and Dwivedi, 2008) at a temperature of 16 °C. The 1-methylcyclopropene was dissolved in warm water, placed in the chamber and the chamber was sealed airtight. However, the available 1-MCP during the treatment was not quantified.

2.6. Storage conditions

After treatment, 'Tommy Atkins' and 'Kent' mangoes were stored in either one of six controlled atmosphere (CA) chambers (90 l) containing two different gas compositions CA-1 (5% O_2 + 5% CO_2) and CA-2 (3% O_2 + 8% CO_2). Initial O_2 and CO_2 levels in the cabinets were established by a flow-through system, mixing N_2 (100%) and O_2 (99.5%) or N_2 and CO_2 via pressure regulators. The temperature and RH within the chamber was monitored with a U23-001 data-logger using Hoboware ® Pro data-logger software (Onset, Maryland, USA). The cold room containing the chambers was set at 10 ±2 ºC.

2.7. In vivo effect of 1-*Methylcyclopropene treatment and/or* Bacillus amyloliquefaciens *PPCB004 under controlled atmosphere conditions on anthracnose and soft brown rot disease development in vivo*

'Tommy Atkins' or 'Kent' mango at commercial harvest maturity (green-purple or green-red with well-developed shoulders) was inoculated with either *C. gloeosporioides* or *B. parva* prior to either biocontrol, 1-MCP or only hot water treatment after inoculation with *C. gloeosporioides*. For each treatment 36 fruit from each cultivar was selected. *In vivo* inoculation of fruit was done as described in 2.4.

After the 6 h incubation period, the inoculated fruit were stored under two different CA conditions for the following treatments; CA-1 + 1-MCP; CA-1+ Biocontrol; CA-1 stand-alone; CA-2 +1-MCP; CA-2 + Biocontrol; CA-2 stand-alone and untreated control fruit. Anthracnose and soft brown rot severity was evaluated by measuring the lesion diameter (mm) after 18 d at 10 ±2 ºC and after ripening (five days for anthracnose and two days for soft brown rot) at 25 °C. The experiment was repeated twice for both cultivars separately.

2.8. Effect of 1-Methylcyclopropene treatment or Bacillus amyloliquefaciens *PPCB004 on control of postharvest decay and retention of fruit quality under controlled atmosphere conditions in naturally infected fruit*

'Tommy Atkins' or 'Kent' mango was subjected to biocontrol or 1-MCP treatment or untreated and subjected to the following treatments; CA-1 + 1-MCP; CA-1+ Biocontrol; CA-1 stand-alone; CA-2 +1-MCP; CA-2 + Biocontrol; CA-2 stand-alone. Untreated fruit not stored in CA was included as comparative control. For each treatment 24 fruit was selected. All the above mentioned treatments had four replicate boxes and each box containing six fruit. The fruit subjected to all treatments were stored for 18 days at 10 ±2˚C and at 90% RH. After 18 days of cold storage, fruit were removed and the effect of all treatments on decay incidence, weight loss, and skin colour was determined. Thereafter, fruit were allowed to ripen at 20 °C, 72% RH under normal atmosphere condition for five days. After ripening, skin and flesh colour, overall quality, incidence and severity of decay, fruit firmness, soluble solid concentration (SSC), titratable acidity (TA) and SSC/TA content were assessed.

2.9. Fruit quality evaluation

2.9.1. Skin and flesh colour

Fruit (replicate boxes) subjected to all the treatments mentioned in Fig. 2 were weighed before and after 18 days storage and data was expressed as percentage weight loss. Fruit surface skin and flesh colour was measured (10 fruit per treatment) using a Hunterlab Miniscan XE Plus (Reston, Virginia, USA), expressing CIELAB Commission International de I Eclairage (CIE) colour space values (*L*, Chroma, hue*^{0}). Two spots on opposite sides of the fruit were measured and the mean of the two measurements were considered as one reading. Skin colour was measured after cold storage and also after ripening. Flesh colour was taken after cutting the fruit from two cut halves (10 fruit per treatment) after ripening. The L* value express light intensity, where 0 = black and 100 = white; hue^{o} is the angle in a colour wheel of 360^{o}, with 0, 90, 180 and 270^{o} representing the hues red, yellow, green and blue, respectively and chroma is the intensity or purity of the hue (Phebe and Ong, 2010).

2.9.2. *Fruit firmness*

Fruit firmness was measured on opposite sides of individual fruit (10 fruit per treatment) with a Chatillon penetrometer (Chatillon and Sons, New York, USA) equipped with a 6 mm diameter plunger capable of penetrating through the peel into the pulp.

2.9.3. *Soluble solid concentration*

A set of 10 fruit per treatment replicate was randomly selected for SSC determination with a digital refractometer (Atago Co., Tokyo, Japan) and expressed in percentages (Sivakumar and Korsten, 2010).

2.9.4. Titratable acidity

Percentage titratable acidity (TA) was determined after ripening by titration of 10 ml fruit juice with 0.04 g/100 ml NaOH, using a 1% solution of phenolphthalein (Saarchem, Midrand). Titratable acidity was calculated as a citric acid (Factor: 0.0064) equivalent from 20 g of flesh obtained from ten fruit separately (Sivakumar and Korsten, 2006).

$$\% \text{ Acid} = \frac{\text{Titre x Acid factor x 100}}{\text{Juice (ml)}}$$

2.10. Biocontrol recovery

Biocontrol and microbial recovery from the mango fructoplane were done for the biocontrol treated fruit according to the methods described by Govender (2005a) and Govender *et al.* (2005b) within 24h after treatment. Three fruit of each treatment were selected and individually placed in a beaker containing sterile ¼ strength Ringers solution (Merck). The beaker containing the fruit were placed in an ultrasonic water bath (Labex, Orange Grove) and sonicated for 30 s. The washing solution, was vacuum filtered through a 0.22 µm filter paper. The filter paper was vortexed in ¼ Ringers solution and then serially diluted into four separate sets. Two sets were plated in triplicate onto Standard 1 (STD1) nutrient agar (Biolab) to recover *B. amyloliquefaciens* PPCB004 and other bacteria. The two remaining sets were plated out in triplicate onto MEA to recover fungi and yeast. Plates were incubated at 25 ºC for four days and biocontrol, other bacteria; fungi and yeast colonies were counted. The same procedure was followed within 24h after fruit were removed from cold storage to observe the population shift on the fruit phyloplane. Biocontrol colonies were visually distinguished from other colonies by comparing it to a STD1 plate containing a pure culture of *B. amyloliquefaciens.* The same recovery method was followed with untreated fruit to provide background microflora.

2.11 Statistical Analysis

The experiment was repeated twice adopting a complete randomized design. Statistical evaluation of the differences was performed using analysis of variance (ANOVA). The mean values of the significant interactions were compared using the Fisher's protected L.S.D (least significant difference) test at the 5% level using the SAS statistical software (Version 9.2, SAS Institution, Cary, NC).

3. RESULTS

3.1. Confirmation of pathogenicity of pathogens

The selected *B. parva* and *C. gloeosporioides* isolates successfully infected mango fruit and pathogenicity was thus confirmed.

3.2. Effect of 1-Methylcyclopropene or Bacillus amyloliquefaciens PPCB004 under controlled atmosphere conditions on postharvest decay incidence and severity in 'Tommy Atkins' and 'Kent' mangoes

3.2.1. Artificial inoculation with Colletotrichum gloeosporioides

'Tommy Atkins' overall had smaller lesions than 'Kent' fruit after ripening. The pre-treatment with biocontrol failed to control anthracnose (Fig. 1). 'Kent' fruit pre-treated with 1-MCP had overall smaller lesions, irrespective of the CA treatment. However, compared to the untreated control, lesions were not significantly smaller. 'Tommy Atkins' fruit subjected to CA-2 overall had smaller lesions than fruit stored under CA-1. The pre-treatment of 'Tommy Atkins' with 1-MCP resulted in significantly smaller lesions for fruit stored under CA-1. Pre-treatment of fruit with biocontrol did produce significantly bigger lesions on fruit stored under CA-2, but no difference could however be observed between stand-alone CA-2 or the combination of CA-2 and 1-MCP.

3.2.2. Artificial inoculation with Botryosphaeria parva

'Kent' fruit was overall more susceptible to soft brown rot than 'Tommy Atkins' (Fig. 2). 'Kent' fruit pre-treated with 1-MCP had slightly smaller soft brown rot lesions in the respective CA treatments. 'Kent' subjected to stand alone CA-1, as well as in combination with biocontrol had significantly bigger lesions than untreated fruit. In 'Tommy Atkins' soft brown rot was controlled the best by stand-alone CA-1. The combination of 1-MCP pre-treatment stored under CA-1 had significantly the largest soft brown rot lesions after ripening. The combination of 'Tommy Atkins' treated with biocontrol and stored under CA-1, as well as the stand alone CA-2 storage, showed significantly bigger lesions than untreated fruit after ripening.

3.2.3. Natural incidence and severity of postharvest diseases of mangoes stored under controlled atmosphere

The incidence of anthracnose was in general higher on 'Kent' than on 'Tommy Atkins' (Fig. 3). The highest incidence of anthracnose on 'Kent' was observed on fruit stored under stand-alone CA-1. Also, pre-treatment with biocontrol did not control incidence or severity (Fig. 4) of anthracnose on 'Kent', but pre-treatment with 1-MCP had a lower incidence of anthracnose, irrespective of the CA used. The incidence of anthracnose on 'Tommy Atkins', was highest on fruit subjected to 1-MCP pre-treatment and CA-1 storage. On 'Tommy Atkins' stand-alone CA-1 and CA-2, and biocontrol pre-treated fruit stored under CA-1 all had no or very low incidences of anthracnose.

On 'Kent' fruit the incidence of soft brown rot was higher on fruit subjected to CA-2 storage (Fig. 5). No soft brown rot was observed on 'Kent' fruit stored under CA-1 and subjected to pre-treatment with either 1-MCP or biocontrol. No soft brown rot could be observed on 'Tommy Atkins' subjected to stand-alone CA-1 or CA-2. The severity of soft brown rot on 'Kent' was the highest in stand alone CA-1 (Fig. 6). The combination of CA-1 and 1-MCP had the highest severity of soft brown rot on 'Tommy Atkins' (Fig. 6).

3.2.4. Biocontrol recovery and microbial population shift

Almost no *B. amyloliquefaciens* could be recovered from the surfaces of either of the cultivars after cold storage (Fig. 7). On 'Tommy Atkins' fruit, an increase in bacterial and fungal populations could be observed after cold storage. On 'Kent', subjected to CA-1 an increase in fungal, bacterial and to a lesser extent yeast populations could be observed after cold storage. On 'Kent' fruit subjected to CA-2 storage an increase in yeast populations could be observed, wile bacterial colonies remained constant (Fig. 8).

Table 1. *Fruit quality parameters at harvest for both export cultivars.*

	External colour			Pulp colour				
Cultivar	**Light intensity**	**Chroma**	**Hue angle**	**Light intensity**	**Chroma**	**Hue angle**	**Firmness**	**SSC/TA[a]**
TOMMY ATKINS	42.86	24.14	73.72	82.13	53.66	86.93	2.13	13.69
KENT	54.04	23.55	93.16	86.37	45.68	86.59	1.95	9.04

a: SSC = Soluble solid concentration / TA = Titratable acidity

3.3. Effect of 1-methylcyclopropene or Bacillus amyloliquefaciens PPCB004 under controlled atmosphere conditions on weight loss, fruit firmness and colour retention in 'Tommy Atkins' and 'Kent' mangoes

3.3.1. Weight loss

The loss of weight is one of the most important factors responsible for fruit quality deterioration. Weight of treatments was taken after cold storage only and not after ripening, since individual fruit severely infected with soft brown rot had to be removed after cold storage (Fig. 9). For 'Tommy Atkins' weight loss was reduced by all treatments when compared to untreated fruit. 'Kent' fruit pre-treated with 1-MCP had the lowest percentage of weight loss for treatments stored under CA-2. However, under CA-1 the opposite was observed in the sense that 1-MCP treated fruit lost the most weight during storage.

3.3.2. Firmness

Fruit firmness was measured after five days of ripening and not after cold storage because of the destructive method used (Fig. 10). 'Kent' fruit, pre-treated with 1-MCP and stored under CA-2 was significantly firmer after the ripening period than any of the other CA-2 treatments and the untreated control. However, 'Tommy Atkins' fruit pre-treated with 1-MCP and stored under CA-1 were significantly softer than any of the other treatments. Pre-treatment with biocontrol resulted in firmer 'Tommy Atkins' fruit after storage under CA-1, compared to untreated fruit.

3.3.3. Skin colour

Different treatments adopted in this study affected the skin and flesh colour changes at different levels in both cultivars. The skin colour change during ripening for 'Kent' is from green - red to yellow-red and for 'Tommy Atkins' from green-purple to yellow-red (Table 1 and 2).

The L value was observed to increase in both cultivars during ripening. Chroma values for 'Kent' decreased during the ripening period while it increased for 'Tommy Atkins'. The 'Kent' mangoes showed reduced chroma values after ripening in fruit subjected to different treatments compared to 'Tommy Atkins' mangoes which could be due to colour

developments observed in both cultivar types. However, the 1-MCP pre-treatment did not show any significant effect on chroma value. Untreated 'Kent' fruit did not show any notable change in chroma value during the ripening period.

Generally higher hue° value means retention of green skin colour and a slight decline in hue° value was noted in Tommy Atkins in fruit under CA storage condition after ripening. In 'Kent' fruit a decline in hue° value was noted, except for fruit pre-treated with 1-MCP and stored under CA-2, where the value did not change during ripening.

3.3.4. Pulp colour

After ripening, the pulp of 'Kent' fruit subjected to the 1-MCP pre-treatment had a significantly higher light intensity compared to the other treatments. No notable differences in light intensity could however be observed for the pulp of 'Tommy Atkins' fruit (Table 3).

Overall, the chroma of 'Kent' pulp stored under CA-1 was higher than fruit subjected to CA-2. Pre-treated 1-MCP 'Kent' fruit stored under CA-1 had a significantly higher chroma value than the same treatment stored under CA-2 (which also had the lowest chroma value overall). 'Tommy Atkins' fruit pulp, pre-treated with 1-MCP, had higher chroma values than any of the other treatments, irrespective of the CA (Table 3).

The pulp hue° value of 'Kent' fruit subjected to 1-MCP pre-treatment were higher than the other treatments within their respective CA treatments, with fruit subjected to CA-2 having a significantly higher pulp hue° value than CA-1 stored fruit. However, the opposite could be observed for 'Tommy Atkins' fruit, where the pulp hue° was lower in 1-MCP pre-treated fruit within their respective CA treatments and fruit stored under CA-2 having an slightly lower value than CA-1 fruit (Table 3).

3.3.5. Soluble solid content / Titrateble acidity ratio

The soluble solid content / titrateble acidity of 'Kent' fruit was overall higher than 'Tommy Atkins' after ripening (Fig. 11). However, no meaningfull differences could be observed between treatments of 'Kent' fruit when compared to the untreated control. 'Tommy Atkins' fruit pre-treated with 1-MCP and subjected to CA-1 had a significantly higher SSC/TA ratio (hence a lower TA) than any of the other CA treatments, but similar to the untreated control. However, all the remaining CA treatments, including 1-MCP pre-

treatment in combination with CA-2 storage of 'Tommy Atkins' fruit resulted in a lower SSC/TA ratio than untreated fruit.

4. DISCUSSION

The effect of *B. amyloliquefaciens* PPCB004 application under CA-1 or CA-2 storage condition was less effective in reducing anthracnose severity for both cultivar types when compared to 1-MCP and CA-2 combination treatment. *B. amyloliquefaciens* PPCB004 was observed to survive in 1-MCP pre-treated papaya fruit during storage (Osman *et al.*, 2010). However, under CA-2, no *B. amyloliquefaciens* PPCB004 could be recovered after cold storage. It is evident from the observations that the efficiency of decay control under CA conditions and adopted treatments in this study is cultivar depended. Also, that pre-treatment with 1-MCP in combination with CA1 seem to stimulate disease formation on 'Tommy Atkins', as can be observed with soft brown rot (natural and artificial infection) and anthracnose (natural infection).

The application of CA with a reduced O_2 concentration of around 3 - 5% and an elevated CO_2 concentration of 5 – 10% are the suggested atmosphere regimes for a successful CA system for mango fruit (Yahia, 1998, Kader, 1994). The use of CA-2 (3% O_2 and 10% CO_2) resulted in lower anthracnose incidence in `Tommy Atkins', and after removal from cold storage to room temperature (25°C) conditions, the residual effect of CA was clearly demonstrated on control of anthracnose (Kim *et al.*, 2007). A reduction in rots could also be observed on 'Kensington Pride' mangoes stored under 2% O_2 and 3-6 CO_2 (Lalel *et al.*, 2001). The 1-MCP treatment delayed the decay development in apricots (Dong *et al.*, 2002). Disease development also increased in strawberries at high (500 and 1000 nl/l) 1-MCP concentrations. However, according to Jiang *et al.* (2001), treatments at 100 and 250 nl/l 1-MCP delayed the development of decay in strawberries. The 1-MCP treatment (25µl) was reported to increase the severity of stem-end rots in 'Kensington Pride' mango (Hofman *et al.*, 2001). Detailed information regarding the severity and incidence of anthracnose in mango pre-treated with 1-MCP needs further investigation with respect to different cultivar types.

The observed decrease in weight loss recorded in this study for 'Tommy Atkins', can be attributed to the reduction in rate of respiration, where the reduction in respiration rate of fruit reduces its loss of organic matter and the metabolic water. Our results coincide with other authors for CA storage of mangoes. Lizana and Ochagavia (1996) reported that after 23

days, 'Kent' fruit lost 2.3 % weight when stored under CA (5% O_2 / 10% CO_2) and 2.7% for the same cultivar stored under 5% O_2 / 5% CO_2. On 'Tommy Atkins', they noted that fruit stored under 5% O_2 / 5% CO_2 lost 33% less weight than control fruit. Lalel *et al.* (2003), also observed a reduction in weight loss for 'Kensington Pride' mangoes stored under CA (2% O_2 / 3 – 9% CO_2) for 23 days. Chaiprasart and Hansawasdi (2009) and Hofman *et al.* (2001), reported that pre-treatment with 1-MCP did not reduce weight loss of 'Namh-dawg-mai-sri-tong' and 'Kensington Pride' mangoes, when compared to untreated fruit for a similar storage period. However, Alves *et al.* (2004) reported that pre- treatment of 'Tommy Atkins' mangoes with 1-MCP did reduce weight loss during storage. This support the theory that for certain cultivars the combination of 1-MCP and CA will have a greater influence on weight loss, than either of the treatments used alone, which support our results for 'Tommy Atkins' under CA-1 and 'Kent' under CA-2.

The loss of firmness is one of the most important factors responsible for fruit quality deterioration. Fruit softening is considered as a result of degradation of protopectin and decrease in protopectin levels. Coinciding with our observations, other authors also reported that CA storage and 1-MCP treatment maintained firmness of mangoes. The increase in soluble pectin levels in mango fruits were observed in 1-MCP treated mango cv Guifei fruit with inhibition of fruit softening (Wang *et al.*, 2006). Osuna-Garcia *et al.* (2009) also reported that pre-treatment with 1-MCP resulted in firmer 'Kent' fruit. Lalel *et al.* (2003) reported that CA storage maintained firmness of 'Kensinton Pride' mangoes. Furthermore, the CA storage conditions with increasing CO_2 composition (<10%) has been shown to decrease ethylene production and ripening by affecting the ethylene biosynthetic pathway (Bender *et al.*, 1995). Therefore, the combination of 1-MCP pre-treatment and CA-2 storage (8% CO_2) had served synergistically to reduce fruit softening via maintaining the firmness in 'Kent' mangoes.

Contradictory to our results, Kim *et al.* (2007) could observe no differences in light intensity (L*) of 'Tommy Atkins' fruit skin for the duration of CA storage as well as at the end of the ripening period. Coinciding with our findings, they observed that the hue^o value decreased during the storage period, which corresponds with an increase in carotenoid synthesis as fruit ripen. However, a higher hue^o value was observed for 'Tommy Atkins' stored under CA conditions than for control fruit (Kim *et al.*, 2007). 'Tommy Atkins' fruit stored under CA eventually matched hue^o of control fruit after a longer ripening period, indicating that CA storage have no residual effect on carotenoid synthesis (Kim *et al.*, 2007).

Coinciding with our results on 'Tommy Atkins' an increase in chroma for the same cultivar could be observed during the storage period (Kim *et al.*, 2007).

However, contrary to our observations, Kim *et al.* (2007) reported that fruit subjected to CA storage had an overall lower chroma value than control fruit. Supporting this, Bender *et al.* (1995) also reported that mature green 'Tommy Atkins' fruit after 21 days of storage under CA (10% CO_2 and 5% O_2) showed an decrease in hue^o value and an increase in chroma, with the hue^o higher and the chroma lower than control fruit after this storage period. A similar increase in chroma and decrease in hue^o was observed for 'Tommy Atkins' and 'Haden' fruit after removal from a 5% O_2 CA to normal atmospheric conditions to ripen (Bender *et al.*, 2000). Plotto *et al.* (2003) reported that fresh-cut slices made 24h after treatment of intact 'Tommy Atkins' fruit pre-treated with 1-MCP and hot water had a higher L* value than control fruit after 14 days of storage. According to Ayala-Silva *et al.* (2005) for mangoes, L* is an indication of the total pigment present, thus a more reddish fruit will have a lower L* and a more greenish fruit will have a higher L*. Lower hue^o values indicate a more reddish colour and a higher chroma will indicate a brighter red colour.

Supporting our results, it was found that 'Tommy Atkins' and 'Kent' fruit stored under CA (5% O_2 / 10% CO_2) at 12 ºC had a higher acid content than control fruit after 23 days, although the acid content gradually decreased (Lizana and Ochagavia, 1996). Our observation for 'Kent' failed to coincide but was supported for 'Tommy Atkins' by other authors. According to Wang *et al.* (2006), 1-MCP treatment was reported to delay the decrease in TA in mango fruit cv. 'Guifei'. The 1-MCP treatment in 'Guifei' showed reduced SSC when compared to the control untreated fruit (Wang *et al.*, 2006). Chaiprasart and Hansawasdi (2009) also observed that the SSC/TA ratio in 'Namh-dawg-mai-sri-tong' mangoes increased slower in 1-MCP treated fruit during storage. Similar observation was reported in cultivar Delta R2E2 under 3% O2 and 8% CO2 CA storage (Lalel *et al.*, 2005). The 3% CO_2 and 8% O_2 storage environment was reported to reduce the rate of respiration in 'Delta R2E2'(Lalel *et al.*, 2005). However, Lalel *et al.* (2001 and 2003) reported that the total acids and TSS/acid ratio on 'Kensington Pride' mangoes was higher than control fruit after CA storage (2% O_2 and 3-9% CO_2).

5. CONCLUSION

'Kent' fruit was overall more susceptible to anthracnose and soft brown rot. The CA-2, in combination with 1-MCP pre-treatment, controlled *in vivo* inoculated anthracnose best

on both cultivars. Stand-alone CA-1 gave the best control of *in vivo* inoculated soft brown rot on 'Tommy Atkins'. Natural infection of anthracnose was best controlled on 'Tommy Atkins' by stand-alone CA-2. The combination of 1-MCP pre-treatment and CA-2 storage gave fairly good control of anthracnose and soft brown rot overall. Weight loss was overall reduced by CA for 'Tommy Atkins'. Weight loss was the lowest in 'Tommy Atkins' subjected to 1-MCP pre-treatment and CA-1 storage, and 'Kent' subjected to the combination treatment of 1-MCP and CA-2. Firmness of 'Kent' fruit subjected to 1-MCP and CA-2 storage remained the firmest after the five day ripening period. Overall, storage under CA resulted in a lower SSC/TA ratio for 'Tommy Atkins', with the exception of fruit subjected to 1-MCP and CA-1, which failed to maintain SSC/TA ratio lower than the untreated control.

Table 2[1]. *The skin colour of mango fruit after cold storage and after ripening, measured according to the light intensity, chroma and hue angle. Values with the same letter do not differ significantly, with respect to cultivar, colour parameter and stage of ripening, according to Fisher's protected test.*

	Skin Colour (After cold storage & After ripening)					
Treatment	**Light Intensity**		**Chroma**		**Hue angle**	
	KENT (ACS)	**TOMMY ATKINS (ACS)**	**KENT (ACS)**	**TOMMY ATKINS (ACS)**	**KENT (ACS)**	**TOMMY ATKINS (ACS)**
CA1	37.591 BC	49.618 AB	84.989 A	28.764 B	78.724 CD	61.307 B
CA1+1-MCP	33.563 BC	49.338 AB	94.609 A	29.584 B	82.511 C	70.289 AB
CA1+BC	48.907 A	53.647 A	69.510 C	36.313 A	117.356 A	63.442 AB
CA2	36.185 BC	45.345 BC	89.957 AB	25.534 BC	101.294 B	59.872 B
CA2+1-MCP	32.860 C	49.189 AB	92.465 AB	30.457 AB	70.046 DE	62.998 AB
CA2+BC	38.698 B	47.591 BC	85.131 B	29.817 B	69.042 E	60.326 B
Untreated	35.882 BC	42.907 C	67.714 C	20.865 C	73.188 DE	74.491 A
p-value	<0.0001	0.0021	<0.0001	0.0004	<0.0001	0.1463
	KENT (AR)	**TOMMY ATKINS (AR)**	**KENT (AR)**	**TOMMY ATKINS (AR)**	**KENT (AR)**	**TOMMY ATKINS (AR)**
CA1	48.449 B	55.502 A	32.256 B	41.905 AB	58.401 B	56.938 AB
CA1+1-MCP	49.208 B	55.313 A	32.329 B	45.149 A	60.176 B	56.467 AB
CA1+BC	48.493 B	53.036 AB	30.987 B	42.226 AB	58.479 B	53.215 BC
CA2	49.043 B	48.943 BC	28.846 B	41.418 AB	60.433 B	41.184 D
CA2+1-MCP	51.968 AB	51.651 AB	25.472 B	42.691 AB	71.062 A	45.531 CD
CA2+BC	47.963 B	45.707 C	27.536 B	39.331 B	57.272 B	41.728 D
Untreated	56.193 A	51.684 AB	69.246 A	41.195 B	69.662 A	63.474 A
p-value	0.0232	0.0012	<0.0001	0.1655	<0.0001	<0.0001

[1] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene; ACS: after cold storage; AR: after ripening

Table 3[2]. *The pulp colour of mango fruit after ripening, measured according to the light intensity, chroma and hue angle. Values with the same letter do not differ significantly, with respect to cultivar and colour parameter, according to Fisher's protected test.*

	Pulp colour (After Ripening)					
Treatment	**Light Intensity**		**Chroma**		**Hue angle**	
	KENT	**TOMMY ATKINS**	**KENT**	**TOMMY ATKINS**	**KENT**	**TOMMY ATKINS**
CA1	62.97 BC	76.487 AB	105.493 AB	62.004 C	73.464 C	80.233 A
CA1+1-MCP	65.896 B	74.191 ABC	110.795 A	70.924 A	74.517 BC	77.850 BC
CA1+BC	63.225 BC	77.04 A	108.665 A	65.301 BC	73.527 C	78.603 ABC
CA2	66.513 B	76.235 AB	100.6 B	63.373 C	76.500 AB	79.930 AB
CA2+1-MCP	74.477 A	73.412 ABC	68.825 E	68.803 AB	77.297 A	77.273 C
CA2+BC	65.186 B	71.398 C	77.025 D	65.698 BC	72.381 C	77.927 BC
Untreated	60.154 C	73.03 BC	84.325 C	64.727 BC	76.136 AB	79.173 ABC
p -value	<0.0001	0.0225	<0.0001	0.0011	0.0018	0.0898

[2] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

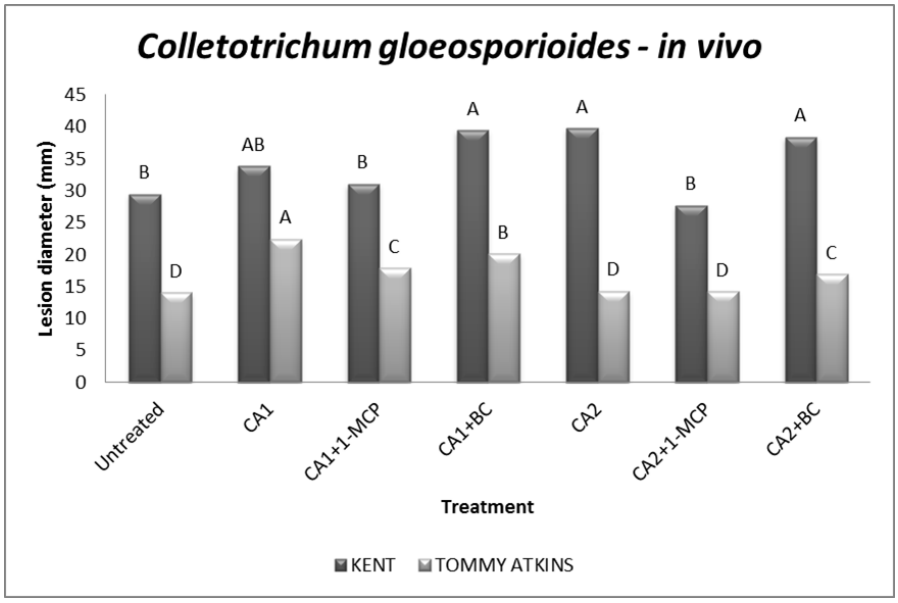

Fig. 1[3] *Lesion diameter of anthracnose lesions on mango fruit artificially inoculated with* <u>*Colletotrichum gloeosporioides*</u> *after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test.*

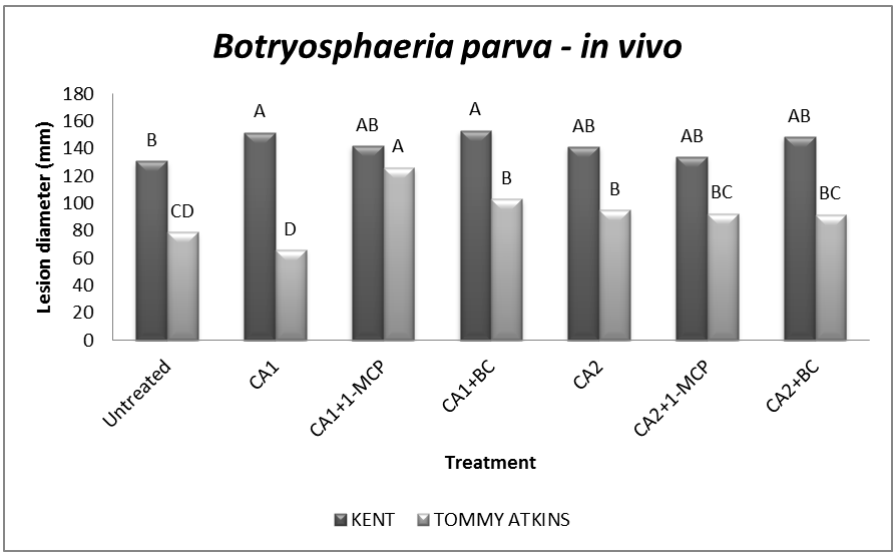

Fig. 2[4] *Lesion diameter of soft brown rot lesions on mango fruit artificially inoculated with* <u>*Botryosphaeria parva*</u> *after ripening, with respect to cultivars. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test.*

[3] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

[4] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

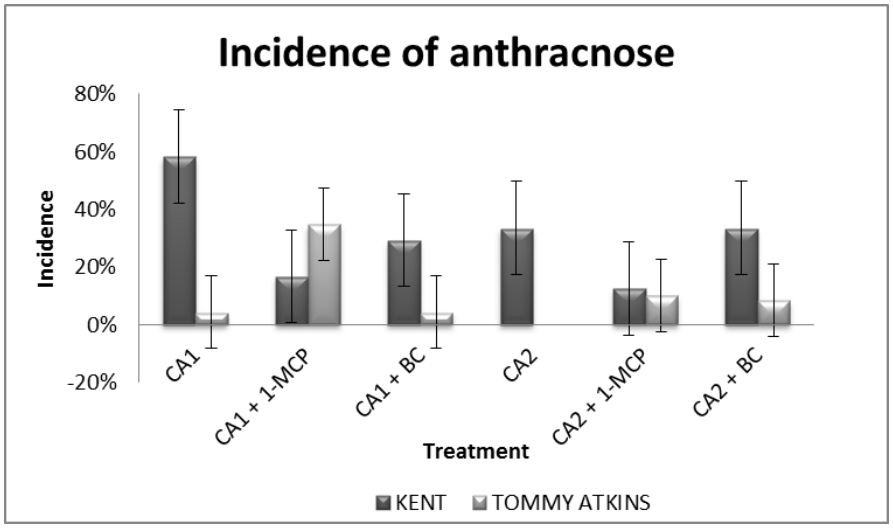

Fig. 3[5] *Incidence of anthracnose on naturally infected fruit from different treatments after cold storage. Error bars indicate standard deviation, with respect to cultivar.*

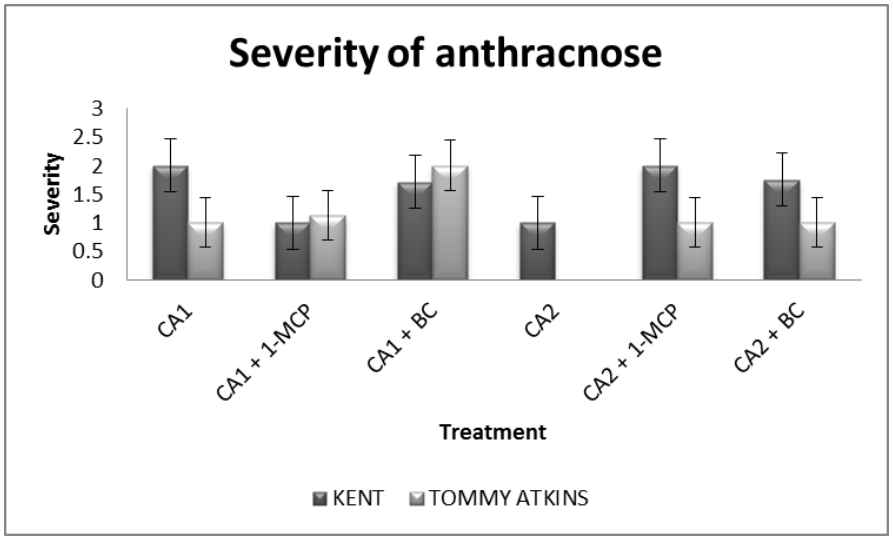

Fig. 4[6] *Severity of anthracnose on naturally infected mango fruit after cold storage. Evaluations were done on a scale of 1-5. Severity was determined by the amount of anthracnose lesions visible on the fruit: 1 – one lesion; 5 – 5+ lesions. Error bars indicate the standard deviation, with respect to cultivar.*

[5] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

[6] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

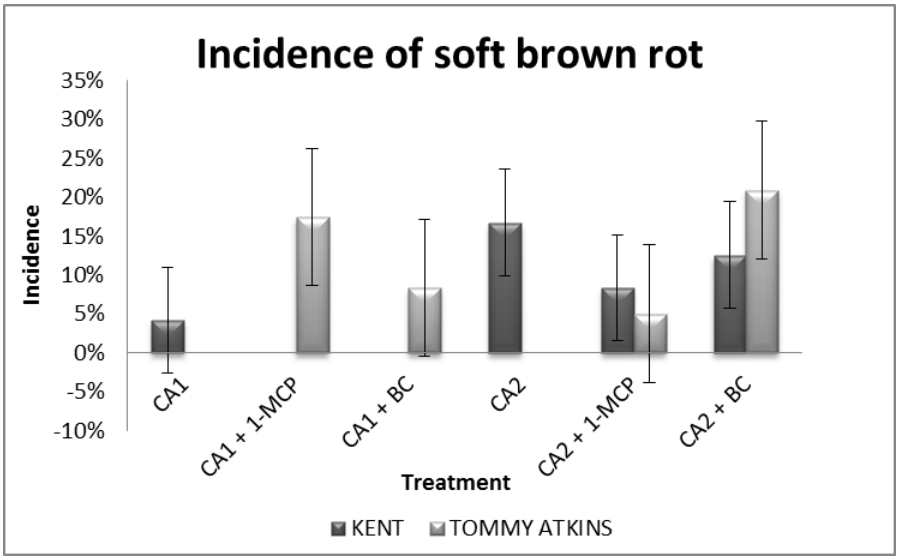

Fig. 5[7] *Incidence of soft brown rot on naturally infected fruit from different treatments after cold storage. Error bars indicate standard deviation, with respect to cultivar.*

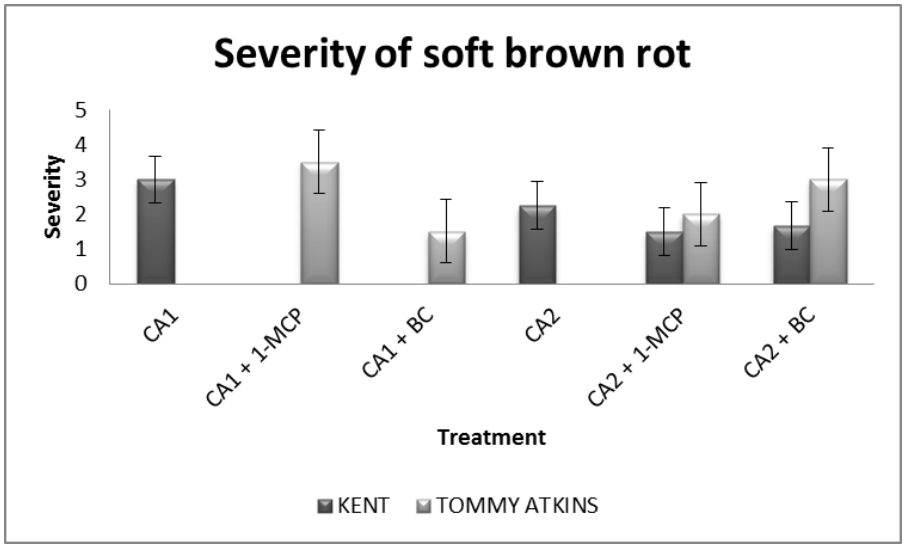

Fig. 6[8] *Severity of soft brown rot, on naturally infected mango fruit after cold storage. Evaluations were done on a scale of 1-5. Severity was determined by the total percentage of surface area the lesion covered; 1: 1-20%; 2: 21-40%; 3: 41-60%; 4: 61-80%; 5: 81-100%. Error bars indicate the standard deviation, with respect to cultivar.*

[7&6] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

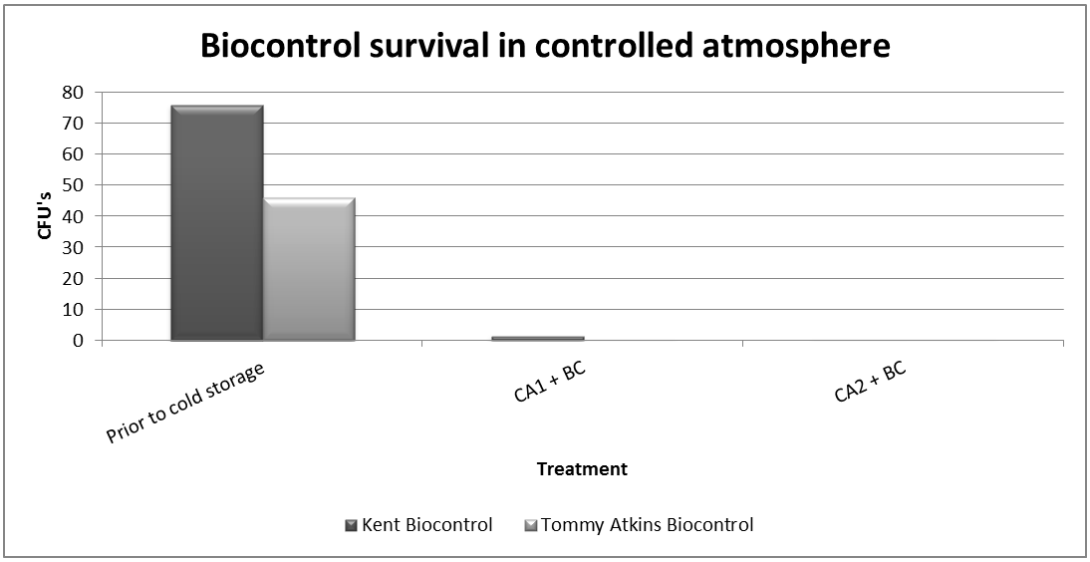

Fig. 7[9]: *The survival of Bacillus amyloliquefaciens PPCB004 on the surface of mangoes after cold storage compared to biocontrol recovery done 24 h after treatment.*

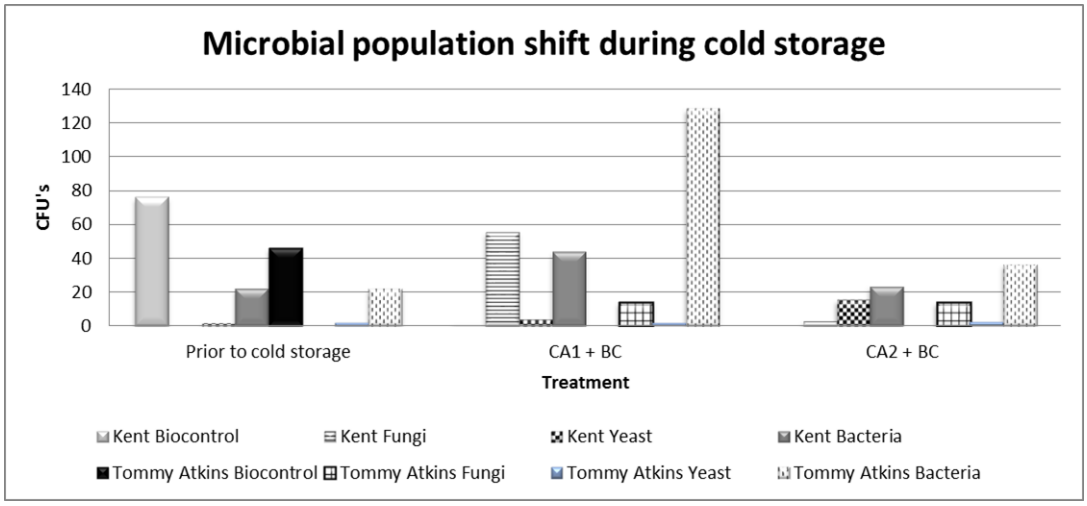

Fig. 8[10] *The microbial population shift observed on the surface of mango fruit treated with Bacillus amyloliquefaciens PPCB004, while in cold storage under controlled atmosphere.*

[9 & 8] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment

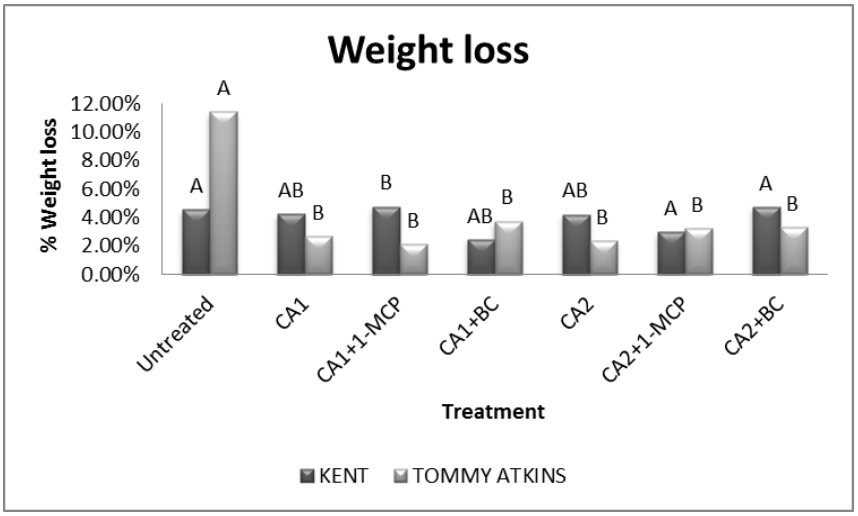

Fig. 9[11] *Percentage weight loss after mango fruit was removed from cold storage. Weight loss was not noted after ripening, because some fruit severely infected with soft brwon rot had to be removed from certain treatments. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (p-value – Kent: 0.0924; Tommy Atkins: 0.1002)*

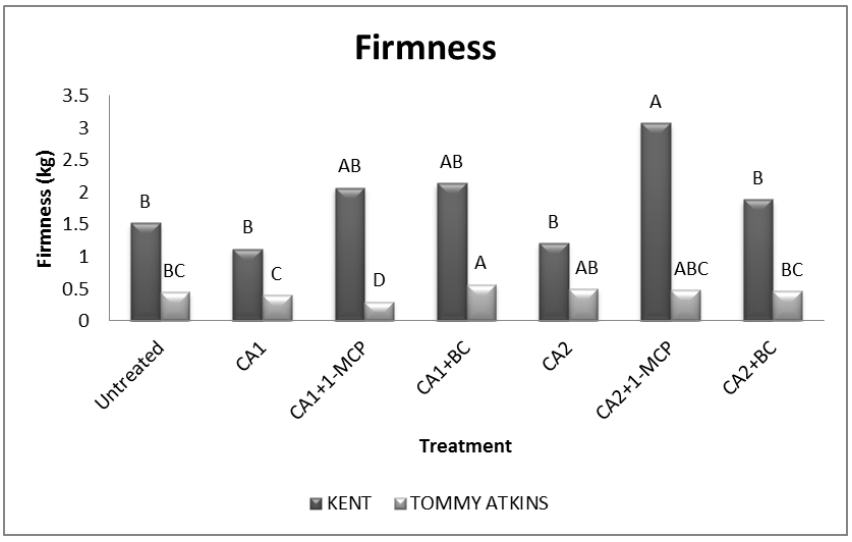

Fig. 10[12] *Firmness of mango fruit after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (p-value – Kent: 0.0123; Tommy Atkins: <0.0001)*

[11] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

[12] CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

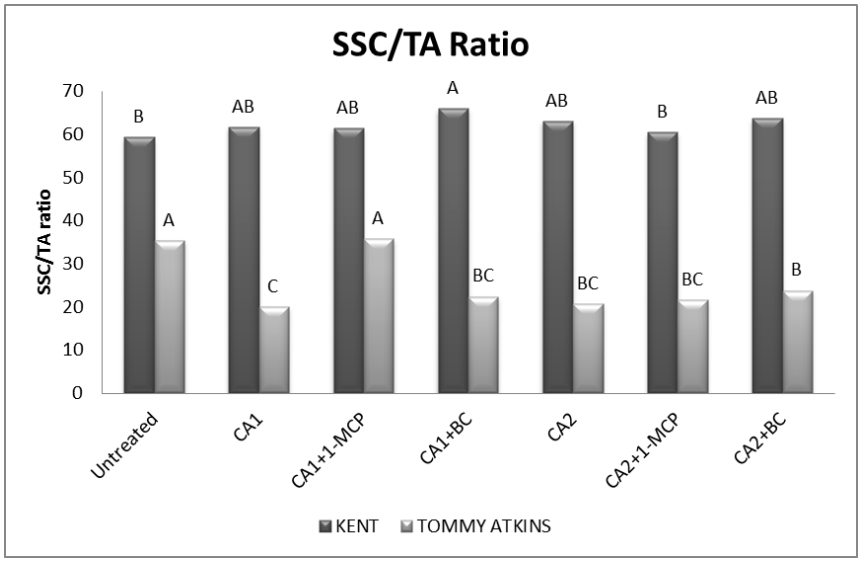

Fig. 11[13] *The sugar – acid ratio of mango fruit subjected to different treatments, after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (p-value – Kent: 0.1032; Tommy Atkins: <0.0001).*

[13]CA1: 5% CO_2 & 5% O_2 controlled atmosphere; CA2: 8% CO_2 & 3 O_2 controlled atmosphere; BC: *Bacillus amyloliquefaciens* PPCB004 treatment; 1-MCP: 1-methylcyclopropene

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CHAPTER 4

Effect of 1-methyl cyclopropene and *Bacillus amyloliquefaciens* on mango cultivars 'Kent' and 'Tommy Atkins' under conventional storage.

ABSTRACT

Postharvest losses of mangoes due to decay remain a problem for exporters. Postharvest treatments with prochloraz applied as a spray or dip give some control of anthracnose and soft brown rot, but not adequate to sustain a commercial viable industry. Alternative options are thus required. The combination of *Bacillus amyloliquefaciens* PPCB004 and 1-methylcyclopropene (1-MCP) to maintain quality and control postharvest diseases have proven to have potential on papaya. 'Tommy Atkins' and 'Kent' mangoes were subjected for two minutes to a 50 ºC hot water treatment, containing the biocontrol agent or not. Fruit were then subjected to a 24h 1-MCP (2mg / kg fruit) treatment at 16 ºC. Fruit artificially inoculated with *Botryosphaeria parva* and *Colletotrichum gloeosporioides* were subjected to the same treatments before storage at 10 ºC. After cold storage and ripening, fruit was evaluated for weight loss, firmness, SSC/TA, skin and pulp colour, and diseases incidence and severity. Although no *B. amyloliquefaciens* could be recovered from the surface of fruit, the combination of 1-MCP and biocontrol controlled anthracnose best in both cultivars. Pre-treatment with 1-MCP resulted in firmer 'Kent' fruit and also reduced weight loss in the same cultivar.

1. INTRODUCTION

The mango (*Mangifera indica* L.) tree originates from the Indo-Burmese region (Mukherjee, 1971) and bears a pleasant tasting fruit with a variety of beneficial health properties (Tharanathan *et al.*, 2006). The major cultivars produced in the Republic of South Africa for export purposes are 'Tommy Atkins', 'Kent' and 'Keitt', but the industry continuously faces challenges, including postharvest decay of fruit at export destinations (Fivaz, 2009). Two of the major postharvest diseases of mangoes include stem end rot / soft brown rot caused by *Botryosphaeria* spp. and anthracnose, caused by *Colletotrichum*

gloeosporioides. Both diseases can result in significant economic losses if not controlled effectively (Jeffries *et al.*, 1990; Arauz, 2000; Johnson, 1994).

Effective control of diseases has been achieved with pre – and – postharvest chemical sprays. For the South African industry however, only prochloraz is registered as a postharvest treatment to control soft brown rot and anthracnose (Nel *et al.*, 2003), with various success. The use of biocontrol agents as an postharvest disease control method in a variety of crops, have been shown (Droby, 2006; Janisiewicz and Korsten, 2002). Osman *et al.* (2010) further showed that *Bacillus amyloliquefaciens* PPCB004 has potential as a biocontrol agent to control anthracnose and phomopsis rot on papaya, pre-treated with 1-MCP.

The chemical product 1 - methylcyclopropene (1-MCP) was discovered in 1991 and have been applied as a gas treatment for fruit to inhibit the ripening process and extend shelf life (US Environmental Protection Agency, 2008). The effect of 1-MCP on mangoes have been described by various authors and the potential of using this product to extend the ripening period of mango cultivars, including 'Namh-dawg-mai-sri-tong', 'Kent', and 'Tommy Atkins' (Chaiprasart and Hansawasdi, 2009; Osuna-Garcia *et al.*, 2009; Alves *et al.*, 2004; Cocozza *et al.*, 2004). Effectiveness of 1-MCP to maintain quality of mango fruit depends on maturity at harvest, and to a lesser extent, cultivar (de Melo Silva *et al.*, 2004; Alves *et al.*, 2004; dos Santos *et al.*, 2004).

Mangoes have a limited postharvest life and although different postharvest treatments such as application of edible coatings, heat treatments, 1-MCP application, and biocontrol agents were researched, the objective of this investigation is to determine the combined effect of *B. amyloliquefaciens* PPCB004 and 1-MCP on mango cultivars 'Tommy Atkins' and 'Kent' on decay control and maintenance of fruit quality after cold storage and ripening.

2. MATERIALS AND METHODS

2.1. Fungal pathogens

During the 2008-2009 season, isolations were made from anthracnose and soft brown rot lesions from symptomatic 'Tommy Atkins' and 'Kent' mangoes as described in Chapter 3, section 2.1.

2.2. Identification of postharvest pathogens using the polymerase chain reaction (PCR)

Identification of pathogens was confirmed using PCR and sequencing as described in Chapter 3, section 2.2.

2.3. Fruit

Mango 'Tommy Atkins' and 'Kent' fruit (360 fruit of each cultivar) were hand harvested from a commercial orchard and treated as described in Chapter 3, section 2.3.

2.4. In vivo inoculation

In vivo inoculation of freshly harvested 'Tommy Atkins' and 'Kent' mangoes with *C. gloeosporioides* or *B. parva* were done as described in Chapter 3, section 2.4.

2.5. Postharvest treatments for naturally infected fruit

Fruit not artificially inoculated with the pathogens, were treated as described in Chapter 3, section 2.5.

2.5.1. Biocontrol treatment

Fruit subjected to the biocontrol treatment, were handled as described in Chapter 3, section 2.5.1.

2.5.2. One- methylcyclopropene treatment

Fruit subjected to a 24h, 1-MCP treatment, were handled as described in Chapter 3, section 2.5.2.

2.5.3. Waxing of fruit

Carnauba Tropical wax (Avello Agrochemicals, Tzaneen) were applied with roller brushes in the packhouse to fruit after treatment and dried under an electric fan.

2.6. Storage conditions

After treatment, 'Tommy Atkin' and 'Kent' mangoes were stored at 10 ºC ±2 (85% RH) on shelves in the cold room. The temperature and RH within the cold room was monitored with a U23-001 data-logger using Hoboware ® Pro data-logger software (Onset, Maryland, USA).

2.7. Effect of 1-methylcyclopropene treatment or biocontrol agent under conventional storage conditions on anthracnose and soft brown rot disease development in vivo

Batches of 216 'Tommy Atkins' or 'Kent' mango fruit at commercial harvest maturity (green-purple or green-red with well-developed shoulders) was inoculated with either *C. gloeosporioides, B. parva* or sterile agar. Artificial inoculation of fruit was done as described earlier.

Before subjection to different treatments (Fig. 1) fruit were kept at 16 ºC for 6 h to allow pathogen establishment. Untreated fruit, fruit subjected to hot water and fruit subjected to hot water and wax were included as comparative controls.

2.8. Effect of 1-methylcyclopropene treatment or biocontrol agent on control of postharvest decay and retention of fruit quality under conventional storage in naturally infected fruit

A total of 144 'Tommy Atkins' or 'Kent' mango fruit was subjected to various postharvest treatments (Fig. 2). Untreated fruit, fruit subjected to hot water and fruit subjected to hot water and wax were included as comparative controls. All the mentioned treatments had four replicate boxes and each box containing six fruit. The fruit subjected to all treatments were stored for 18 days at 10 ±2˚C and at 85% RH. At completion of 18 days storage, fruit were removed from cold storage and the effect of all treatments on decay incidence, weight loss, and skin colour was determined. Thereafter, fruit were allowed to ripen at 20 °C, 72% RH under normal atmosphere condition for five days. After ripening, skin and flesh colour, overall quality, incidence and severity of decay, fruit firmness, soluble solid concentration (SSC), titratable acidity (TA) and SSC/TA content were assessed.

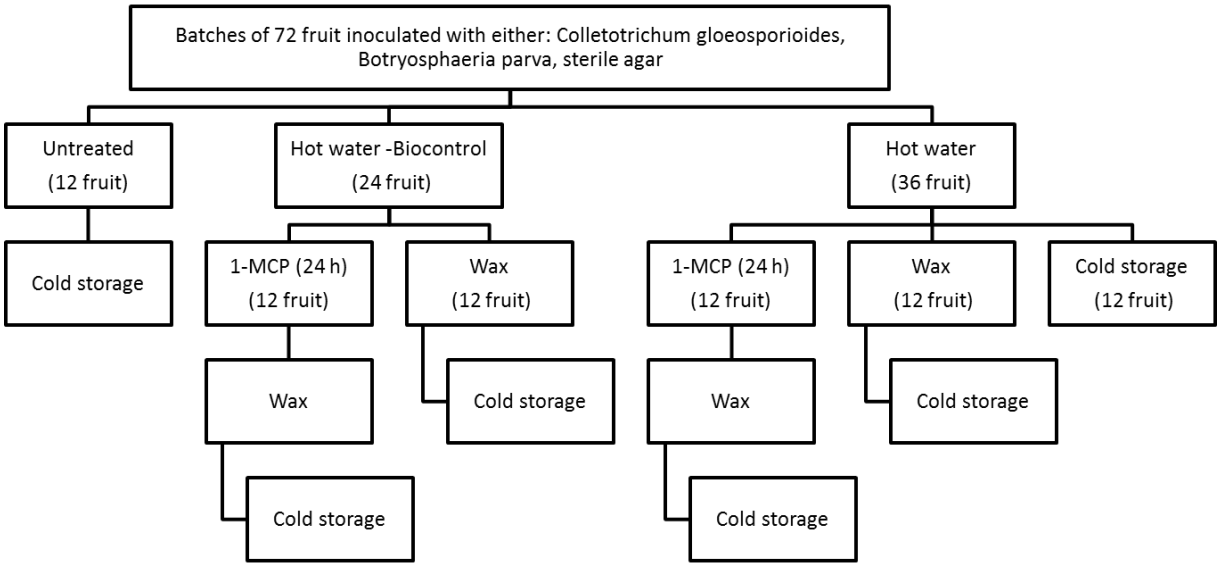

Fig. 1 *The sequence of events for fruit treated after inoculation with either Colletotrichum gloeosporioides, Botryosphaeria parva or sterile agar.*

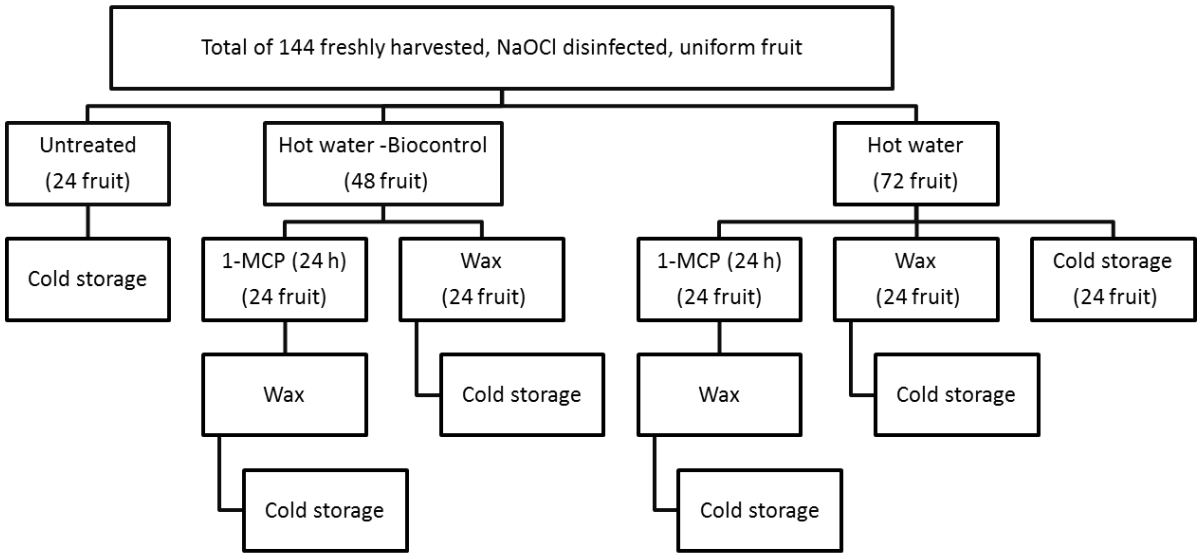

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Fig. 2 *Sequence of treatments applied to fruit prior to cold storage. Each treatment was separated into four replicates, each replicate consisting of six fruit.*

Table 1. *Fruit quality parameters at harvest for both export cultivars*

	External colour			Pulp colour				
Cultivar	**Light intensity**	**Chroma**	**Hue angle**	**Light intensity**	**Chroma**	**Hue angle**	**Firmness**	**SSC/TA[a]**
TOMMY ATKINS	42.86	24.14	73.72	82.13	53.66	86.93	2.13	13.69
KENT	54.04	23.55	93.16	86.37	45.68	86.59	1.95	9.04

a: SSC = Soluble solid concentration / TA = Titratable acidity

2.9. Fruit quality evaluation

2.9.1. Skin and flesh colour

Fruit (replicate boxes) subjected to all the treatments mentioned in Fig. 2 were weighed before and after 18 days storage and data was expressed as percentage weight loss. Fruit surface skin and flesh colour was measured (10 fruit per treatment) using a Hunterlab Miniscan XE Plus (Reston, Virginia, USA), expressing CIELAB Commission International de I Eclairage (CIE) colour space values (L^* *Chroma* h^0). Two spots on opposite sides of the fruit were measured and the mean of the two measurements were considered as one reading. Skin colour was measured after cold storage and also after ripening. Flesh colour was taken after cutting the fruit from two cut halves (10 fruit per treatment) after ripening.

The L* value express light intensity, where 0 = black and 100 = white; h^o is the angle in a colour wheel of 360º, with 0, 90, 180 and 270º representing the hues red, yellow, green and blue, respectively and C* is the intensity or purity of the hue (Phebe and Ong, 2010).

2.9.2. Fruit firmness

Fruit firmness was measured after ripening, on opposite sides of individual fruit (10 fruits per treatment) with a Chatillon penetrometer (Chatillon and Sons, New York, USA) equipped with a 6 mm diameter plunger capable of penetrating through the peel into the pulp.

2.9.3. Soluble solid concentration

A set of 10 fruit per replicate per treatment was randomly selected after ripening for soluble solid content (SSC) determination using a digital refractometer (Atago Co., Tokyo, Japan) and expressed in percentages (Sivakumar and Korsten, 2010).

2.9.4. Titratable acidity

Percentage titratable acidity (TA) was determined after ripening by titration of 10 ml of fruit juice with 0.04 g/100 ml NaOH, using a 1% solution of phenolphthalein (Saarchem, Midrand, South Africa) and calculated as citric acid (Factor: 0.0064) equivalent from 20 g flesh obtained from ten fruit separately (Sivakumar and Korsten, 2006).

$$\% \text{ Acid} = \frac{\text{Titre x Acid factor x 100}}{\text{Juice (ml)}}$$

2.10. Biocontrol recovery

Biocontrol (BC) and microbial recovery from the mango fruit skin surface was done according to the method by Govender (2005a) and Govender *et al.* (2005b), on treatments that received a biocontrol treatment (*B. amyloliquefaciens* PPCB004) within 24h after treatment as well as untreated fruit. Three fruit of each treatment were selected and individually placed in a beaker containing sterile ¼ strength Ringers solution (Merck). The beaker containing the fruit were placed in an ultrasonic water bath (Labex, Orange Grove) and sonicated for 30 s. The washing solution was vacuum filtered through a 0.22 µm filter paper. The filter paper was vortexed in ¼ Ringers solution and then serially diluted into four separate sets. Two sets were plated in triplicate onto Standard 1 (STD1) nutrient agar (Biolab, Wadeville, Gauteng) to recover *B. amyloliquefaciens* PPCB004 and other bacteria. The two remaining sets were plated out in triplicate onto malt extract agar (MEA) (Biolab) to recover fungi and yeast. Plates were incubated at 25 ºC for four days and biocontrol, other bacteria; fungi and yeast colonies were counted. The same procedure was followed within 24h after fruit were removed from cold storage to observe the population shift on the fruit phyloplane. Biocontrol colonies were distinguished from other colonies by comparing it to a STD1 plate containing a pure culture of *B. amyloliquefaciens.*

2.11. Statistical analysis

The experiment was repeated twice adopting a complete randomized design. Statistical evaluation of the differences was performed using analysis of variance (ANOVA). The mean values of the significant interactions were compared using the Fisher's protected t-

test L.S.D (least significant difference) at the 5% level using the SAS statistical software (Version 9.2, SAS institution, Cary, NC).

3. RESULTS

3.1. Confirmation of pathogenicity of pathogens

The selected *B. parva* and *C. gloeosporioides* isolates successfully infected mango fruit and pathogenicity was thus confirmed.

3.2 Effect of 1-methylcyclopropene treatment or biocontrol agent on postharvest decay incidence and severity in 'Tommy Atkins' and 'Kent' mangoes

3.2.1. Artificial inoculation with Colletotrichum gloeosporioides

From Fig. 3 it is clear that 'Tommy Atkins' fruit artificially inoculated with *C. gloeosporioides* showed to be less susceptible to anthracnose overall than 'Kent'. 'Kent' fruit artificially inoculated with *C. gloeosporioides* and subjected to the 1-MCP treatment generally had significantly smaller lesions than all the other treatments. The 1-MCP treated 'Kent' fruit in combination with biocontrol was found to have significantly smaller anthracnose lesions than any of the other treatments.

For 'Tommy Atkins' fruit, the opposite effect was observed. The addition of biocontrol to 1-MCP treated fruit reduced the size of anthracnose lesions significantly more than 1-MCP alone. However, both treatments had significantly larger lesions than fruit subjected to hot water and wax or the hot water-wax-biocontrol combination treatments. Both treatments that included 1-MCP failed to control the development of anthracnose lesions more effective than either of the hot water treated or untreated control fruit.

3.2.2 Artificial inoculation with Botryosphaeria parva

'Tommy Atkins' fruit were overall less susceptible to *Botryosphaeria parva* infection than 'Kent' (Fig. 4). No significant differences could be observed between treatments from 'Kent' fruit artificially inoculated with *B. parva* (p-value: 0.4425). However, 'Tommy Atkins' fruit were significantly more susceptible to soft brown rot after treatment with 1-

MCP than control fruit. Although not significant, the combination of 1-MCP and biocontrol controlled soft brown rot better than 1-MCP treatment alone in 'Tommy Atkins'. The control treatment consisting of hot water and wax controlled soft brown rot lesion size significantly better and performed the best on 'Tommy Atkins fruit'.

3.2.3. Natural incidence and severity of postharvest diseases on mango

The incidence of anthracnose was overall higher on 'Tommy Atkins' fruit (Fig. 5 A). 'Kent' fruit subjected to treatments that included wax had overall a lower incidence of anthracnose. No incidence of anthracnose could be found on 'Tommy Atkins' fruit subjected to the combination pre-treatment of biocontrol and 1-MCP. Incidence of anthracnose on 'Tommy Atkins', with respect to treatment was in the following order: Untreated > HW > HW+wax > HW+BC+wax > HW+1-MCP+wax > HW+1-MCP+BC+wax.

Incidence of soft brown rot was overall higher on 'Tommy Atkins' fruit (Fig. 5 B). 'Tommy Atkins' fruit subjected to biocontrol pre-treatment showed no incidence of soft brown rot. 'Kent' fruit subjected to 1-MCP pre-treatment or the combination of 1-MCP and biocontrol pre-treatment had no incidence of soft brown rot.

3.2.4. Biocontrol survival and fruit surface micro-population shift

From Fig. 7 it can be seen that almost no *B. amyloliquefciens* were recovered from the fruit surface of both cultivars after cold storage.

In Fig. 8 the microbial population shift on fruit surface of both cultivars can be seen. On 'Tommy Atkins' fruit the bacterial population increased during cold storage on fruit treated with the combination of 1-MCP and biocontrol. The fungal population increased on both cultivars fruit subjected only to biocontrol, but no increase in the fungal population was observed on 'Tommy Atkins' fruit subjected to the combination treatment and only a slight increase for 'Kent' fruit subjected to the combination treatment. Yeast populations did not change for both cultivars during the cold storage period. No real differences could be observed on the bacterial population shift for 'Kent' fruit.

3.3.1. Weight loss

Weight loss of treatment replicates was evaluated directly after cold storage but not after ripening (Fig. 9). This was done because some fruit had to be removed after cold storage because of total destruction by soft brwon rot. Weight loss in untreated 'Tommy Atkins' fruit was significantly higher than all the other treatments. No significant differences could however be observed between any of the other treatments (p-value: 0.0525).

In 'Kent' fruit weight loss were significantly higher for the untreated fruit and fruit subjected to a hot water treatment and weight lost was least in fruit treated with 1-MCP only. Fruit subjected to biocontrol only lost significantly more weight than fruit treated with 1-MCP + biocontrol, which again lost significantly more weight than fruit treated with 1-MCP only. Fruit subjected to hot water and wax only, did, although not significantly, lose less weight than the BC+1-MCP treatment but lost more weight than fruit subjected to 1-MCP only (p-value: <0.0001).

3.3.2. Firmness

Firmness of fruit was measured after ripening, so no correlation can be drawn between weight loss and firmness (Fig. 10). No significant differences in firmness could be observed between 'Tommy Atkins' treatments (p-value: 0.5682). However, 'Kent' fruit subjected to a 1-MCP treatment prior to cold storage remained significantly firmer after five days of ripening than any of the control treatments. The combination of 1-MCP and biocontrol caused fruit to remain significantly firmer than fruit subjected to either 1-MCP or biocontrol. No significant difference could however be observed between fruit treated with either 1-MCP or biocontrol (p-value: <0.0001).

3.3.3. Sugar/Acid ratio

'Tommy Atkins' mangoes subjected to 1-MCP only treatment had a significantly higher sugar/acid ratio after ripening than any of the other treatments (Fig. 11). The addition of the biocontrol agent to the 1-MCP treatment significantly reduced the SSC/TA ratio compared to 1-MCP treatment alone, although it is still significantly higher than the hot water control treatments. Untreated fruit were the only fruit with a significantly lower SCC/TA ratio than the 1-MCP + biocontrol or biocontrol only treatments (p-value: <0.0001).

For 'Kent' fruit the opposite was observed in the sense that fruit subjected to only a 1-MCP treatment had a significantly lower SSC/TA ratio than any of the other treatments after ripening. Also, fruit subjected to a combination treatment of 1-MCP and biocontrol or biocontrol only had a significantly lower SSC/TA ratio than the comparative hot water + wax control treatment and the untreated fruit. (p-value: <0.0001).

3.3.4. Pulp colour

For 'Kent' fruit no differences could be observed in light intensity (Table 2) of fruit pulp (p-value: 0.1928). The light intensity of the pulp of 'Tommy Atkins' fruit subjected to 1-MCP was significantly lower than untreated fruit, fruit subjected to hot water or fruit treated with biocontrol (p-value: 0.0003). The chroma (Table 2) intensity of the pulp of both cultivars did not give any meaningful results (p-value Kent: 0.2256 and Tommy Atkins: 0.0107). No differences in the pulp colour according to the hue angle (Table 2) could be observed for 'Kent' fruit, except that all treatments pulp changed colour, according to the hue^{o}, from yellow to a more yellow red (orange) (p-value: 0.8785).

In general, the pulp colour of 'Tommy Atkins' were more yellow than 'Kent' fruit after ripening. No meaningful significant differences could be observed between 'Tommy Atkins' treatments, but fruit treated with 1-MCP had a lower hue^{o} pulp colour than all the other treatments (p-value: 0.2131).

3.3.5. Skin colour

For both cultivars the hue angle value gradually decreased during storage. No significant differences could however be observed between the skin colour of fruit subjected to the different treatments for 'Tommy Atkins' according to the hue angle (Table 3) after cold storage (ACS) or after ripening (AR) (p-values: ACS: 0.3177 and AR: 0.3702). 'Tommy Atkins' fruit treated with 1-MCP in this study had a slightly lower hue angle colour value than control fruit.

'Kent' fruit treated with 1-MCP or 1-MCP + biocontrol had a significantly higher hue angle value after cold storage, hence a greener colour than any other treatment. After ripening only the combination treatment of 1-MCP and biocontrol maintained a significantly greener colour than any of the other treatments (p-value: ACS and AR: <0.0001). It has to be noted that the light intensity 'Kent' fruit had decreased during ripening, whereas that of

'Tommy Atkins' have increased during ripening. The chroma of 'Kent' and 'Tommy Atkins' fruit, treated with the combination of 1-MCP and biocontrol, was also lower than other treatments.

4. DISCUSSION

Results of this study coincides with reports by Auraz (2000) and Nelson (2008) that 'Tommy Atkins' is less susceptible to anthracnose than 'Kent', which is known to be very susceptible. Hofman *et al.* (2001) also observed in Kensington Pride mangoes an increase in stem rots on fruit treated with 1-MCP which is similar to what was found in this study for 'Tommy Atkins'. Janisiewicz *et al.* (2003), found that treatment with 1-MCP increased bitter rot and blue mould on apples after cold storage, but also that the addition of an antagonist (*Metchnikowia pulcherrima* T5-A2) were able to sufficiently control both pathogens, irrespective of the treatment. Contradictory to this, Osman *et al.* (2010) observed that papaya pre-treated with 1-MCP only or in combination with *B. amyloliquefaciens* PPCB004 had a significantly lower incidence of anthracnose and *Phomopsis* rot after cold storage.

Contradictory to what was observed in this study, Govender *et al.* (2005a; 2005b) used *B. licheniformis* as biocontrol agent against anthracnose and soft brown rot on 'Keitt' and 'Tommy Atkins' mangoes and was able to recover the biocontrol agent from the fruit surface after 21 days of cold storage. Osman *et al.* (2010), was also able to recover *B. amyloliquefaciens* PPCB004 from 'Solo' papaya pre-treated with 1-MCP, after cold storage and ripening. It has to be noted that the *B. licheniformis* isolate used by Govender was originally isolated from mango, whereas the *B. amyloliquefaciens* used in this study was originally isolated from citrus. Govender (2005a) noted that on 'Tommy Atkins' mangoes an increase of yeast populations could be observed during storage, which is contradictory to what was found in this study for both cultivars. However, similarly to what was found in this study on 'Tommy Atkins', fruit subjected to biocontrol treatment only, Govender (2005a) noted an increase in background fungal populations during the storage period on 'Tommy Atkins'.

In this study mangoes were subjected to wax in combination with 1-MCP, but similarly in unwaxed fruit, Chaiprasart and Hansawasdi (2009) found that treatment with 1-MCP had no effect on weight loss in 'Namh-dawg-mai-sri-tong' mangoes. Hofman *et al.* (2001) also observed no difference in weight loss on 'Kensington Pride' mangoes treated with 1-MCP only and stored at 20 ºC, when compared to untreated fruit. However, Alves *et*

al. (2004) found that weight loss in unwaxed 'Tommy Atkins' fruit treated with 1-MCP was less than the control fruit. This reduction in weight loss due to 1-MCP in combination with wax treatment has also been reported for avocado, whereas unwaxed avocado treated with 1-MCP showed increased weight loss after storage for seven days at 20 ºC (Jeong *et al.*, 2003), which coincides with the findings of our study on mangoes.

The results found in this study on 'Tommy Atkins' was contradictory to observations made by Alves *et al.* (2004), who reported that this cultivar treated with 1-MCP remained firmer for a longer period than control fruit, when stored at 25 ºC. However, similary to our observations Chaiprasart and Hansawasdi (2009) reported that the treatment of 'Namh-dawg-mai-sri-tong' mangoes with a lower concentration 1-MCP (500ppb) were not able to maintain firmness better than control fruit when stored at 25 ºC. They did however observe that fruit subjected to a higher concentration 1-MCP (1000ppb) maintained firmness for three days before starting to deteriorate.

In this study, similar results for 'Kent' fruit to that reported by Osuna-Garcia *et al.* (2009) was observed for the same cultivar. They found that after 20 days of cold storage, fruit treated with 1-MCP were significantly firmer than control fruit. Also, they found that fruit treated with 1-MCP reached eating ripe firmness four days later than control fruit, once removed from cold storage. Wang *et al.* (2006) also found that treatment with 1-MCP significantly kept 'Guifei' firmer than the control and for a longer period.

Contradictory to what was found on 'Tommy Atkins' fruit but similar to results obtained on 'Kent', it was observed the SSC/TA ratio increased slower in fruit treated with 1-MCP compared to control fruit on 'Namh-dawg-mai-sri-tong' mangoes (Chaiprasart and Hansawasdi, 2009). Wang *et al.* (2006) found that 1-MCP treatment delayed the decrease of titratable acidity in 'Guifei' mangoes after storage at 20 ºC and Hofman *et al.* (2001) failed to find any treatment differences for brix and TA on 'Kensington Pride' mangoes.

Similar results obtained for 'Tommy Atkins' in this study was described by Chaiprasart and Hansawasdi (2009) who also found on 'Namh-dawg-mai-sri-tong' no significant differences in the colour change according to the hue angle after storage at room temperature. However, fruit treated with 1-MCP had a slightly higher hue angle value than control fruit. Hofman *et al.* (2001) found in 'Kensington Pride' mangoes that a significant lower light intensity of fruit skin could be observed, compared to control fruit. This observation coincides with others made in this study with 'Kent' fruit after a 1-MCP, biocontrol treatment. They could however find no treatment difference for hue angle or chroma, whereas in this study with 'Kent', meaningful significant differences could be

observed between 1-MCP treated and control fruit after ripening. Plotto *et al.* (2003) reported that fresh-cut slices made 24h after treatment of intact 'Tommy Atkins' fruit pre-treated with 1-MCP and hot water had a higher L* value than control fruit after 14 days of storage.

5. CONCLUSION

It can be concluded that after being subjected to a 1-MCP treatment, 'Tommy Atkins' fruit were more susceptible to artificially inoculated soft brown rot and anthracnose. However, the addition of biocontrol to the 1-MCP treatment reduced the severity of artificially inoculated anthracnose and soft brown rot in this cultivar. The combination of 1-MCP and biocontrol controlled artificially inoculated anthracnose on 'Kent' the best. The application of 1-MCP prior to cold storage to 'Tommy Atkins' had no effect on weight loss, but significantly reduced weight loss in 'Kent'. Treatment with 1-MCP also resulted in firmer 'Kent' fruit after ripening and the combination of 1-MCP and biocontrol which resulted in fruit with a higher hue angle skin colour, after ripening. Overall, the addition of 1-MCP in combination with biocontrol maintained quality of 'Kent' mangoes best.

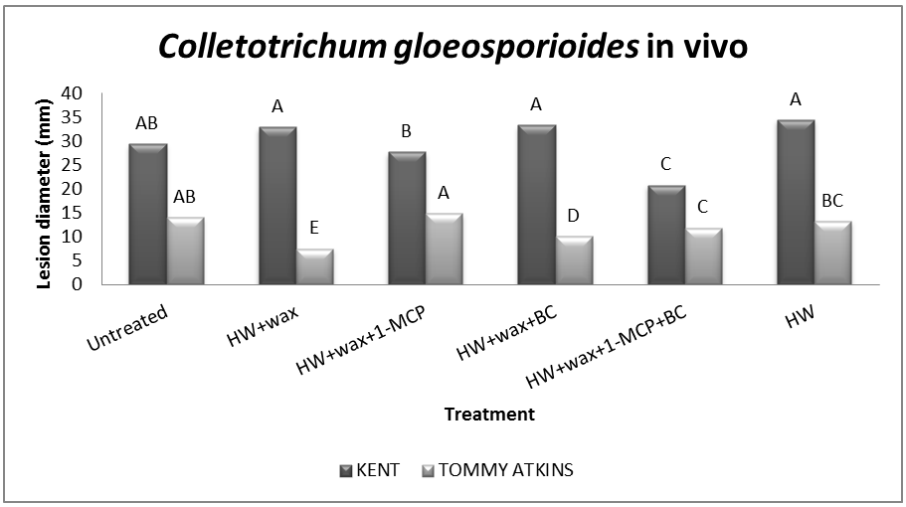


Fig. 3 [14]*Lesion diameters of anthracnose lesions on mango fruit artificially inoculated with Colletotrichum gloeosporioides after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (Kent: p<0.0001; Tommy Atkins: p<0.0001).*

[14] BC – Biocontrol; HW – Hot water treatment

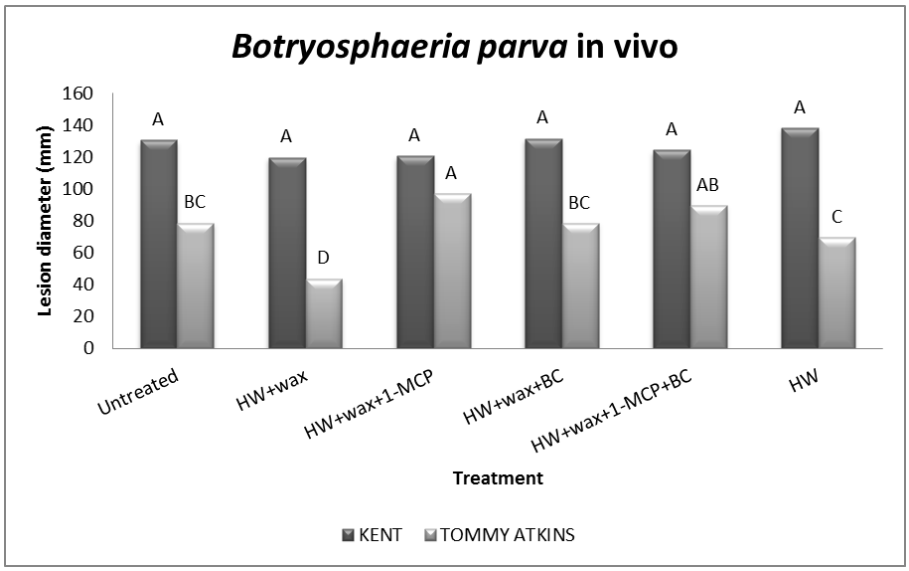


Fig. 4[15] *Lesion diameters of soft brown rot lesions on mango fruit artificially inoculated with* <u>*Botryosphaeria parva*</u> *after ripening, with respect to cultivars. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (Kent: p= 0.4425; Tommy Atkins: p<0.0001).*

[15] BC – Biocontrol; HW – Hot water treatment

Table 2[16]. *The pulp colour of mango fruit after ripening, measured according to the light intensity, chroma and hue angle.*

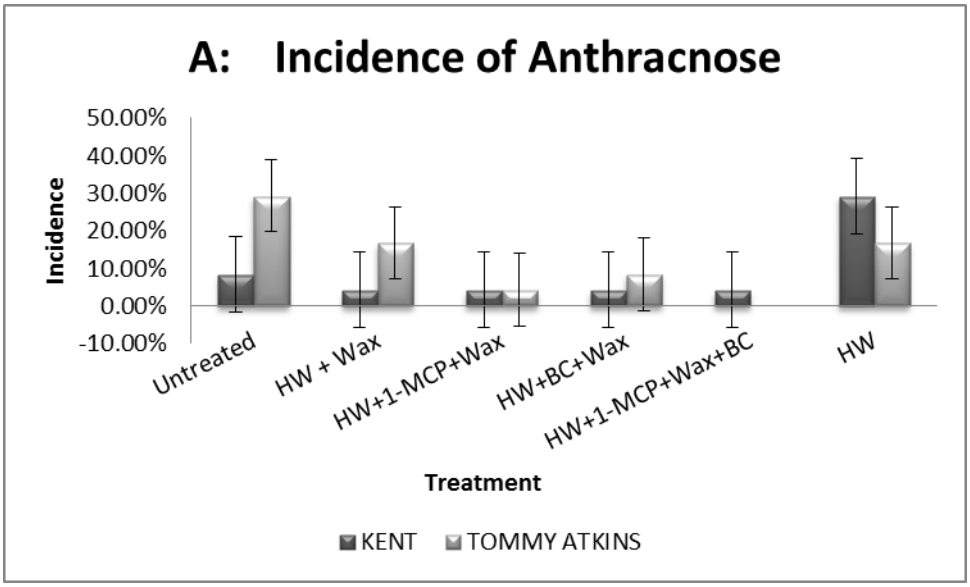
	Pulp Colour (After Ripening)					
Treatment	**Light intensity**		**Chroma**		**Hue angle**	
	KENT	**TOMMY ATKINS**	**KENT**	**TOMMY ATKINS**	**KENT**	**TOMMY ATKINS**
Untreated	60.154 A	73.030 B	84.325 B	64.727 AB	76.136 A	79.173 AB
HW+wax	59.833 A	70.621 BC	88.240 AB	61.478 B	75.311 A	78.532 AB
HW+wax+1-MCP	63.003 A	68.529 C	85.909 AB	66.934 A	75.584 A	76.586 B
HW+wax+BC	63.517 A	72.155 B	88.822 AB	67.551 A	75.038 A	77.527 AB
HW+wax+1-MCP+BC	65.340 A	71.464 BC	84.893 B	67.441 A	75.566 A	78.927 AB
HW	65.326 A	77.453 A	94.734 A	60.251 B	75.204 A	80.213 A
p-value	0.1928	0.0003	0.2256	0.0107	0.8785	0.2131

[16] Values with the same letter do not differ significantly, with respect to cultivar and colour parameter, according to Fisher's protected test. HW - Hot water; BC – Biocontrol (*Bacillus amyloliquefaciens* PPCB004).

Table 3[17]. *The skin colour of mango fruit after cold storage and after ripening, measured according to the light intensity, chroma and hue angle.*

	Skin Colour (After cold storage & After ripening)					
Treatment	**Light Intensity**		**Chroma**		**Hue angle**	
	KENT (ACS)	**TOMMY ATKINS (ACS)**	**KENT (ACS)**	**TOMMY ATKINS (ACS)**	**KENT (ACS)**	**TOMMY ATKINS (ACS)**
Untreated	59.392 CD	42.907 B	35.882 C	20.865 B	67.714 C	74.491 A
HW+wax	65.114 AB	45.324 AB	48.951 A	25.159 AB	72.906 C	73.837 A
HW+wax+1-MCP	58.085 D	47.470 A	37.286 C	28.107 A	95.812 A	73.234 A
HW+wax+BC	62.332 BC	45.496 AB	45.676 AB	26.222 A	82.571 B	66.748 A
HW+wax+1-MCP+BC	59.231 CD	44.987 AB	37.635 C	27.378 A	93.743 A	58.496 A
HW	67.184 A	44.683 AB	44.683 B	23.608 AB	78.710 B	64.305 A
p-value	<0.0001	0.4893	<0.0001	0.089	<0.0001	0.3177
	KENT (AR)	**TOMMY ATKINS (AR)**	**KENT (AR)**	**TOMMY ATKINS (AR)**	**KENT (AR)**	**TOMMY ATKINS (AR)**
Untreated	56.193 A	51.684 AB	69.246 B	41.195 A	69.662 B	63.474 A
HW+wax	53.442 AB	49.501 AB	81.677 A	39.947 A	72.089 B	51.369 A
HW+wax+1-MCP	54.693 AB	54.562 A	81.521 A	42.307 A	72.697 B	61.037 A
HW+wax+BC	51.303 B	50.930 AB	82.118 A	38.77 A	72.083 B	55.356 A
HW+wax+1-MCP+BC	45.257 C	45.904 B	59.028 C	33.951 B	86.205 A	53.408 A
HW	53.269 AB	51.163 AB	67.591 BC	40.601 A	71.540 B	54.133 A
p-value	0.0007	0.1419	<0.0001	0.0071	<0.0001	0.3702

[17] Values with the same letter do not differ significantly, with respect to cultivar, colour parameter and stage of ripening, according to Fisher's protected test. ACS: after cold storage; AR: after ripening.



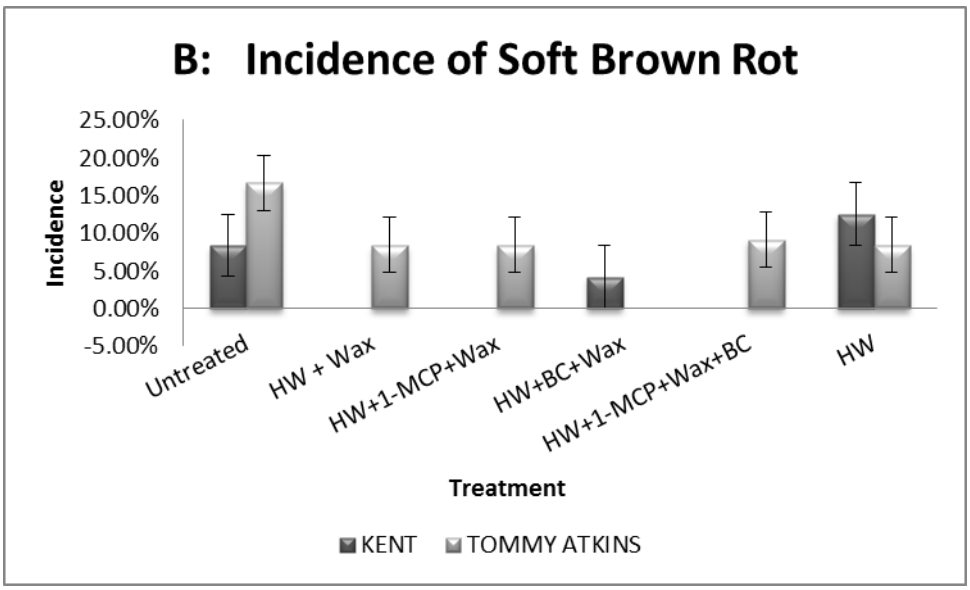


Fig. 5 A & B[18] *Natural incidence (percentage of fruit infected per treatment) of anthracnose (A)and soft brown rot (B) after cold storage on mango fruit subjected to different treatments, with respect to cultivar.*

[18] BC: Biocontrol; HW: Hot water treatment

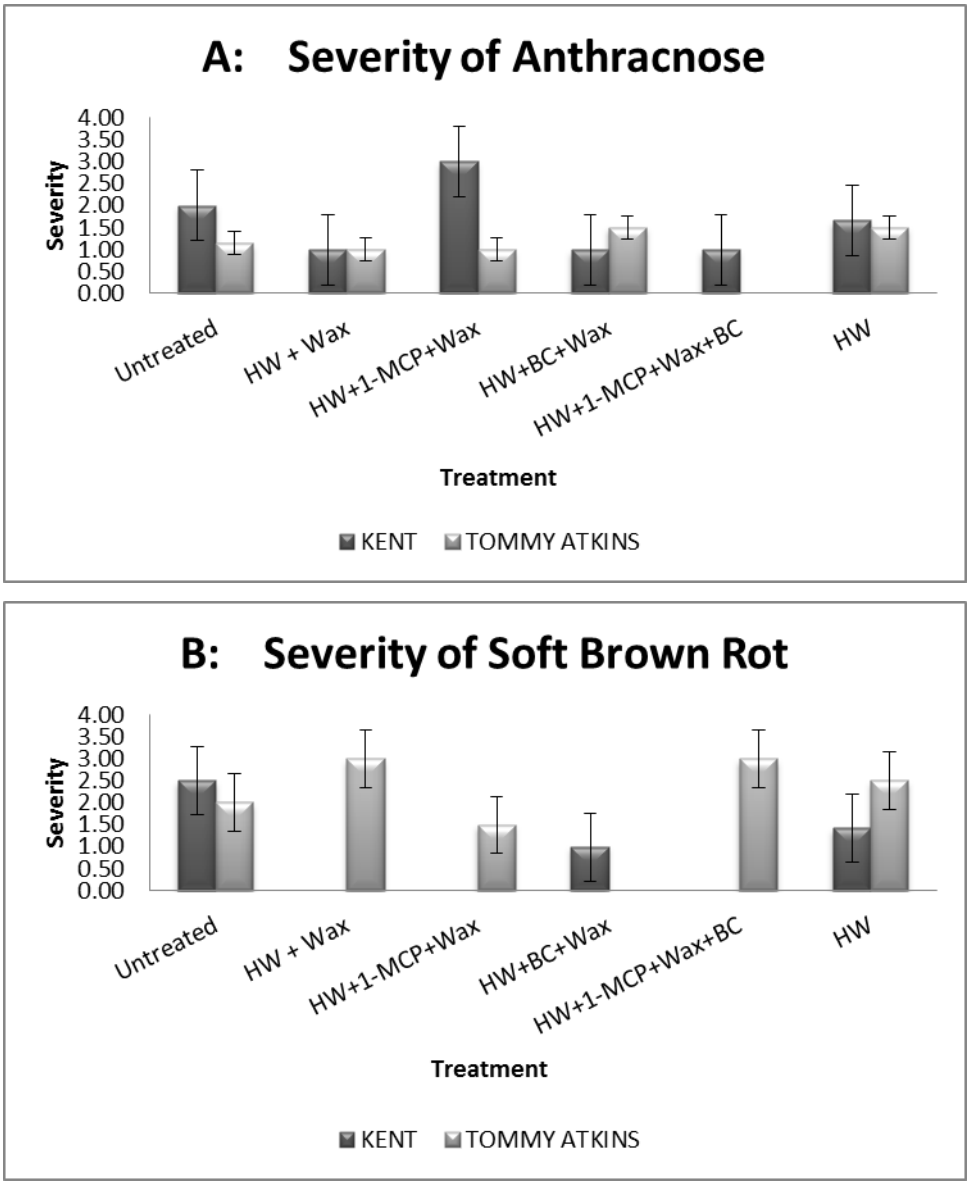


Fig. 6 A & B[19] *Severity of the postharvest diseases, anthracnose and soft brown rot, on naturally infected mango fruit after cold storage. Evaluations were done on a scale of 1-5. For anthracnose the severity was determined by the amount of anthracnose lesions visible on the fruit: 1 – one lesion; 5 – 5+ lesions. For soft brown rot severity was determined by the total % of surface area the lesion covered; 1: 1-20%; 2: 21-40%; 3: 41-60%; 4: 61-80%; 5: 81-100%.*

[19] BC: Biocontrol; HW: Hot water treatment

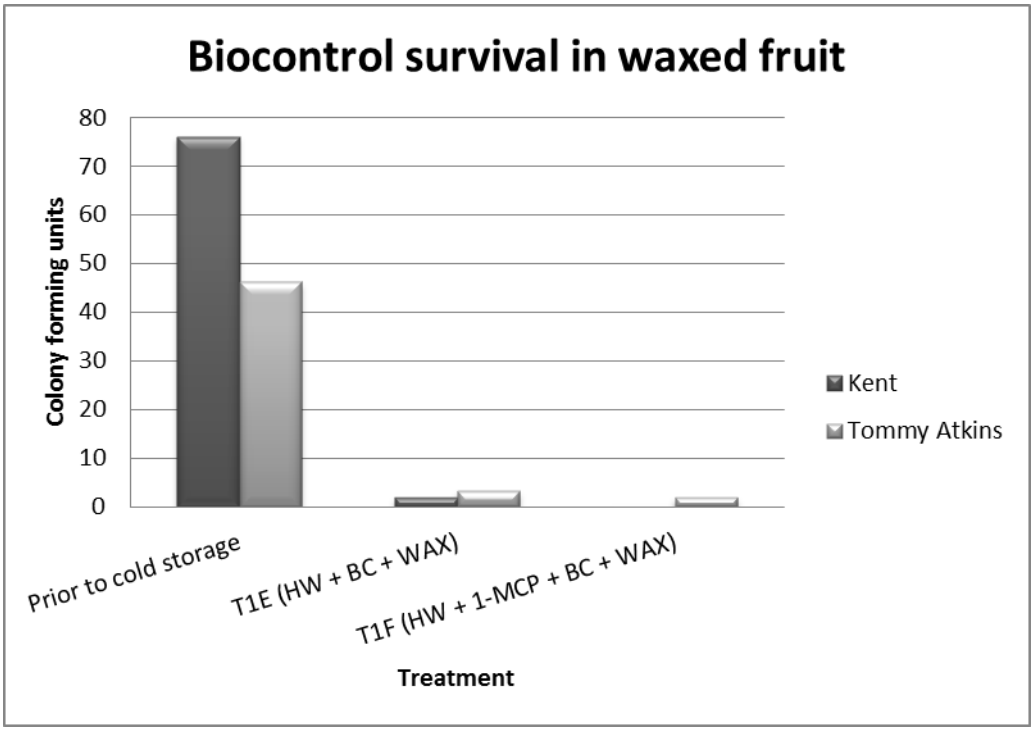


Fig. 7[20] *The survival of Bacillus amyloliquefaciens PPCB004 on the surface of mangoes after cold storage compared to biocontrol recovery done 24 h after treatment.*

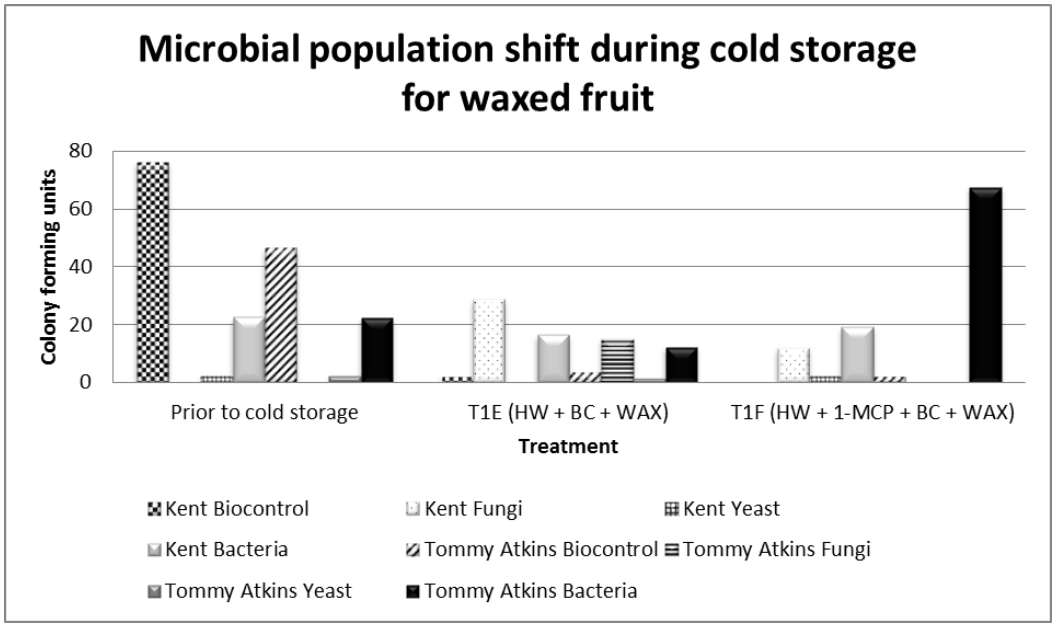


Fig. 8[21] *The microbial population shift observed on the surface of mango fruit treated with Bacillus amyloliquefaciens PPCB004, while in cold storage.*

[20]BC: Biocontrol; HW: Hot water treatment

[21] BC: Biocontrol; HW: hot water treatment

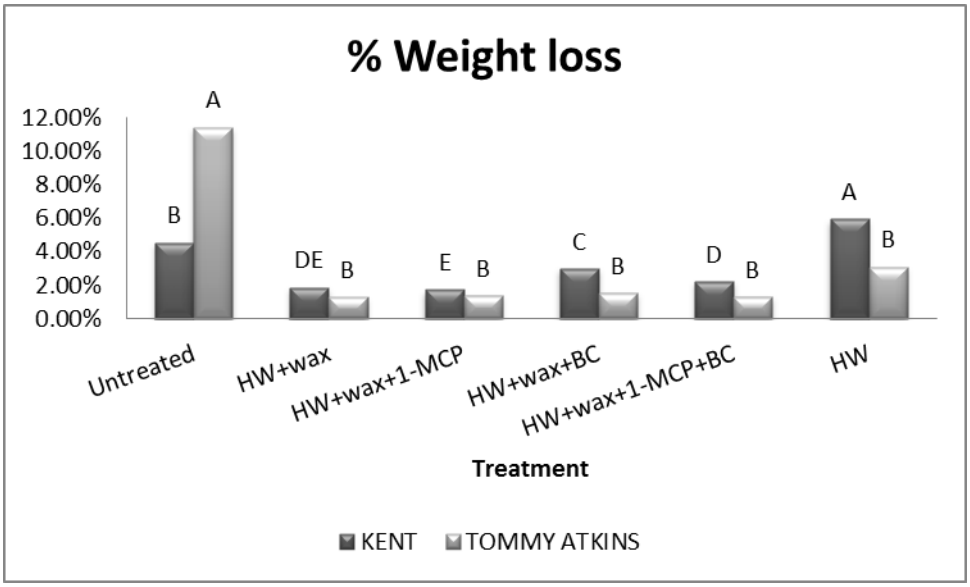


Fig. 9 [22] *Percentage weight loss after mango fruit was removed from cold storage. Weight loss was not noted after ripening, because some fruit severely infected with soft brown rot had to be removed from certain treatments. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (Kent: p <0.0001; Tommy Atkins: p= 0.0525).*

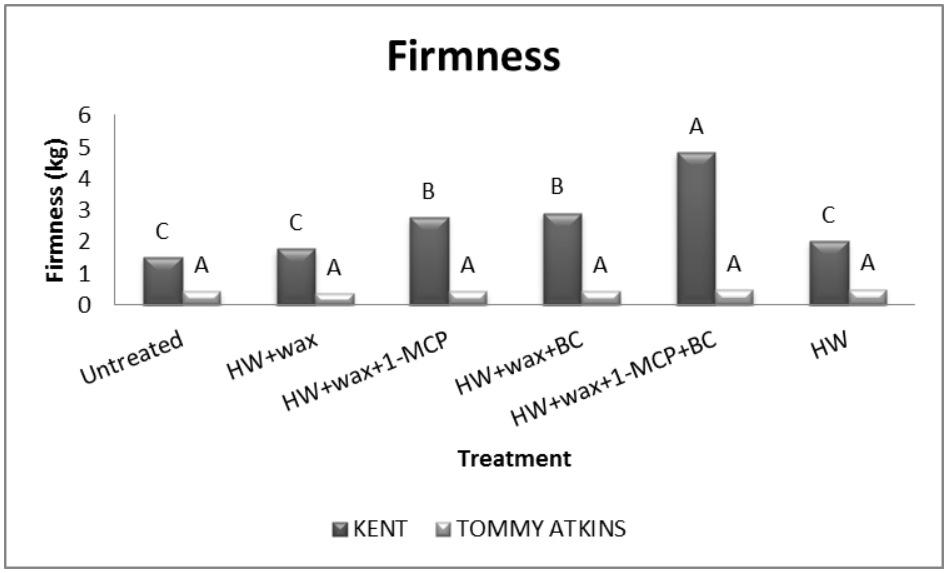


Fig. 10[23] *Firmness of mango fruit after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (Kent: p <0.0001; Tommy Atkins: p= 0.5682).*

[22] BC: Biocontrol; HW: Hot water treatment

[23] BC: Biocontrol; HW: Hot water treatment

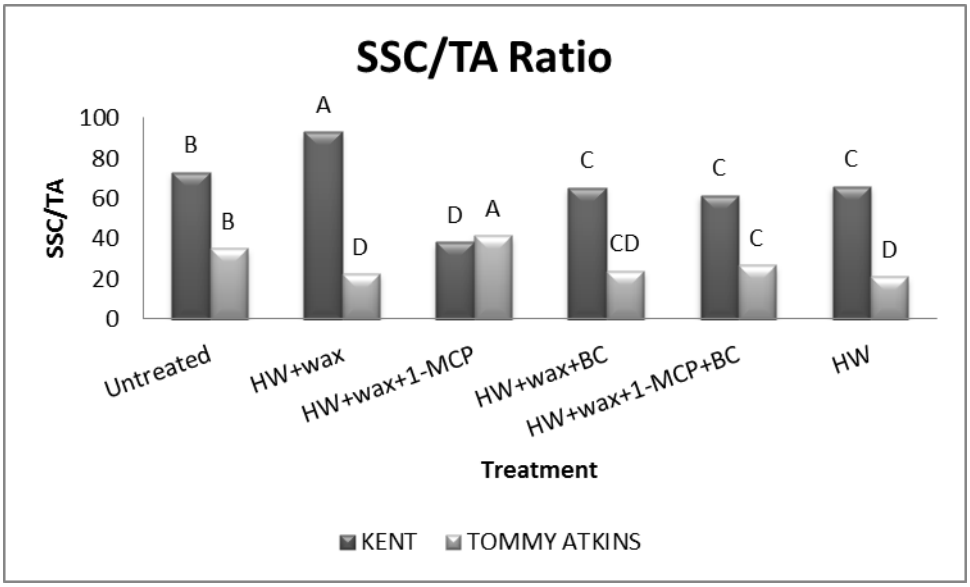


Fig. 11[24] *The sugar – acid ratio of mango fruit subjected to different postharvest treatments and after ripening. Columns with the same letter do not differ significantly, with respect to cultivar, according to Fisher's protected test (Kent and Tommy Atkins: $p < 0.0001$).*

[24] BC: Biocontrol; HW: Hot water treatment

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CHAPTER 5

Effect of modified atmosphere packaging in combination with *Bacillus amyloliquefaciens* on postharvest quality of 'Tommy Atkins' mango

ABSTRACT

Economic losses due to poor quality and postharvest diseases of mango fruit during export are some of the problems South African exporters face. Modified atmosphere packaging (MAP) has been shown to be successful in addressing these problems in some fruit and vegetables. *Bacillus amyloliquefaciens* PPCB004 in combination with MAP was able to retain quality and control disease in peaches during cold storage. Freshly harvested 'Tommy Atkins' mangoes were packed in four different film type bags untreated or after being subjected to a cold, *B. amyloliquefaciens* (8.3×10^6 cells/ml) dip treatment. After cold storage for 23 days, severe damage caused by low O_2 or high CO_2 conditions could be observed on fruit from certain bags. The incidence of anthracnose was completely inhibited by two of the bag types. None of the MAP used were able to retain quality of mango fruit at a level fit for either human consumption or marketing.

1. INTRODUCTION

The mango (*Mangifera indica* L.), originates from the Indo-Burmese region and belongs to the Anacardiaceae family. It bears a large drupe fruit (Tharanathan *et al.*, 2006), that are very susceptible to damage caused by improper handling (Yahia, 1998). Consumer acceptance of this fruit is highly influenced by taste, flavour, skin colour and pulp colour (Tharanathan *et al.*, 2006; Barrett *et al.*, 2010).

The Republic of South Africa contributes only 0.22% to world production, producing mainly 'Florida' originating cultivars 'Kent', 'Keitt' and 'Tommy Atkins' for export purposes. Exporters however, remain under pressure because of various factors, one of them being the economically important postharvest diseases anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. in Penz.) and soft brown rot (*Botryosphaeria* spp.). Unfortunately, control strategies against these pathogens are limited and, increasing limitations on the use of synthetic fungicides enforced by export destinations, are not favourable for exports (Fivaz, 2009).

Modified atmosphere packaging (MAP), is the storage of fruit and vegetables in plastic film that allows the exchange of gases and vapour, so that a specific equilibrium of CO_2 and O_2 is maintained inside the packaging (Mir and Beaudry, 2002). The use of MAP in mangoes have shown some potential to maintain quality, specifically by reducing weight loss, pathological or physiological disorders and delayed ripening (Reddy and Raju, 1988; Gonzalez et al., 1990; Castro *et al.*, 2005). The retention of mango fruit quality in MAP can be ascribed to the reduced O_2 and elevated CO_2 levels, which suppress respiration and water loss (Ben-Ari *et al.*, 2001). Kader (1994), describes mangoes as moderately susceptible to damage caused by low O_2 and high CO_2.

Some researchers have changed their focus to the development of biocontrol agents as viable alternatives to control postharvest diseases (Wisniewski *et al.*, 2007). The driving force behind this was the increasing negative perception amongst consumers about the use of synthetic fungicides on fresh produce. Given the potential alternative strategy, another motivation was the always present potential of resistant pathogen strains evolving after continues use of pesticides (Wisniewski *et al.*, 2007). Various biological products are commercially available to control postharvest diseases on amongst others citrus, apples, pears and avocado (Janisiewicz and Korsten, 2002).

The use of *Bacillus amyloliquefaciens* PPCB004 in combination with lemon grass oil and modified atmosphere packaging on peaches showed a reduction of disease after cold storage as well as retention of overall fruit quality (Arrebola *et al.*, 2009). Arrebola *et al.* (2010) concluded that the iturin lipopeptide family, specifically iturin A are the major metabolites responsible for the antagonistic properties of *B. amyloliquefaciens* PPCB004.

The combination of MAP and biological control on mangoes to control disease and maintain quality of fruit during export may prove beneficial to producers and consumers alike. The aim of this study was to evaluate the potential of four different films used as MAP, in combination with *Bacillus amyloliquefaciens* PPCB004, to maintain postharvest quality of 'Tommy Atkins' mangoes.

2. MATERIAL AND METHODS

2.1 Fruit

Mango 'Tommy Atkins' fruit (360 fruit in total) were hand harvested at commercial maturity by the orchard workers during the early morning from a commercial orchard (Farm

Moria in the Hoedspruit area (Limpopo Province, South Africa)). After harvest, fruit were de-sapped and transported to the on farm laboratory at the Lushof farm in Tzaneen (Limpopo Province), within an hour. Thereafter, fruit were sorted for uniform size and absence of mechanical damage or disease. Fruit were then surface disinfected with 0.05 µl 1^{-1} NaOCl solution dip for 2 min and rinsed with clean tap water.

2.2 Storage of 'Tommy Atkins' fruit in modified atmosphere packaging only or in combination with Bacillus amyloliquefaciens PPCB004.

Bags consisting of four different film types were used (Refer to Table 1 for treatment codes). Ten replicates of ± 2kg fruit (± 4 fruit) were weighed and packed in each of the four bag types. The biocontrol agent, *B. amyloliquefaciens* PPCB004 (8.3 x 10^9 cells/ml) (formulated by Stimuplant Pty. Ltd (Boschkop, SA) was incorporated into a cold water bath (20 dm^3) to give a final concentration of 8.3 x 10^6 cells/ml. Fruit were dipped for two minutes in this suspension before being allowed to dry and packed. Ten replicates of ± 2kg (± 4 fruit) biocontrol treated fruit were weighed and packed in each of the four bag types. Bags were sealed with a commercial heat sealer and treatments were stored at 10 °C (± 2 °C) for 23 days after which fruit were evaluated for quality and decay. Untreated fruit packed in boxes were included as comparative control.

Table 2. *Treatment codes and commercial bag codes*

Treatment Code:	**Bag commercial code**	**Company**
Kn1	PFEB:180-80-400-PR30	Knilam Packaging (Westlake, South Africa)
Kn2	PFEB: 280-80-400-PR20	Knilam Packaging
Kn3	PFSB: 280-80-400	Knilam Packaging
BP	Biodegradable film	

2.3 Quality evaluations

2.3.1 Colour

Fruit surface skin colour (L, chroma, hue angle; L^* = lightness coefficient, C^* = chroma and h° = hue angle) was measured on opposite sides of an individual fruit (10 fruit per treatment) using a Hunterlab Miniscan XE Plus (Reston, Virginia, USA).

The L* value express light intensity, where 0 = black and 100 = white; h^o is the angle in a colour wheel of 360º, with 0, 90, 180 and 270º representing the hues red, yellow, green and blue, respectively and C* is the intensity or purity of the hue (Phebe and Ong, 2010).

2.3.2 Firmness and weight loss

Fruit firmness was measured after cold storage, on opposite sides of individual fruit (10 fruit per treatment) with a Chatillon penetrometer (Chatillon and Sons, New York, USA) equipped with a 6 mm diameter plunger capable of penetrating through the peel into the pulp. Weight loss was determined after cold storage.

2.3.3 Gas composition

Carbon dioxide (CO_2) and oxygen (O_2) composition inside individual bags were measured directly after cold storage using a checkpoint model gas meter (PBI Dansensor, Elemental Analytical, Denmark) and expressed as a percentage.

2.4 Evaluation of the postharvest diseases anthracnose and soft brown rot, caused by natural infection

Anthracnose and soft brown rot incidence were determined by counting fruit infected with either or both anthracnose and soft brown rot, irrespective of severity, in a treatment and expressing it as a percentage. Anthracnose or soft brown rot severity on individual fruit was evaluated by using a scale of 1 - 5. For anthracnose severity was determined by the number of the anthracnose lesions visible on the fruit: 1 = one lesion; 5 = 5+ lesions. For soft brown rot severity was determined by the total % surface area lesion; 1: 1-20%; 2: 21-40%; 3: 41-60%; 4: 61-80%; 5: 81-100%.

2.5 Statistical analysis

The experiment was repeated once and fruit was stored during the trial in a complete randomized design. Statistical evaluation of the differences was performed using analysis of variance (ANOVA). The mean values of the significant interactions were compared using the Fisher's protected t-test L.S.D (least significant difference) at the 5% level using the SAS statistical software (Version 9.2, SAS institution, Cary, NC).

3. RESULTS

3.1. Effect of modified atmosphere packaging on external colour

Except for Kn1, all other treatments had a significantly lower light intensity (Fig. 1), compared to untreated. Light intensity was the lowest for the Kn2 and Kn3 treatments with or without the biocontrol agent and the BP treatment. All treatments except for the Kn1 treatment, with or without the biocontrol agent, recorded lower chroma levels. By adding the biocontrol agent to the biodegradable film resulted in a higher lightness and chroma level (Fig. 2). Also, fruit stored in Kn1 with or without biocontrol and Kn3 had a lower hue angle colour than fruit stored in the other MAP (Fig. 3). Visually, fruit stored in BP, Kn2 and Kn3 were brown – black after cold storage. Fruit stored in Kn1 remained greener than untreated fruit, but brown damage spots could be observed.

3.2. Effect of modified atmosphere packaging on fruit firmness

Fruit stored in the biodegradable packaging, irrespective of the treatment, were significantly firmer than all the other treatments (Fig. 4). Although not significant, fruit stored in Kn1 were the softest after cold storage, but firmer than untreated fruit.

3.3 Effect of modified atmosphere packaging on weight loss

All treatments resulted in significantly less weight loss compared to the untreated fruit (Fig. 7). Of these treatments the Kn1 treatment with or without biocontrol resulted in the least weight loss, while the BC treatment (with or without biocontrol) was the highest.

3.4 Effect of the different films on the gas composition inside bags

Overall, the percentage of CO_2 in all the packed fruit treatments was extremely high after cold storage. The highest levels of CO_2 was recorded in the Kn3 bags, with or without the biocontrol treatment (Fig. 5), which also had the lowest O_2 levels (Fig. 6).

The MAP bags Kn3 (with or without biocontrol) and BP all had a O_2 composition of below 2% after cold storage. The level of O_2 was however not significantly lower than the Kn2 treatment. Although the Kn1 treatment (with or without biocontrol) resulted in the highest O_2 level, it was not significantly higher than the biocontrol combinations of Kn2 and BP.

3.5. Effect of modified atmosphere packaging and Bacillus amyloliquefaciens PPCB004 on the incidence and severity of postharvest diseases

All treatments reduced anthracnose and soft brown rot incidence and severity (Fig. 8 and 9). Treatments Kn2, Kn3 on its own or in combination with biocontrol totally controlled anthracnose incidence. Accept for Kn1 in combination with the biocontrol treatment, all other biocontrol combined treatments effectively controlled soft brown rot, similar to BP on its own. Anthracnose severity was increased by the Kn1 treatment. The treatments found to be most effective in controlling soft brown rot severity was BP, Kn1+BC and Kn2+BC (Fig. 8 and 9).

4. DISCUSSION

Contradictory to what was found in this study with Kn2, Kn3, and BP, Castro *et al.* (2005) reported that, visually, the storage of 'Tommy Atkins' mangoes in low density polyethylene bags containing potassium permanganate remained 'greener' after 21 days of storage at 12 ºC. Similarly, reduced chlorophyll breakdown (more greener fruit) in the skin of 'Tommy Atkins' and 'Keitt' were reported by Ben-Ari *et al.* (2001) after cold storage in MAP for 21 days at 12 ºC or 8 ºC and ripening for five days at 20 ºC. Reduced skin colour development was also reported by Pesis *et al.* (2000) on 'Tommy Atkins' and 'Keitt' stored in MAP for 21 or 19 days at 12 ºC plus five days at 20 ºC after removal from MAP.

Similarly to our results, reduced softening of 'Tommy Atkins' and 'Keitt' were reported by Ben-Ari *et al.* (2001) for fruit stored in MAP. Gonzalez *et al.* (1990) reported that the wrapping of individual 'Keitt' mangoes with either low or high density polyethylene bags had no effect on fruit firmness, when compared to untreated fruit. However, Castro *et al.* (2005) reported that 'Tommy Atkins' fruit stored in low density polyethylene bags containing 2.5g potassium permanganate remained firmer after 21 days of storage at 12 ºC.

The threshold value, at which CO_2 damage and other disorders like softening and off flavour developed on mango, was set at >10% (Kader, 1994). The O_2 threshold values according to Kader (1994) where injury occurred was found to be <2%. Damage can be characterised as greyish flesh colour, skin discolouration and off flavours. Sornsrivichai *et al.* (2000) found that for 'Nam Dok Mai' mangoes stored at 13 ºC, a minimum O_2 concentration of 19-20% is required for the development of acceptable skin (after 26 days) and flesh (after 13 days) colour. Gonzalez *et al.* (1990), found that individual 'Keitt' mangoes wrapped in low and high density polyethylene films showed a reduction in O_2 in the packaging to around 10 and 14 % respectively and an increase in CO_2 levels of 8 -10 % after storage at 20 ºC for two weeks.

Elevated levels of CO_2 and reduced levels of O_2 result in a drop in respiration rate of fruit, inducing fermentation. The products of fermentation (ethanol, acetaldehyde, ethyl-acetate and lactate) can all cause damage to plant tissue and can also result in off-flavour development (Ben-Yehoshua *et al.*, 2005). An important factor to consider when selecting a film for MAP for a specific crop is the respiration rate of that commodity. To develop a successful MAP system, permeability of the film and respiration rates need to be balanced, which is also both influenced by temperature. This is important to maintain desirable levels of CO_2 and O_2 necessary for respiration and preventing anaerobic conditions from developing (Geeson, 1989; Ben-Yehoshua *et al.*, 2005).

Coinciding with our results, reduction in weight loss by MAP in general has been confirmed by others on different cultivars stored in various types of packaging (Gonzalez *et al.*, 1990; Pesis *et al.*, 2000; Ben-Ari *et al.*, 2001; Castro *et al.*, 2005). Weight loss of fruit during storage is mainly the result of moisture loss and MAP is usually a good barrier to water vapour, resulting in reduced moisture (weight) loss, when compared to fruit stored in open packaging (Ben-Yehoshua *et al.*, 2005).

From our results it could be observed that the use certain MAP in combination with biocontrol could reduce the incidence of soft brown rot even further, when compared to using MAP only. Coinciding with our results for total anthracnose control in certain MAP, Castro

et al. (2005), reported that the disease was sufficiently controlled on 'Tommy Atkins' mangoes during cold storage in low density polyethylene bags containing potassium permanganate. However, an increase in disease development was observed on these fruit after removal from cold storage and MAP to ripen. Singh *et al.* (2000), found that the treatment of 'Haden' mangoes with $CaCl_2$ in combination with MAP had a lower incidence of fruit rots compared to fruit subjected to MAP only, after cold storage.

It is estimated that at a concentration of above 10%, CO_2 become fungistatic or fungitoxic to various fungal pathogens and can effectively stop or slow down the development of disease. Elevated CO_2 levels controls decay by directly influencing the microorganisms involved, rather than changing the physiology of plant tissue. However, according to Ben-Yehoshua *et al.* (2005) different commodities are adversely affected. Immature avocado and mango fruit have high concentrations of antifungal compounds, which decrease during ripening. On avocado it was found that a high CO_2 treatment for 24h delayed the rate at which antifungal compound concentrations decreased on fruit, resulting in lower incidence of antharacnose (Arauz, 2000).

Fruit failed to ripen after cold storage to an acceptable level, fit for human consumption or marketing, after being removed from bags. Severe damage because of low O_2 or high CO_2 levels as described by Kader (1994) could be observed on fruit stored in Kn2, Kn3 and BP and to a lesser extent on fruit stored in Kn1. Grey flesh and grey skin colour could be observed in Kn2, Kn3 and BP and to a lesser extent on fruit stored in Kn1. When bags from all treatments were opened in this study, a strong fermenting smell were noted and flavour development not characteristic of mangoes were noted. Subtropical fruit such as mangoes are known to be more sensitive to anaerobic damage, exhibiting lower tolerances towards low O_2 atmospheres (Pesis, 2005). Reduced O_2 levels inhibit respiration in fruit, resulting in an energy shortage that leads to undesirable metabolic processes such as fermentation (Ben-Yehoshua *et al.*, 2005). Similarly, elevated levels of CO_2 can also induce the fermentation pathway, producing ethanol and acetaldehyde, which can result in off flavours and tissue damage. Also, it has been found that higher levels of CO_2 make plant tissue more sensitive to low levels of O_2, resulting in fermentation occurring at higher O_2 levels (Ben-Yehoshua *et al.*, 2005).

Also, in conjunction with Kader (1994), fruit of treatments that had very high CO_2 values after ripening (Kn2 and Kn3), were less firm than fruit stored in BP, which had a lower CO_2 composition after storage. The only exception on this was fruit stored in Kn1, which did not react with O_2 and CO_2 levels as described by Kader (1994).

5. CONCLUSION

Three of the four films used as MAP in this study were unable to maintain the quality of mangoes at a level acceptable to consumers. This can mainly be attributed to extremely high CO_2 and very low O_2 concentrations in the bags, creating an anaerobic environment. The result of these anaerobic conditions prevailing in the bags was induction of the fermentation metabolic pathway, resulting in off-flavours and tissue damage of fruit. Although fruit stored in Kn1 had the lowest weight loss and acceptable skin colour retention after removal from cold storage, fermentation of fruit resulted in an off-flavour. Anthracnose was completely inhibited in Kn2 and Kn3 treatments. However, the use of biocontrol in combination with Kn2 and Kn3 reduced the incidence of soft brown rot even more, compared to Kn2 and Kn3 used on its own. It can be concluded that none of the films used in this study had sufficient perforation to maintain an acceptable balance between gas exchange and respiration rate in order to maintain the quality of mangoes. From our study it can be concluded that *B. amyloliquefaciens* PPCB004 might prove beneficial to control soft brown rot on 'Tommy Atkins' in combination with MAP, given that the packaging is optimized for the respiration rate of mangoes.

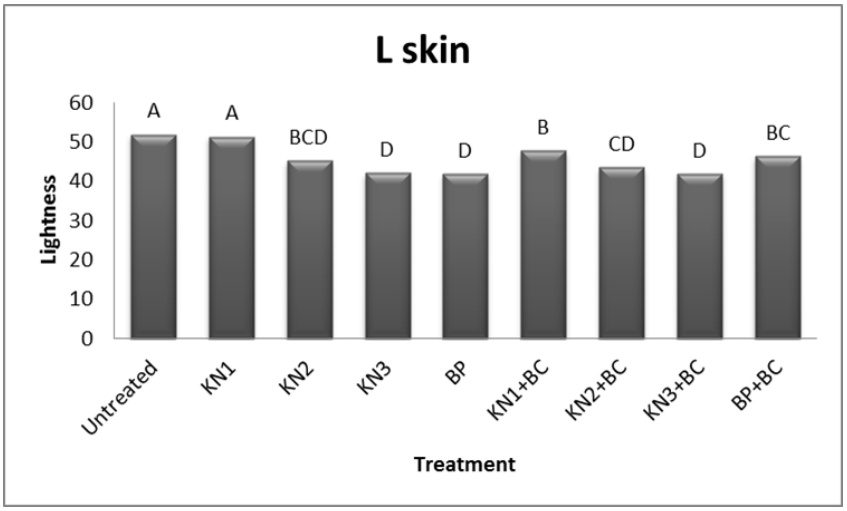

Fig. 1[25] *Light intensity of 'Tommy Atkins' fruit skin stored in four different modified atmosphere packaging (Harvest values – 44.13). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

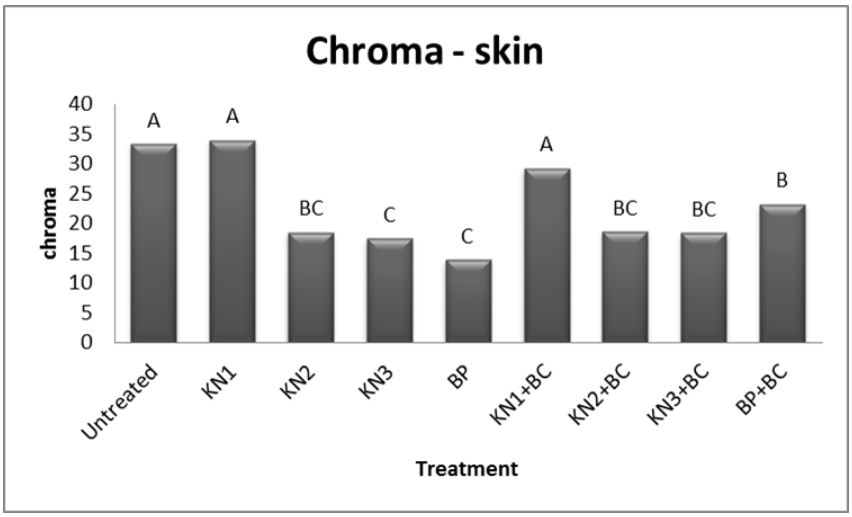

Fig. 2[26] *Chroma of 'Tommy Atkins' fruit skin stored in four different modified atmosphere packaging, after cold storage (Harvest values – 18.18). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

[25] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

[26] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

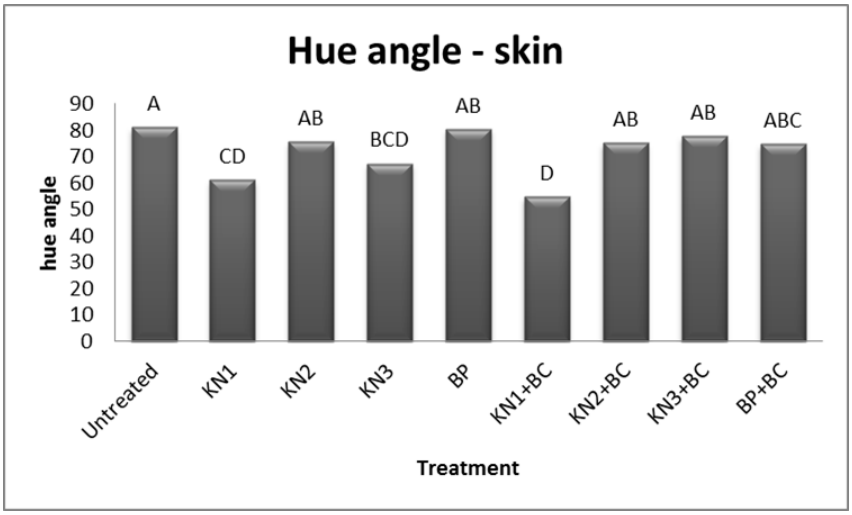

Fig. 3[27] *Hue angle colour of Tommy Atkins fruit skin stored in four different modified atmosphere packaging, after cold storage (Harvest value – 80.21). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: 0.0018).*

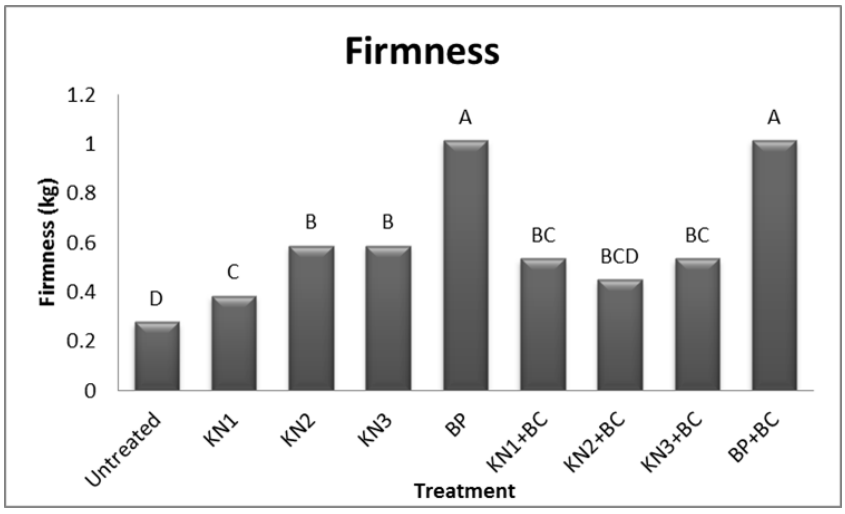

Fig. 4[28] *Firmness of Tommy Atkins fruit stored in four different modified atmosphere packaging, after cold storage (Harvest value – 1.13). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

[27] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

[28] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

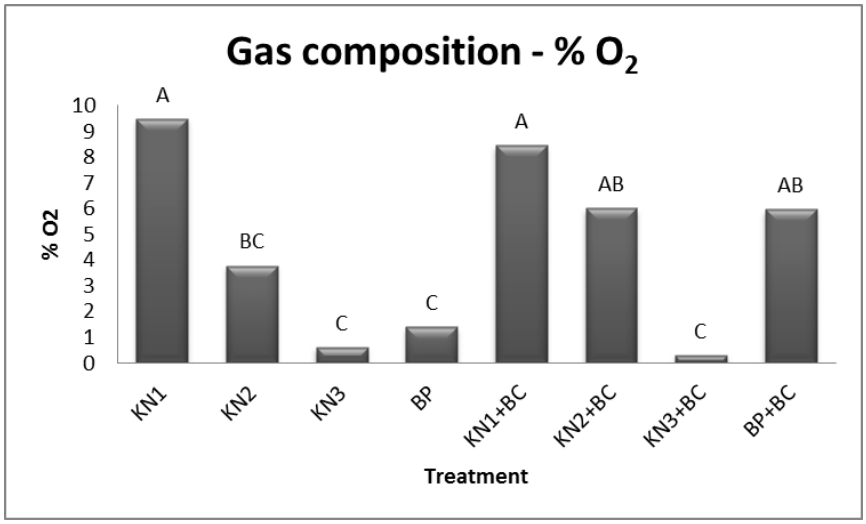

Fig. 5[29] *Composition of CO_2 in bags of Tommy Atkins fruit stored in four different modified atmosphere packaging, after cold storage. (Original value – 0.6). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

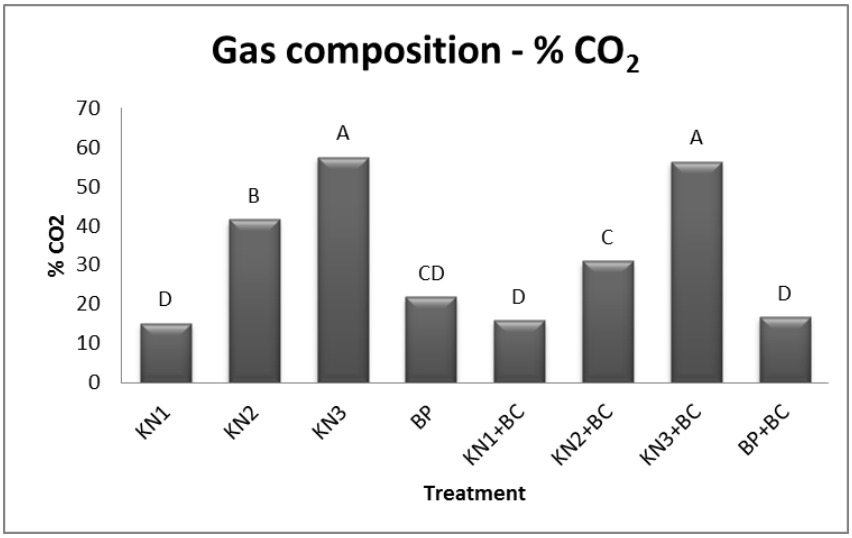

Fig. 6[30] *Composition of O_2 in bags of Tommy Atkins fruit stored in four different modified atmosphere packaging, after cold storage. (Original value – 21.7%). Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

[29] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

[30] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

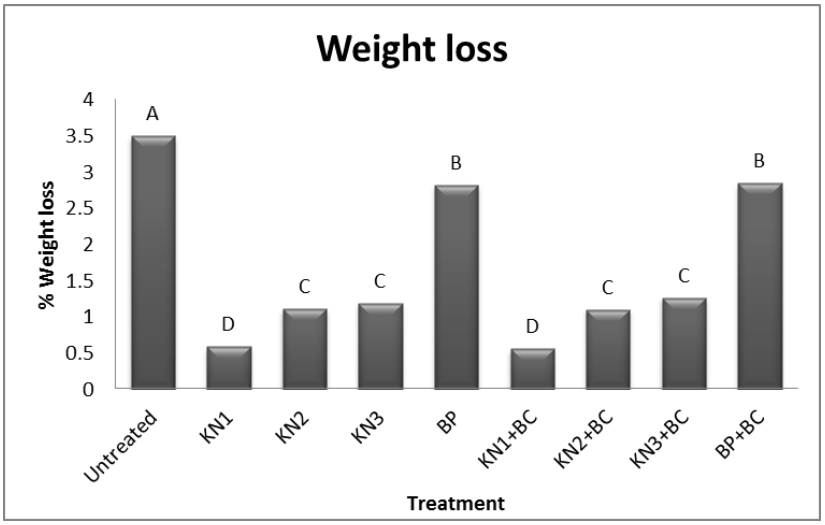

Fig. 7[31] *Percentage weight loss of Tommy Atkins fruit stored in four different modified atmosphere packaging, after cold storage. Treatments with the same letter do not differ significantly at a confidence level of 0.05 (p-value: <0.0001).*

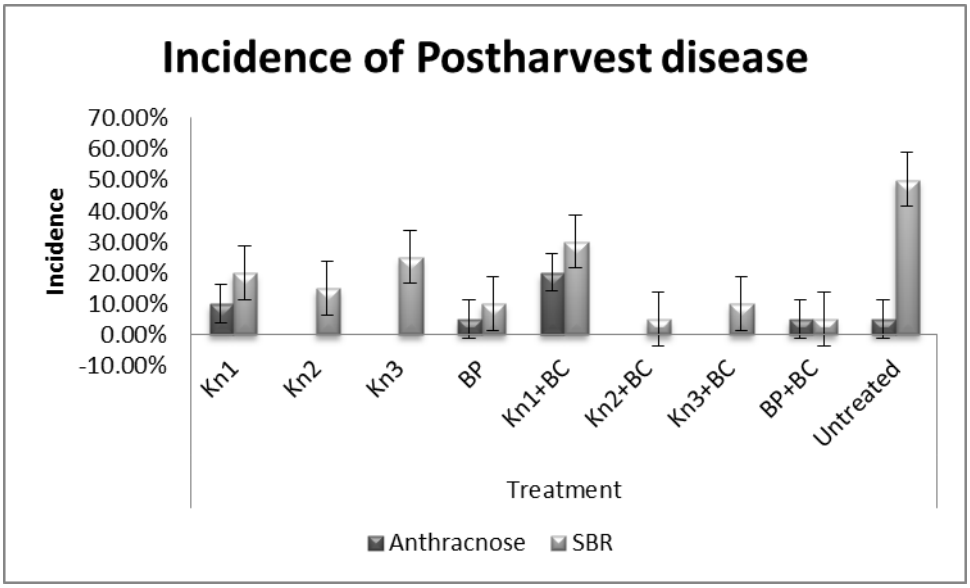

Fig. 8[32] *The natural incidence of the postharvest diseases soft brown rot and anthracnose on fruit stored in modified atmosphere packaging, after cold storage. Error bars indicate standard deviation, with respect to disease.*

[31] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004.

[32] KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004; SBR: Soft brown rot

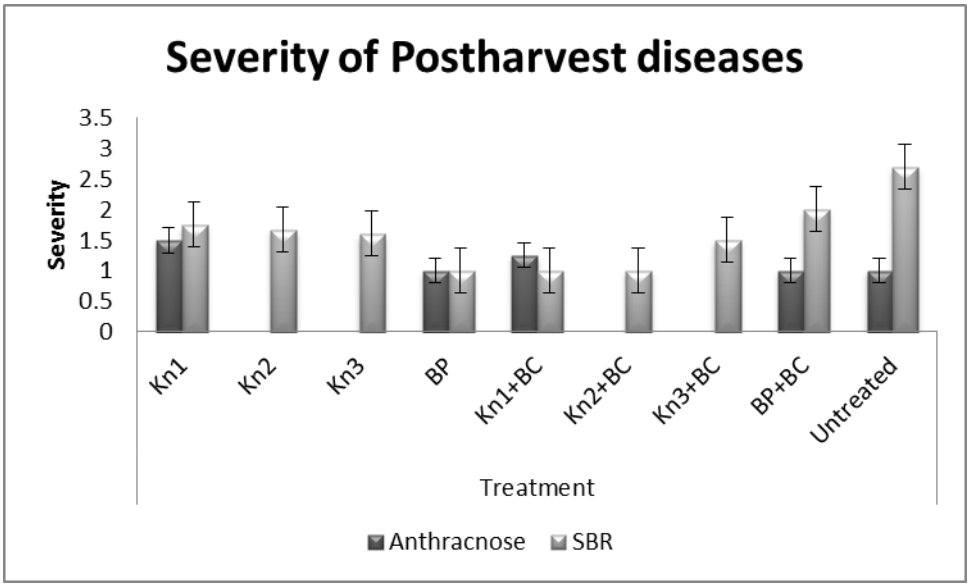

Fig. 9[33] *Severity of the postharvest diseases, soft brown rot and anthracnose, on naturally infected fruit after cold storage. Evaluations were done on a scale of 1-5. For anthracnose the severity was determined by the amount of anthracnose lesions visible on the fruit: 1 – one lesion; 5 – 5+ lesions. For soft brown rot severity was determined by the total % of surface area the lesion occupy; 1: 1-20%; 2: 21-40%; 3: 41-60%; 4: 61-80%; 5: 81-100%. Error bars indicate standard deviation, with respect to disease.*

[33]KN1: Knilam PFEB: 180 – 80 – 400 PR30; KN2: Knilam PFEB: 280 – 80 – 400 – PR20; KN3: Knilam PFSB: 280 – 80 -400; BP: Biodegradable Packaging; BC: *Bacillus amyloliquefaciens* PPCB004; SBR: Soft brown rot.

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CHAPTER 6

General Discussion

Developing effective postharvest treatments to control anthracnose and soft brown rot on 'Tommy Atkins' and 'Kent' mangoes postharvestly, were investigated in this study. The aim was to use specific, more environmentally acceptable technologies alone or as combination treatments.

In this study, the technologies used included biocontrol (*Bacillus* amyloliquefaciens PPCB004), 1-methylcyclopropene (1-MCP), controlled atmosphere (CA) and modified atmosphere packaging (MAP). The use of biocontrol products has gained momentum in the fresh fruit industries over the past years. As a result, a variety of products have been registered for use on different crops and to an lesser extent for postharvest applications (Janisiewicz and Korsten, 2002). Since the discovery of 1-MCP as a ethylene inhibitor, it has been used effectively on various crops to maintain quality of fresh produce postharvestly (Watkins, 2008). The metabolism of certain fresh fruits can be manipulated by storing it in MAP or CA to extend the postharvest storage life of the product and control diseases (Irtwange, 2007).

In South Africa, a hot water dip followed by a cold prochloraz treatment is the recommended procedure for mango producers when exporting fresh fruit to the European Union (EU) (Nel *et al.*, 2003). This treatment regime is effective in controlling anthracnose, but variable results are reported for soft brown rot (Saaiman, 1997). The increasing lobby of consumers opposing the use of synthetic fungicides to control diseases in general (Wisniewski *et al.*, 2007), are forcing fruit producers to consider more 'environmentally acceptable' control methods. However, the introduction of a new technology to the industry must provide better or equal postharvest diseases control as well as ensure quality retention. Ideally, no logistical difficulties in handling large volumes of fruit should be experienced with the introduction of a new technology, given that implementation and running costs are low.

In this study, weight loss was overall reduced for 'Tommy Atkins' stored under CA. On 'Tommy Atkins', CA storage resulted in a lower SSC/TA ratio after ripening. The combination of 5% O_2 and 5% CO_2 (CA-1) storage gave the best control of soft brown rot, artificially inoculated on 'Tommy Atkins' mangoes after ripening. The natural incidence of

anthracnose was also lower on 'Tommy Atkins' fruit subjected to a 3% O_2 and 8% CO_2 (CA-2) after cold storage. In a CA system used for fresh produce storage, the O_2 and CO_2 composition within the chamber are changed and maintained at desired levels, known to maintain quality of a specific product (Kader, 2002). This type of system have been shown to be beneficial to stored produce with high respiration rates as well as produce, such as subtropical fruit, sensitive to the effect of ethylene. The selection of a gas composition is based on the effect that CO_2 and O_2 have on different physiological activities. The beneficial effect of a lower O_2 level is the inhibition of ethylene action as well as the suppression of oxidative reactions responsible for tissue browning. A higher CO_2 level on the other hand might have a suppressing effect on decay development and also inhibit ethylene action (Kader, 2002; Kader, 1994; Beaudry *et al.,* 2006).

In this study it was found that the pre-treatment of 'Kent' fruit with 1-MCP only or in combination with storage under CA-2, maintained firmness significantly better after ripening and also controlled weight loss best during cold storage. Anthracnose severity, resulting from artificial inoculation of fruit with *Colletotrichum gloeosporioides*, was reduced on both cultivars subjected to 1-MCP pre-treatment and CA-2 storage. The novel compound, 1-MCP is able to inhibit the effect of ethylene on various ornamentals, fruit and vegetables at low concentrations, by binding irreversibly to ethylene receptor sites. The delay of colour change, respiration rate, softening and ethylene production are the main advantages associated with 1-MCP application to fresh produce, with the added benefit that it has a non-toxic mode of action and leaves virtually no residue. However, more mature fruit and vegetables or those that are rapid ethylene producers, are less affected by 1-MCP (Watkins and Miller, 2005). Variable results were also observed amongst cultivars (Watkins and Miller, 2005). The effect of 1-MCP on decay development have not been studied extensively, but an increase in disease incidence have been observed on mango, strawberry, avocado, apple, citrus, papaya and custard apple (Hofman *et al.,* 2001; Jiang *et al.,* 2001). However, a decrease in the incidence of decay was also observed on apple, apricot, melon, peach, plum, strawberry and tomato (Watkins, 2008).

The application of *Bacillus amyloliquefaciens* PPCB004 to artificially inoculated 'Tommy Atkins' fruit prior to pre-treatment with 1-MCP, was found in this study, to reduce the severity of anthracnose and soft brown rot. The severity of anthracnose on 'Kent' fruit artificially inoculated with *C. gloeosporioides* was also lower after pre-treatment with biocontrol and 1-MCP. Variable successes have been achieved with the use of biocontrol agents to successfully control postharvest diseases of fruit and vegetables. However, the

effectiveness of these agents may be improved by environmental manipulations to favour the antagonist or combining these biocontrol agents with other antagonists or softer chemicals (Janisiewicz and Korsten, 2002; Droby *et al.*, 2009; Droby, 2006).

In this study, four different films were used as MAP. All of the bags used were able to reduce weight loss and maintain firmness better than untreated fruit. However, none of the films were able to retain fruit quality at a level suitable for human consumption. Low O_2 and extremely high CO_2 levels could be linked to severe damage on most of the fruit because of anaerobic fermentation. Modified atmosphere packaging has been applied with success to a variety of crops to maintain quality and control disease and pests during postharvest storage. None of the films used for MAP in this study can be recommended as an alternative to export 'Tommy Atkins' or 'Kent' mangoes. It is suggested that further studies can be conducted to optimize the perforation of a film that will allow suitable gas exchange to store the various cultivars of mangoes.

When fruit and vegetables are stored in MAP, the perforation in the film allows an exchange of O_2 and CO_2 (opposite to CA where the gas composition is manually maintained) and equilibrium is reached and the gas composition is maintained. The effectiveness of a MAP film is dependent on how effective the perforation in the film can allow for the change in the atmospheric composition around a specific commodity. When the perforation in a film is unable to allow for the sufficient exchange of CO_2 at a desired rate (determined by the weight and respiration rate of the specific commodity), anaerobic conditions are created. These anaerobic conditions sets of undesired anaerobic metabolic fermentation conditions, producing metabolites and negatively affecting produce quality (Kader, 1994; Ben-Yehoshua *et al.*, 2005; Beaudry *et al.*, 2006).

From the *in vivo* results obtained in this study it can be concluded that 'Kent' mangoes are more susceptible to soft brown rot and anthracnose than 'Tommy Atkins'. It can also be concluded that storage of 'Tommy Atkins' fruit under CA-1 was able to give some control of *in vivo* soft brown rot, but that a pre-treatment with 1-MCP before CA-1 storage made fruit more sussceptible to soft brown rot. Although not supported by *in vivo* results, the natural incidence of anthracnose was controlled on 'Tommy Atkins' by storage under stand alone CA-2. Pre-treatment of 'Kent' with 1-MCP prior to storage under CA-2 suppresses (natural incidence as well as lesion size of *in vivo* inoculated) anthracnose better than stand-alone CA-2. Pre-treatment of 'Kent' with 1-MCP or the biocontrol agent on its own controled natural incidence of soft brown rot under CA-1 storage, but this is not supported by results obtained from *in vivo* inoculated fruit.

Although CA storage, combined with or without 1-MCP pre-treatment, were able to improve the quality of 'Kent' or 'Tommy Atkins' for certain individual quality parameters, no specific combination were able to improve the quality of fruit during storage when all parameters were taken into consideration. Therefore, from this study it can be concluded that factors such as 1-MCP treatment (treatment temperature, concentration and duration) and CA conditions (atmospheric composition, RH and temperature) need to be optimised for storage of these spesific cultivars.

The addition of *B. amyloliquefaciens* PPCB004 as a pre-treatment to 'Tommy Atkins' and 'Kent' mangoes prior to 1-MCP treatment resulted in reduced lesion size of anthracnose (arteficially inoculated), compared to fruit subjected to 1-MCP pre-treatment only. On 'Tommy Atkins', pre-treatment with the biocontrol agent only did however result in significantly smaller *in vivo* anthracnose lesions, compared to untreated fruit or those pre-treated with biocontrol and 1-MCP or 1-MCP only. The combination treatment of warm water and wax did however reduce the lesion size of *in vivo* soft brown rot significantly on 'Tommy Atkins'.

Future research should focus on optimising the CA storage conditions for 'Kent' and 'Tommy Atkins' mangoes. The concentration of 1-MCP used during treatment must also be optimsed and factors like temperature during treatment and duration of treatment must be evaluated. The effect that 1-MCP treatment has on postharvest disease development also needs further investigation. Also, further studies need to be conducted to optimize the perforation of a film that will allow suitable gas exchange to store the various cultivars of mangoes in MAP.

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APPENDIX 1

Table 1: *The top ten major mango producers in the world, rated according to production area, volumes and productivity compared to South Africa. Source: Food and Agriculture Organisation Statistics (2008). (1: Production area in 2008 according to the South African Department of Agriculture, Forestry and Fisheries (Mango market chain profile document - 2009))*

MAJOR MANGO PRODUCERS (2008)

Rank/Country	Production area (ha)	Production volume (Tonnes)	Productivity (t / ha)
1: India	2138500	13649400	6.4
2: China	452663	3976716	8.8
3: Thailand	285000	1800000	6.3
4: Philippines	186770	884011	4.7
5: Indonesia	185196	2013123	10.9
6: Mexico	177308	1855359	10.5
7: Pakistan	166223	1753686	10.6
8: Nigeria	126500	734000	5.8
9: Bangladesh	84500	802750	9.5
10: Brazil	75911	1272180	16.8
35: South Africa	9500 (7600[1])	89005	9.4

Table 2: *The ten major global mango exporters, rated according to volume of fruit exported.*
Source: Food and Agriculture Organisation Statistics (2008).

TEN MAJOR MANGO EXPORTERS AND IMPORTERS 2008

EXPORTERS			IMPORTERS	
Rank	**Country**	**Quantity (tonnes)**	**Region**	**Quantity (tonnes)**
1	India	240858	USA	295231
2	Mexico	236004	Netherlands	111830
3	Brazil	116271	United Kingdom	57381
4	Peru	82512	UAE	47038
5	Netherlands	80598	Germany	46762
6	Pakistan	62057	Saudi Arabia	45660
7	Thailand	61026	France	39397
8	Ecuador	41379	Belgium	23739
9	Philippines	27068	Malaysia	23087
10	Guatemala	20490	Yemen	22891

Table 3: *The estimated spreading of freshly harvested mangoes produced in South Africa to different sectors from 2001 - 2008. Source: South African Mango Growers Association / Subtrop and South African Department of Agriculture, Forestry and Fisheries (Mango market chain profile document - 2009).*

SOUTH AFRICAN MANGO PRODUCTION AND DISTRIBUTION

Season	Dried (t)	Achar (t)	Juice (t)	Local (t)	Export (t)	Total (t)
2001/2002	5271	23561	15153	24513	16406	84904
2002/2003	6090	15025	11761	16564	17337	66777
2003/2004	7228	22143	19868	17033	7584	73856
2004/2005	11894	16939	34922	19653	6056	89464
2005/2006	11606	12456	16174	16133	2355	58724
2006/2007	16841	15695	18550	21161	4409	76656
2007/2008	14560	11758	32473	18654	2759	80204

Table 4: *Volumes of fresh mangoes sold on the major local markets in South Africa and the prices obtained (South African Rand / ton). Source: Directorate: Agricultural Statistics of the National Department of Agriculture (2008) and South African Department of Agriculture, Forestry and Fisheries (Mango market chain profile document - 2009)*

SOUTH AFRICAN MANGO PRODUCTION AND LOCAL MARKET

	Total production (t)	Local market (t)	Price (R/t) on local market
2001/2002	95558	24504	2625
2002/2003	74033	16562	3592
2003/2004	79943	16988	3780
2004/2005	93420	18276	3694
2005/2006	50965	16169	4269
2006/2007	62258	21822	3472