

# **Evaluation of biocontrol and sunprotectors to control mango fruit diseases and disorders**

by

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## DECLARATION

I, the undersigned, hereby declare that the work reported herein is the result of my original research findings and has not previously been submitted by me for a degree at any other university or institution of higher education.

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## DEDICATION

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## SUMMARY

### EVALUATION OF BIOCONTROL AND SUNPROTECTORS TO CONTROL MANGO FRUIT DISEASES AND DISORDERS

**Promoter: Prof. L. Korsten**

The first objective of this trial was to test the efficacy of plastic caps (made from polyethylene foam) with added inner wool linings (woolly caps) to provide sun protection and additional control of diseases through a slow release activity. Facet one of this trial was to determine the ability of the antagonist *Bacillus licheniformis* to attach and survive on the woolly base of the caps and facet two was to evaluate the efficacy of woolly caps to prevent sunburn damage and disease control on mango fruits. Woolly caps impregnated with *B. licheniformis* or copper oxychloride, were placed on fruits three to four weeks after fruit set. The caps were also compared to a sun-protector sprayed on its own or inoculated with *B. licheniformis*. Antagonist could effectively attach to woolly caps and could survive for up to three months in the field. Plastic woolly caps were the most effective treatment in reducing sunburn damage to mango fruits. Although sun-protector was effective in reducing sunburn damage on mango fruits, it could not achieve the same level of protection compared to the currently used commercial plastic caps. None of these treatments had any significant effect on pre- and post-harvest diseases except for one treatment where woolly caps impregnated with copper or antagonist and sun-protector sprays, could reduce the incidence of soft brown rot.

The second objective of this study was to evaluate alternative disease control options to protect mango fruit against pre- and post-harvest diseases using *B. licheniformis*. Facet one of this trial was to evaluate the ability of different spreaders, stickers, wetters and a biostimulants to enhance attachment, colonisation and growth of the antagonist on mango leaves. Facet two of this trial was to evaluate *B. licheniformis* and copper fungicides applied as pre-harvest sprays on its own or alternated with copper on mango trees from flowering until harvest at three weekly intervals at two geographical distinct regions and over two seasons. The results showed that Nufilm-P, Biofilm, Agral 90 and Bioboost had no negative effect on the attachment, colonisation and growth of the antagonist. Copper fungicides and Superfilm proved to have an inhibitory effect on the growth of the antagonists. Alteration of antagonists and copper sprays showed equal control of anthracnose as compared to commercial copper

sprays when used on its own. Antagonist on its own or alternated with copper showed potential for the control of bacterial black spot and soft brown rot. Future studies should focus on large-scale commercial trials to address inconsistency of biocontrol performance. Pre-harvest biocontrol treatments should also be evaluated in combination with post-harvest applications of the antagonist, taking into account possible build up of pathogen resistance.

## CHAPTER 1

### GENERAL INTRODUCTION

Mango (*Mangifera indica* L.) is currently rated as the world's third most important crop in the tropics preceded by citrus and banana (Nakasone & Paull, 1998). The global growing demands for the fruit on first world markets have recently increased and now provide greater export opportunities for countries within tropical and subtropical regions and specifically for developing countries (Dodd *et al.*, 1991). Annual world mango production is estimated at over 28 million tons of which India and Mexico are the greatest producers. Currently, the major mango producing countries include Bangladesh, Brazil, China, Haiti, India, Indonesia, Madagascar, Mexico, Pakistan, Philippines, South Africa and Thailand. Although India produces roughly 62 % of the total world production, it exports less than one percent of its crop. Mexico is the biggest mango exporter in the world, followed by Brazil, Madagascar, Mali, Pakistan, Peru, South Africa and Venezuela. The most important mango importing countries are Belgium, Denmark, France, Germany, Great Britain, Italy and the Netherlands (Snyman, 1998).

Although mango was first introduced into South Africa in 1920, the industry only started to grow rapidly from 1960 (Snyman, 1998). Currently, mango is rated as the fifth most important subtropical fruit cultivated in South Africa after citrus, banana, avocado and pineapple. More than 100 commercial mango cultivars are available world wide, with eighteen being under cultivation in South Africa. However, of these, only a few cultivars are suitable for export, namely Zill, Tommy Atkins, Kent, Keitt, Haden and Irwin (Sanders, 1993). South African mango production has increased from 32 080 to 87 583 tons between the 1989/90 to 2001/02 season. Of this, eight percent was exported during 1989/90 and 19.8 % during 2001/02, showing an increase of 12 % over a 12 year period.

Like many other crops, mango is subject to various diseases and other factors that affect fruit, foliage, roots, branches and trunks of the bearing trees (De Jager, 1999). Pre- and post-harvest disease, insect infestation, physiological disorders and abiotic factors are major concerns resulting in significant fruit losses. Pre-harvest diseases, which cause fruit damage and affect production in South Africa include bacterial black spot, blossom blight and tree dieback (Ridgway, 1989; Johnson *et al.*, 1991; Jacobs, 2002). Post-harvest diseases, which include

anthracnose, soft brown rot and stem-end rot also has a great impact on reducing fruit quality (Ridgway, 1989).

Management of diseases rely primarily on the application of fungicides based on pre-harvest sprays and post-harvest hot water dips incorporating fungicides (Pelser & Lesar, 1989; Lonsdale & Kotzé, 1993; Johnson *et al.*, 1997; Ippolito & Nigro, 2000; Janisiewicz & Korsten, 2002a). These practices have proven to be relatively effective in maintaining a lower rate of pre-harvest pathogen infection and in extending the shelf life of mango fruit during overseas shipment (De Jager, 1999; Gerhardson, 2002). Continuous application of copper is uneconomical, and results in build up of copper in soils. Build up of pathogen resistance to the target fungicides has been reported (Jeffries *et al.*, 1990; Spurrier, 1990; Schumann, 1991; Estrada *et al.*, 1996; Gerhardson, 2002). Furthermore, pesticide residues on food are a major concern for many people. European consumers, which represent South Africa's main export market, are demanding safe food produced within a framework of good agricultural practices which is focused on environmental protection and reduced use of pesticides (Flora, 1990; Hall, 1995; Nautiyal, 2000; Saxena *et al.*, 2000; Vidhyasekaran *et al.*, 2000).

Sunburn is another major problem facing mango producers, especially in tropical regions where temperatures can reach more than 42°C in mid-summer. Mangoes are summer crops and the main growth period for fruit development is during peak summer when high temperatures prevail. Due to excessive temperatures and high ultra violet radiation exposure, fruits tend to be damaged by sunburn especially those that are situated on the western and north western side of the trees in the Southern Hemisphere, or those exposed to sunlight when branches are broken or leaves have been shed (Ridgway, 1989).

More recently, fruit bagging were implemented to reduce sunburn damage and prevent pathogen infection, although not used widely. Bagging is used successfully in banana production to prevent fruit from blemishing due to dust and damage caused by birds. Similarly, paper bags have been used on guava to reduce insect damage and maintain fruit quality (Parreira, 1990; Johnson *et al.*, 1997). Rain shield made of polyethylene or other waterproof, light transmitting materials over the tree canopies prevent cracking due to rain for sweet cherry production (Meland & Skjervheim, 1998). In addition, rain shields during rainy periods prior to harvesting, reduce the need for fungicide treatments (Børve & Stensvand,

2003). Previously, mango fruits were covered with brown paper bags to protect them against sunburn, skin blemish and pathogen attack (Parreira, 1990). However, these methods were shown to be ineffective, since heavy rains caused paper bags to become translucent and cling to fruit when wet. This promoted pathogen infection and development of diseases on fruits (Bugante & Lizada, 1997). Currently, in South Africa, plastic caps (made from polyethylene foam) are commercially used on certain farms to prevent sunburn damage on fruit. A new innovative approach of using plastic caps with inner wool linings proved to be more effective in reducing sunburn on mango fruits than commercial caps (Silimela & Korsten, 2001).

The development of alternative techniques to reduce disease and improve quality and shelf life of fruit is becoming increasingly important as global chemophobia and environmental social awareness increases (Emmert & Handelsman, 1999; Kondoh *et al.*, 2001; Janisiewicz & Korsten, 2002a). It is therefore of importance that new innovative methods are developed to provide the industry with alternatives to reduce reliance on chemicals, while still ensuring sound quality fruit. More emphasis should also be placed on reducing the use of pesticides to ensure workers safety, consumer health and environmental protection (Flora, 1990; Joubert *et al.*, 1999; Gerhardson, 2002).

Integrated pest management, which includes the use of biological products, can result in reduced chemical sprays, considered as an effective alternative to prevent build up of pathogen resistance and chemical residues in the environment (Baker *et al.*, 1974; Chet, 1987; Spurrier, 1990; Schumann, 1991; Gunasekaran & Weber, 1996; Ippolito & Nigro, 2000). It can also improve agroecosystems to ensure sustainable agriculture and can better maintain the natural microbial ecological balance (Noiaium & Soyong, 1999; Ippolito & Nigro, 2000; Shtienberg, 2000; Kondoh *et al.*, 2001; Gerhardson, 2002). Biological control used on its own is however, often less effective than commercial fungicides currently in use (Obagwu & Korsten, 2003). Biological control of plant diseases has provided a relative recent alternative strategy for disease control particularly when used pre-harvestly to protect against post-harvest diseases (Korsten, 1993; Gunasekaran & Weber, 1996; Janisiewicz & Korsten, 2002b). Previously, the use of the biocontrol agent *Bacillus licheniformis* proved to be effective for control of anthracnose and SBR on mango (Korsten *et al.*, 1992; Korsten & Lonsdale, 1993). Pre-harvest *B. licheniformis* applications also showed promise in controlling BBS and anthracnose (Korsten *et al.*, 1992; Silimela & Korsten, 2002). The mode of action for *B. licheniformis* has not yet been determined.

Before commercialising biological control, it is important to have a sound knowledge of the relationship between the biological products, the host and environmental conditions suitable for host-pathogen-antagonist interactions (Emmert & Handelsman, 1999; Janisiewicz & Korsten, 2002a, 2000b). Effects of alternative fungicides and other additives applied to the crop while managing biocontrol programmes are of importance in ensuring antagonist survival. Additives such as stickers, spreaders or wetters are commercially applied with fungicides to enhance deposition and adherence. These products were previously evaluated and found not to have any negative effect on the growth and survival of *B. licheniformis* (Korsten *et al.*, 1992).

The first objective of this study was to evaluate alternative disease control options combining several innovative approaches that include the use of plastic caps with inner wool linings (woolly caps) impregnated with either copper oxychloride or *B. licheniformis* to prevent sunburn damage and simultaneously control disease. In order to study this, the ability of the biocontrol agent to attach and survive on woolly caps first had to be determined. The second objective of this study was to evaluate pre-harvest spraying of mango trees with copper oxychloride or *B. licheniformis* or alternating them at three weekly intervals from flowering until harvesting, to control pre- and post-harvest diseases. In order to ensure effective application, different additives (stickers, spreaders, wetters and biostimulant) was first evaluated to determine its ability to enhance antagonist attachment, growth and colonisation. The compatibility of *B. licheniformis* and copper oxychloride was also tested *in vitro* on mango leaves prior to initiating the field spray trials. The mode of action of *B. licheniformis* on different pathogens was not determined in this study.

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## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 ORIGIN OF MANGO

Mango (*Mangifera indica* L.) belongs to the family *Anacardiaceae*, also known as the cashew family, which consists of 62 species in the genus of which 15 bear edible fruits (Snyman, 1998a). The cultivated mango has other related plant species of agronomic importance e.g. the cashew (*Anacardium occidentale* L.), pistachio (*Pistachio vera* L.) and marula (*Sclerocarya birrea* L.) (Nakasone & Paull, 1998).

Mango was previously thought to have originated from India, but it is now generally accepted that it originated from the Burma-Malaysian region (Popenoe, 1920; Singh, 1960; Samson, 1986; Snyman, 1998a; Nakasone & Paull, 1998). It has been grown in India for more than 4 000 years, where it has been associated with Hindu and Buddhist religion.

From India, mango was disseminated to Africa, Asia, Australia and the rest of the world by sailors, traders and missionaries (Kwee & Chong, 1985). Indian traders and Buddhist priests probably introduced the mango into Malaysia and other East-Asian countries during the 4<sup>th</sup> to 5<sup>th</sup> century BC and to the Philippines between 1400 to 1450 AD. The Portuguese were the first Europeans to establish trade routes with India, transporting mangoes to East Africa and Brazil. Spanish traders took these fruit from the Philippines to the West Coast of Mexico before the English arrived on the Hawaiian Island in 1778. Since then, the mango has been introduced into every tropical and subtropical country around the world (Nakasone & Paull, 1998).

#### 2.2 COUNTRIES WHERE MANGO IS COMMERCIALY CULTIVATED

Mango can grow in tropical and subtropical climates, where conditions vary from hot, humid to cooler, less humid and very hot and arid. The cultivation of mango varies from subsistence farming to large, highly organised commercial production where the best available technology is applied (Snyman, 1998b).

Mango is cultivated in more than 111 tropical and subtropical countries around the world (Snyman, 1998b). Of these, the largest plantings are from India with  $\pm$  1.3 million hectares followed by Thailand, Mexico, Brazil, Haiti, Taiwan, Australia, Peru, Venezuela, South Africa and others (Snyman, 1998b).

### **2.3 MOST IMPORTANT MANGO CULTIVARS**

Hundreds of mango cultivars exist throughout the tropics and subtropics (Nakasone & Paull, 1998). Some of the most important cultivars worldwide include: Banganapalli, Bombay green, Bourbon, Cambodiana, Chansa, Dashehari, Glen, Golek, Haden, Irwin, Itamaraka, Keitt, Kensington Pride, Kent, Madu, Mallika, Maya, Naomi, Osteen, Pairi, Rosa, Sabina, Sabre, Sensation, Tahar, Tommy Atkins, Van Dyke, Zill and others. Of these, Kent, Sensation, Keitt, Tommy Atkins, Irwin, Haden, Zill, Carabao and Kensington Pride are of commercial importance (Knight Jr, 1997; Human & Snyman, 1998; Nakasone & Paull, 1998).

### **2.4 ECONOMIC ASPECTS OF MANGO PRODUCTION**

Mango is one of the most important fruits in the world and currently ranks fifth in global production amongst major crops such as banana, citrus, grapes and apples (Mukherjee, 1997). The biggest mango producer in terms of volume is India, which produces 9 million tons per annum. However, it is one of the smallest exporters of fresh mango with less than one percent of its total crop being exported. The bulk of their annual production is consumed locally (Snyman, 1998b).

Mexico is the world's largest exporter with annual volumes of 194 540 tons for 2001 ([www.fao.org](http://www.fao.org)). Other major exporters include Brazil (94 291 tons), Pakistan (52 465 tons), Philippines (38 523 tons), Peru (26 543 tons), South Africa (13 947 tons) and Thailand (10 829 tons) during 2001 ([www.fao.org](http://www.fao.org); 2002). Fresh mango is exported mainly to Europe from both northern and southern hemisphere countries. The largest importers of mango are the United States of America, Netherlands, France, China, Germany, Belgium, Italy and Denmark (Colyn, 1998).

Mango is an important part of man's diet in less developed countries, particularly in the tropics and subtropics. Consumers' preferences for mangoes vary, with colour and flavour

being some of the most important criteria for selection. Amongst others, approximately one percent of mango is utilised for juice processing, nectars, preserves (including chutney), frozen pulp, ice cream, yoghurt, fruit salads and fruit cocktails (Mukherjee, 1997; Nakasone & Paull, 1998). The seeds are used for extraction of starch 'anchur' and the peels (skin) can be used for biogas production by anaerobic digestion. Mango wood is used as a low quality timber and the bark of the tree is an important source of tannins for curing leather (Mukherjee, 1997; Nanjundaswamy, 1997).

## **2.5 PROBLEMS AFFECTING MANGO PRODUCTION**

Like with any other crop, mango producers and marketers around the world are facing increasing commercial pressure to present quality fruit to consumers. Factors that affect fruit quality include damage to fruit by insects, mechanical injuries, sunburn, spray residues, bruising, cold damage, latex damage and plant diseases. In mangoes, diseases affect all parts of the tree including roots, stem, leaves, flowers and fruit (Table 2.1).

## **2.6 SOUTH AFRICAN MANGO PRODUCTION**

### **2.6.1 History**

Mango was introduced into South Africa in early 1920 in the Ofcolaco and Malelane areas, in the Northern Province. Larger scale plantings started in 1962 with the introduction of Florida fibrous cultivars (Finnemore, 1999). Currently, the most important cultivars that are planted in South Africa are Tommy Atkins (26 %), Sensation (13 %), Kent (12 %), Heidi (9 %), Keitt (8 %) and Zill (8 %). A tree census conducted in 1995 showed that 8 000 hectares have been planted in SA, with three million trees giving a total production of 40 000 tons. Currently, production has increased from 45 757 tons in the production year 1995/1996 to 87 583 tons for the year 2001/02 (South African Mango Growers' Association, (SAMGA) report). Today, mangoes are mainly grown in the northern and eastern provinces of South Africa. The elevation of the mango growing areas varies from 300 to 950 meters above sea level. Major regions for mango production are Tzaneen (36 %), Hoedspruit (28 %) and Malelane and Komatipoort (20 %) (Finnemore, 1999).

## 2.6.2 Economic aspects

South African mango production is focused on processing, exports of fresh fruits and to a lesser extent local marketing. In the year 2000/01, 26.7 % (23 366 tons) of fresh fruits were sold on the local market, 26.9 % (23 561 tons) were used for making achar, 19.8 % (17 375 tons) were exported, 17.3 % (15 153 tons) were used for sap or juice while 9.3 % (8 128 tons) were used for dried fruits (SAMGA report).

The South African mango season stretches from January to middle March. During January, mango volumes on the export markets compete with fruit from South American countries, while countries from the west coast of Africa such as the Ivory Coast, Gambia, Burkina Faso and Mali supply fruits from the middle of March (Finnemore, 1999). South Africa has become a major competitor on the European market and export volumes have more than doubled over the past five years from 8 603 tons in 1997/98 to 17 375 tons in 2001/02 (SAMGA report).

## 2.7 MANGO DISEASES

The most important pre-harvest diseases which cause fruit damage and affect production include bacterial black spot (BBS) caused by *Xanthomonas campestris* pv. *mangiferaeindicae* and anthracnose caused by *Colletotrichum gloeosporioides* (Penz & Sacc). Important post-harvest diseases are soft brown rot (SBR), the anamorph of *Botryosphaeria* spp. previously described as *Nattrassia mangiferae* (Sydow et Butler) and stem-end rot (SER) *Dothiorella dominicana* (Pet. et Cif.) (Ridgway, 1989, Johnson et al., 1991; Jacobs, 2002). Additionally, anthracnose also causes post-harvest disease.

### 2.7.1 Bacterial black spot

Direct losses on fruit being rejected for export due to BBS on susceptible cultivars such as Kent and Keitt may be as high as 80 % (Boshoff *et al.*, 1998; Gagnevin & Pruvost, 2001). Heavy losses have also been reported on cultivars such as Tommy Atkins, Haden, Irwin, and Smith (Ridgway, 1989; Sanders *et al.*, 1992; Kotzé & Visser, 1997). Indirect losses such as premature fruit drop, and induction of severe defoliation, especially when storms or hurricanes were involved, are associated with BBS infection (Gagnevin & Pruvost, 2001).

The disease occurs in both low and high rainfall areas and is more severe in windy areas in the absence of windbreaks. Persistent dew in combination with strong winds permit extensive infection and disease development (Persley, 1993).

Bacterial black spot symptoms occur in all aerial parts of the tree, with leaves and fruit being most severely affected (Gagnevin & Pruvost, 2001; Swings and Civerolo, 1993). Infected leaves show raised, black, angular elongated lesions with greasy margins (Lelliott & Stead, 1987; Ridgway, 1989; Persley, 1993). Lesions are surrounded by chlorotic halos, which becomes grey and cracked with age. Older lesion turns black and appears slightly raised. Yellowish halos usually form around each lesion and some exudates occur (Swings & Civerolo, 1993). Lesions also occur on leaf stalks and twigs. Stem lesions appear as blackened cankers that form longitudinal cracks and also exude gum. Stem lesions are rare on tolerant cultivars.

Symptoms on the fruit appear as small, irregular, water soaked specks around the lenticels (Ridgway, 1989; Persley, 1993). As lesions enlarge, they become raised, blackened and cracks appear (Swings & Civerolo, 1993). Raised, black spots with greasy margins develop later and bacterial laden sap oozes from the cracks (Ridgway, 1989; Persley, 1993). Skin-deep symptoms are visible on fruit at harvest. Infected fruit drop, especially when infection occurs at the young fruit stage or when the fruit stalk is infected.

*Xanthomonas campestris* pv. *mangiferaeindicae* is a non-pigmented, Gram-negative bacteria, straight rod, 0.4 to 0.7  $\mu\text{m}$  wide and 0.7-1.6  $\mu\text{m}$  long and motile by a single polar flagellum (Dodd *et al.*, 1997; Ploetz & Prakash, 1997). The pathogen lives all year round as an epiphyte on mango trees, particularly on the leaves and in stem lesions. Susceptibility increases as fruit enlarge and mature, with lenticels and wounding providing primary entry sites.

Bacterial black spot is more serious on late maturing varieties, particularly if wind protection is inadequate (Lonsdale, 1993a). Wet conditions are essential for dispersal of the bacterium, which is washed down from the tree and easily dispersed through wind driven rain (Oosthuysen, 1997a). Other dispersal mechanisms include wind, rain, water splash, insects, propagating materials (such as budwood, rootstock, seedlings and infected fruits) and equipment (Ridgway, 1989; Persley, 1993; Swings & Civerolo, 1993; Coates *et al.*, 1995).

The bacteria enter through natural openings on leaves (stomata and hydathodes) and fruit (lenticels) or through abrasions and wounds. Disease incidence is greater on exposed and abraded leaf and fruit surfaces or where fruit are in direct contact causing abrasions. This pathogen can survive in soil for only a few days, but for several months on contaminated plant debris (Gagnevin & Pruvost, 2001).

### 2.7.2 Anthracnose

Anthracnose is one of the most important fungal mango diseases in the world. The pathogen *C. gloeosporioides*, is believed to have co-existed with mango throughout the world and is a major constraint for fruit production and marketing (Jeffries *et al.*, 1990; Estrada *et al.*, 1996). The pathogen infects leaves, flowers and fruit. The post-harvest phase of the disease is economically the most important, because the fruit loses its natural resistance during ripening which ultimately results in decay at the market end (Ridgway, 1989; Arauz, 2000).

Kwee & Chong (1985) indicated that young mango leaves particularly at the bronze to light green stage are more susceptible to anthracnose than older leaves. The disease starts on the leaves as tiny, necrotic black spots. Later, spots enlarge into discrete, roundish or angular spots. Under humid or wet conditions, the spots coalesce to form larger irregularly shaped necrotic patterns (Fitzel & Peak, 1984; Kwee & Chong, 1985; Ridgway, 1989). The spots are typical light brown to greyish brown with a dark brownish-black margin surrounded by light green to yellowish green areas. During dry weather, lesions become dry and fall out giving a shot hole appearance on the leaves.

Flower symptoms begin as tiny, black necrotic spots on the flower buds, opened blossoms, flower pedicels and on the main and secondary stalks of flower panicles (Kwee & Chong, 1985). The spots enlarge, coalesce and cause blighting of clusters of flowers or the entire inflorescence becomes black and dry, which results in shedding and death of the flowers (Ridgway, 1989).

Fruit infection can occur when the fruit is still young, causing fruit drop. Symptoms start as small, necrotic dark spots on the fruit surface. Spots enlarge to form irregular, dark brown to black areas on ripening fruit. In humid atmospheres, pink spore masses appear towards the

centre of the infected area (Ridgway, 1989). The fleshy tissues beneath the necrotic area decay, resulting in a characteristic brownish black, moist and firm rot (Kwee & Chong, 1985).

The pathogen, *C. gloeosporioides*, can infect a wide range of hosts and can live part of its life cycle as an endophyte. Orange, shiny, conidial masses can be formed as the acervuli mature. Conidia are hyaline and unicellular and cylindrical to ellipsoidal, 7 to 20 µm long and 2.5 µm wide. The colour of the fungal mycelium growth on potato dextrose agar range from white to grey (Ploetz & Prakash, 1997).

Conidia germinate and form germ tubes within three to eight hours at a temperature between 25 to 30°C. The optimum temperature for production of appressoria is 20 to 25°C. During light rain, conidia tend to collect in reservoirs around the pedicel and are redistributed down the fruit in subsequent rainfalls (Ploetz & Prakash, 1997).

The pathogen requires a high humidity of more than 80 %, high rainfall and temperature range from 25 to 30°C to infect plant material (Persley, 1993; Coates *et al.*, 1995; Sanders & Korsten, 1996). Conidia as a source of inoculum are produced on infected dead twigs, branches, leaves and old inflorescences where the pathogen survives throughout the year. The pathogen is spread by means of water splash, wind, propagative materials (seeds, rootstock and scions) and infected fruits (Fitzel & Peak, 1984).

Development of post-harvest symptoms result from fruit being infected pre-harvestly. The pathogen remains dormant or latent in unharvested green fruit. Disease development occurs mostly after harvest because the fruit loses its natural resistance during ripening (Persley, 1993; Coates *et al.*, 1995).

### **2.7.3 *Botryosphaeria* diseases on mango**

Many diseases of various woody plants are commonly caused by the anamorph species of the telemorph *Botryosphaeria* (Johnson *et al.*, 1991; Slipper *et al.*, 2001; Jacobs, 2002). The anamorph state of the pathogen that causes SBR and SER of mango has previously been known as *Hendersonia creberrima* (Sydow & Butler) (Johnson *et al.*, 1991). The anamorph genus name has however, been changed incorrectly to *Nattrassia mangiferae* (H. Sydow &

Sydow) Sutton and Dyko and *Dothiorella dominicana*. Pet. et Cif., but it is currently classified as *Fusicoccum* (Johnson *et al.*, 1991; Slippers *et al.*, 2001; Jacobs, 2002).

*Botryosphaeria* spp. affecting mangoes are mainly saprophytic and endophytic, and can attack different parts of the mango tree and fruit, resulting in pre- and post-harvest diseases (Jacobs, 2002). The pathogen colonises the blossom as an endophyte, often resulting in blossom blight. Blossom blight, tree dieback and cankering are also associated with the same endophytic *Botryosphaeria*, which infects fruit from flowering, moving into fruit during its development. Infections of unripe fruit remain latent in the orchard until harvest. After harvesting when fruit ripen, invasion continues and colonisation takes place giving rise to SBR and SER (Lonsdale, 1993b; Jacobs, 2002).

Recent studies by Grobler *et al.* (2002) indicated that *Botryosphaeria* spp. are present in nursery trees, mother and scion material. Once infected, the pathogen spreads systemically through the inflorescence into the fruit where it remains latent until the fruit begins to ripen (Lonsdale, 1993b). The first symptom expressed on the tree is dead blossoms (blossom blight) caused by the endophytic movement of *Botryosphaeria* into the stem. Other symptoms can only be seen once the fruit start to ripen. Symptoms on the harvested mature fruit appear as skin darkness around the base of the pedicel more commonly known as SER or on the body of the fruit also referred to as body rot or SBR. The lesion enlarges rapidly to form a circular brownish-black water soaked area, which can extend over the whole fruit (Ploetz & Prakash, 1997). Necrosis generally remains below the cuticle but may penetrate into the flesh within a week.

The pathogen survives on dead twigs, leaves, litter and peduncles where they produce large numbers of spores. Spores are spread to the flowers and developing fruit where infection occurs. Rainfall plays an important role in the spreading of the pathogen (Saaiman & Smith, 1995; Saaiman, 1996). The exact mode of entry of *Botryosphaeria* on mango trees is not known, but natural openings and wounds caused by pruning, insects and sunburn are considered the most likely route of infection (Lonsdale, 1993b). The fungus that causes SBR and SER occurs as an endophyte on mature stem tissues, and colonises inflorescences once it reaches the peduncle and pedicel after flowering, where it will remain quiescent until fruit ripening (Roux, 1993; Ploetz & Prakash, 1997). The fungi grow down stem tissue and then colonise the inflorescence giving rise to blossom blight, or continue to grow down the

inflorescence, reach the peduncle and infect fruit through the stem-end giving rise to SBR (Lonsdale, 1993b). Fruit may also be infected at the stem-end by soil borne *Lasiodiplodia theobromae* (Pat.) if it is placed on the ground for sap bleeding (Coates *et al.*, 1995; Persley, 1993). The pathogen *L. theobromae* is now generally accepted to be an anamorph of *Botryosphaeria rhodina* (Cooke) Von Arx (Jacobs, 2002). Initial systemic infection plays a role in the cause of blossom blight, but secondary infection is more important in SBR and SER development. In the case of secondary infection, spores are being washed down from various inoculum sources in the tree onto the fruit where it can cause infection (Saaiman, 1996).

## 2.8 FRUIT DISORDERS

Fruit disorders are an important factor that affects mango production. Fruit disorders are ailments not caused by a pathogenic organism, and are mostly the result of some form of physical or environmental damage, or a physiological disorder. These blemishes downgrade the fruit quality and render fruit unacceptable for export (Ridgway, 1989).

Sunburn causes great losses on mango fruit. Damage result from exposure of fruit to high temperature and ultra violet radiation, particularly when branches are broken or when leaves are arranged as such that the fruit are exposed. Fruit exposed to direct high temperature and ultra violet radiation after harvesting may also be affected. Fruit of late ripening cultivars are more prone to sunburn damage.

Symptoms of sunburn appear as bleached or yellow patches to the skin, especially with slight damage. In severe cases, the skin becomes leathery yellow-brown to black (Ridgway, 1989).

Pre-harvest control of sunburn damage on fruit is not essential. After harvest, fruit must be prevented to directly expose to sunlight (Ridgway, 1989). The use of plastic caps with inner wool linings or commercial plastic caps proved to be the most effective method to reduce sunburn on mango fruits (Silimela & Korsten, 2001).

## 2.9 MANGO DISEASE CONTROL

Mango as an export fruit has a relatively high value with a premium price being paid for safer, blemishes free and high quality fruit. Pest and disease are the most important factors to ensure excellent fruit quality and therefore, effective control measures are required to control mango diseases.

### 2.9.1 Cultural and physical control

Field sanitation that include removal of dead twigs and branches, removal of fallen branches, leaves and fruit before flowering are essential in order to reduce the source of inoculum for pre- and post-harvest disease on mango fruit. Weeds and other undergrowth beneath the trees should be controlled to reduce humidity and increase ventilation in the tree canopy (Persley, 1993; Lonsdale, 1993b; Kwee & Chong, 1985; Saaiman, 1997a).

The most effective cultural way of controlling bacterial black spot (BBS) is through the use of disease free rootstock and scion combinations in a plant improvement program. For field planting, a selected site protected from strong wind, which also provides adequate wind breaks around and within the orchard, is essential (Gagnevin & Pruvost, 2001; Ridgway, 1989; Persley, 1993; Visser, 1995).

Planting of susceptible cultivars such as Kent, Keitt, Erwin, Zill and Ruby should be avoided; cultivars such as Sensation, Carabao and Tommy Atkins are less susceptible to BBS (Boshoff *et al.*, 1998). Orchard maintenance should not be performed while trees are wet (Persley, 1993; Swings & Civerolo, 1993). Good site selection for mango orchards is required and young trees should be planted in an area protected from strong wind (Ridgway, 1989). Cultural practices, which include the use of windbreaks, reduce the incident of BBS (Ridgway, 1989; Visser, 1995; Boshoff *et al.*, 1998).

Physical control methods, including dipping of fruit in hot water, proved to be effective in controlling post-harvest disease, but the temperature must be controlled carefully (Ridgway, 1989; Barkai-Golan & Phillips, 1991; Bugante *et al.*, 1997; Arauz, 2000).

## 2.9.2 Chemical control

Management of mango diseases rely primarily on both pre- and post-harvest applications of pesticides, which have been successful. Copper oxychloride is the pre-harvest mostly used registered chemical to control mango diseases from flowering until harvesting at three-week intervals (Boshoff *et al.*, 1998; Silimela & Korsten, 2001). However, copper oxychloride leaves residues, which have to be removed manually at the pachhouse after harvest. Post-harvest fruit dipped in prochloraz showed reduced development of post-harvest diseases on mango (Oosthuysen, 1997b).

## 2.9.3 Biological control

Current control measures for both pre- and post-harvest diseases rely on extensive pre-harvest sprays of copper fungicides. However, extensive copper sprays are providing only limited control (Korsten *et al.*, 1995). Build up of copper levels in soils have reached alarming proportions and recent restrictions by the European Union in terms of allowable copper levels in soils have forced the mango industry to reduce the number of sprays. In addition, increased pressure to decrease the maximum residual levels of pesticides as well as the chemical industry's failure to re-register key fungicides for smaller niche industries such as mango have resulted in an urgent need to develop alternative disease control strategies.

Biological control of plant diseases has provided a relatively recent alternative strategy for disease control particularly when used pre-harvestly to protect against post-harvest diseases (Korsten *et al.*, 1993; Gunasekaran *et al.*, 1996; Janisiewicz & Korsten, 2002).

Biological ways of controlling plant disease existed as long as hosts and plant pathogens. It had been the only way of disease limitation before, until the last few years when chemicals became available (Campbell, 1989). Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organism accomplished naturally or through manipulation of the environment, host or antagonist or by mass introduction of one or more antagonist (Baker & Cook, 1974). An antagonist is an organism exerting a damaging effect on another by producing lytic enzymes or antibiotics, competition and parasitism (Campbell, 1989).

Mechanisms of biological control include rapid colonisation in advance, competition for nutrients and space, release of antibiotics and endolysin, induction of resistance mechanisms in the host and direct interaction with the pathogen (parasitism) (Campbell, 1989; Droby & Chalutz, 1994).

*Bacillus* spp. have been used to control leaf spot and post-harvest diseases because of its capacity to form endospores that facilitate long term storage and ensure survival under adverse field conditions (Collins & Jacobsen, 2003). *Bacillus subtilis* was effective in the control of *Cercospora* spot on avocado (Korsten *et al.*, 1997) and *Bacillus licheniformis* has previously reported to control mango fruit diseases such as anthracnose and SBR (Korsten *et al.*, 1992; Korsten *et al.*, 1993). Some examples of potential biological agent for the control of fruit diseases are indicated in table 2.2.

In many cases, biological control strategies have been effective, but they have been slow, inconvenient, uneconomical and often unpredictable and too variable for large-scale use (Gunasekaran *et al.*, 1996). This is predominantly because of limitation of antagonist growth and survival due to water and nutrient deficiencies, harmful radiation, fluctuating climatic conditions and competition with indigenous microorganisms (Pusey, 1994). There is also a lack of sound knowledge of the biological control systems and difficulty in obtaining a successful commercial formulation (Emmert & Handelsman, 1999).

In order to initiate a successful biological control program, fundamental information concerning the relationship between biological control agents and the pathogen, and how the environment affects this relationship is required. The technologies and techniques that are presently used for production must be taken into consideration when developing effective biological control strategies. Biocontrol agents must be resistant to chemicals used to control bacteria and fungal diseases as well as physiological disorders. It must also be compatible with commercial handling systems that include dump tanks, flumes, drenches, line spray applications and must have a wide tolerance range of temperature and storage atmosphere (Spotts & Sanderson, 1994). To be effective in disease control, biological agents must grow faster and have undemanding nutrient and environmental requirements. Environmental conditions play an important role in the attachment, survival and colonisation of biological control agents (Guetsky *et al.*, 2001). Under commercial or semi-commercial conditions, plants are subjected to fluctuating temperatures, relative humidity, surface wetness periods,

gases and air movement. These conditions can affect the biological control agent directly and indirectly.

#### 2.9.4 Integrated control

Adequate suppression of plant pathogens is achieved by integration of diverse control measures, which may differ in efficacy, duration of effectiveness and cost (Shtienberg, 2000). Integrated Pest Management (IPM), has proven an effective alternative diseases and pests control method and has been shown to be an economically sound method of crop protection. This approach combines several techniques that include cultural, mechanical, biological and chemical control methods to sustain productivity with minimum adverse effects on the environment (Baker & Cook, 1974; Ragunatham & Divacar, 1996). The primary impact of IPM is rapid reduction of pesticide use, which helps to conserve biodiversity and consequently lessens the incidence of pest outbreaks (Ragunatham & Divacar, 1996).

Pre-harvest sprays of *B. licheniformis* integrated with copper fungicide from flowering until harvesting were found to reduce the incidence of anthracnose, soft brown and stem-end rot (Korsten *et al.*, 1992; Silimela & Korsten, 2001). Post-harvest control by dipping fruit in hot water followed by application of unheated prochloraz is effective to control mango post harvest diseases (Ploetz & Prakash, 1997; Pelsler, 1987). Post-harvest treatment of dipping fruit in *B. licheniformis* (isolates B250 and B251) was effective for the control of anthracnose and SBR (Korsten & Lonsdale, 1993). Similarly, hot water treatment mixed with *B. licheniformis* or hot water integrated with wax and prochloraz proved to be similarly effective (De Villiers & Korsten, 1994).

#### 2.10 CONCLUSION

Mango as an export fruit has a relatively high value, with a premium price being paid for safe, blemish-free and high quality fruit. In order for the South African growers to remain internationally competitive, growers have to ensure that they produce fruit that will be sought after in the international markets.

Pests and diseases are the most important factors that affect fruit quality worldwide. Management of plant diseases rely on the application of chemical pesticides. Pesticides

contributed to environmental pollution and destruction of natural microbial ecological balances and soil microflora, and have been linked to serious health problems in humans (Gunasekaran *et al.*, 1996). European Union and United States legislation requires all pesticides to be re-registered, a process that resulted in the dumping of less profitable and smaller niche industry products.

In order for producers to address food security needs and maintain their competitive edge on the global market, they have to ensure that food is produced in a healthy environment, using complementary pest control regime strategies and new alternatives. Adequate suppression of plant pathogens is achieved by integration of diverse control measures, which may differ in efficacy, duration of effectiveness and cost (Shtienberg, 2000). Integrated Pest Management has proven an effective alternative disease control method.

One promising strategy in controlling diseases of plants is the use of biological methods, because it can be used both as an alternative to pesticides or in conjunction with pesticides where necessary (Gunasekaran *et al.*, 1996; Emmert & Handelsman, 1999; Janisiewicz & Korsten, 2002; Obagwu & Korsten, 2003). The applications of naturally occurring suppressing organisms or biological control agents also form part of organic farming methods (Gerhardson, 2002).

Table 2.1 Important mango diseases and their causal agents (extracted from Ridgway, 1989; Dodd *et al.*, 1997; Ploetz & Prakash, 1997; Jacobs, 2002)

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### SOIL BORNE DISEASES

Disease	Casual agent
Phytophthora	<i>Phytophthora palmivora</i> Butler.
Damping off	<i>Pythium vexans</i> De Bary.
Sclerotium rot	<i>Sclerotium rolfsii</i> Sacc
Verticillium wilt	<i>Verticillium alboatrum</i> Reinke & Berth.

### FOLIAR AND FLORAL DISEASES

Disease	Casual agent
Anthracnose and blossom blight	<i>Colletotrichum gloeosporioides</i> Penz. & Sacc.
Bacterial black spot	<i>Xanthomonas campestris</i> pv. <i>mangiferaeindicae</i>
Powdery mildew	<i>Oidium mangiferae</i> Berthet.
Flower malformation	<i>Fusarium subglutinans</i> Wollenweb & Reinking.

### FRUIT DISEASES

Disease	Casual agent
Anthracnose	<i>Colletotrichum gloeosporioides</i> Penz. & Sacc.
Alternaria rot	<i>Alternaria alternata</i> Keissel.
Black mould rot	<i>Aspergillus niger</i> Tieghem.
Bacterial black spot	<i>Xanthomonas campestris</i> pv. <i>mangiferaeindicae</i>
Stem end rot	<i>Botryosphaeria</i> spp. Telemorph of <i>Dothiorella dominicana</i> Pet. et Cif.
Soft brown rot	Anamorph of <i>Botryosphaeria</i> spp. previously known as <i>Nattrassia mangiferae</i> (Sydow et Butler)

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Table 2.2 Some examples of potential biological control agents for control fruit diseases

Crop	Target pathogen	Organisms/antagonists	References
Apple	<i>Botrytis cinerea</i>	<i>Acremonium breve</i>	Janisiewicz, 1987
	<i>Penicillium expansum</i>	<i>Pseudomonas</i> spp. <i>Pseudomonas</i> spp. (L-22-64) White yeast (F-43-31)	Janisiewicz, 1988
	<i>P. expansum</i> <i>B. cinerea</i> <i>Rhizopus nigricans</i>	<i>Candida sake</i>	Vinas <i>et al.</i> , 1998
	<i>P. expansum</i>	<i>C. sake</i>	Usall <i>et al.</i> , 2000
	<i>P. expansum</i> <i>B. cinerea</i> <i>Mucor</i> spp.	<i>Pseudomonas cepacia</i>	Janisiewicz & Roitman, 1987
	<i>P. expansum</i> <i>B. cinerea</i> <i>Pezicula malicorticis</i>	<i>Aureobasidium pullulans</i> <i>Rhodotorula glutinis</i> <i>Bacillus subtilis</i>	Leibinger <i>et al.</i> , 1997
	<i>B. cinerea</i>	<i>Cryptococcus laurentii</i>	Roberts, 1990a
	<i>B. cinerea</i> <i>P. expansum</i>	<i>Cryptococcus albidus</i>	Fan & Tian, 2001
	<i>P. expansum</i>	<i>Candida sake</i>	Usall <i>et al.</i> , 2001a
	<i>P. expansum</i> <i>B. cinerea</i>	<i>Aureobasidium pullulans</i>	Ippolito <i>et al.</i> , 2000
	<i>P. expansum</i>	<i>A. pullulans</i>	Janisiewicz <i>et al.</i> , 2000

Table 2.2 continued

Apple	<i>P. expansum</i>	<i>Pseudomonas</i> spp.	Bedford <i>et al.</i> , 2001
	<i>B. cinerea</i>		
	<i>P. expansum</i>	<i>Pseudomonas syringae</i>	Janisiewicz &
	<i>B. cinerea</i>	(Bio-Save 110)	Jeffers, 1997
	<i>P. expansum</i>	<i>P. syringae</i>	Janisiewicz & Bors,
		<i>Sporobolomyces roseus</i>	1995
	<i>B. cinerea</i>	<i>P. cepacia</i>	Janisiewicz &
			Roitman, 1988
Apricot	<i>P. expansum</i>	<i>Candida oliophila</i>	El-Neshawy, 1999
Avocado	<i>Akaropeltopsis</i> spp.	<i>B. subtilis</i>	Korsten <i>et al.</i> ,
	<i>Pseudocercospora purpurea</i>		1991; Korsten <i>et</i>
	<i>Colletotrichum gloeosporioides</i>		<i>al.</i> , 1994
	<i>Dothiorella</i> spp.		
Citrus	<i>Penicillium digitatum</i>	<i>Debaryomyces hansenii</i>	Chalutz & Wilson,
	<i>Penicillium italicum</i>		1990
	<i>P. digitatum</i>	<i>B. subtilis</i>	Obagwu & Korsten,
	<i>P. italicum</i>		2003
	<i>P. digitatum</i>	<i>Aureobasidium pullulans</i>	Wilson & Chalutz,
	<i>P. italicum</i>	<i>P. cepacia</i>	1989
		<i>P. syringae</i>	
		Yeasts	Kinay <i>et al.</i> , 2001
	<i>P. digitatum</i>	<i>Pantoea agglomerans</i>	Usall <i>et al.</i> , 2001b

Table 2.2 continued

Citrus	<i>P. digitatum</i>	<i>P. cepacia</i>	Smilanick & Denis- Arrue, 1992
		<i>P. corrugata</i>	
		<i>P. aeruginosa</i>	
		<i>P. flourescens</i>	
Grape fruit	<i>P. digitatum</i>	<i>Candida famata</i>	D'hallewin <i>et al.</i> , 1999
Peach	<i>Monilinia fructicola</i>	<i>B. subtilis</i>	Pusey <i>et al.</i> , 1988
Pear	<i>P. expansum</i>	<i>P. cepacia</i>	Janisiewicz & Roitman, 1988
	<i>P. expansum</i>	<i>Erwinia spp.</i> <i>Rhodotorula spp.</i>	Kampp, 1994
	<i>P. expansum</i> <i>B. cinerea</i> <i>Phialophora malorum</i> <i>Alternaria alternata</i>	<i>Cryptococcus infirmo- miniatus</i> <i>C. laurentii</i> <i>R. glutinis</i> <i>Candida oleophila</i>	Benbow & Sugar, 1999
	<i>Mucor piriformis</i>	<i>Cryptococcus flavus</i> <i>C. albidus</i> <i>C. laurentii</i>	Roberts, 1990b
	<i>P. expansum</i> <i>B. cinerea</i> <i>Rhizopus stolonifer</i>	<i>Pantoea applomerans</i>	Nunes <i>et al.</i> , 2001
	<i>P. expansum</i> <i>B. cinerea</i>	<i>P. syringae</i>	Janisiewicz & Marchi, 1992

Table 2.2 continued

Mango	<i>C. gloeosporioides</i> <i>Nattrassia mangiferae</i> <i>Alternaria alternata</i>	<i>Bacillus licheniformis</i>	Korsten <i>et al.</i> , 1992
Strawberry	<i>B. cinerea</i> <i>R. stolonifer</i>	<i>Aureobasidium pullulans</i> <i>Candida oleophila</i> <i>Candida vanderwaltii</i>	Lima <i>et al.</i> , 1997
Table & wine grapes	<i>B. cinerea</i> <i>R. stolonifer</i> <i>Aspergillus niger</i>	<i>Candida guilliermondii</i> <i>Acremonium cephalosporium</i>	Zahavi <i>et al.</i> , 2000

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## CHAPTER 3

### EVALUATION OF ATTACHMENT, COLONISATION AND SURVIVAL OF *BACILLUS LICHENIFORMIS*

#### ABSTRACT

Biological control programmes aimed at reducing mango diseases depend on the successful attachment, colonisation and survival of the biocontrol agent. In this study, different additives including Biofilm and Superfilm (spreader-sticker), Agral 90 (wetter-spreader), Nufilm-P (wetter-sticker), biostimulant Bioboost and copper oxychloride were evaluated *in vitro* and *in vivo* for their effect on the attachment, colonisation and growth of the antagonist *Bacillus licheniformis*. Additionally, plastic woolly caps impregnated with *B. licheniformis* were evaluated for the antagonists' ability to also attach, colonise and survive on the woolly base prior to and after being used in the field to protect fruit from sunburn damage. Attachment and colonisation of the antagonists on mango leaves and woolly caps were evaluated using the scanning electron microscopy and colonisation, growth and survival were determined using a standard dilution series method. *In vitro* studies indicated that copper oxychloride, Superfilm and Agral 90 inhibited growth of *B. licheniformis* on agar and copper and Superfilm reduced growth in liquid medium after eight hours. Additives had no negative effect on the ability of the antagonist to grow and colonise mango leaves. Irreversible attachment of the antagonists to mango leaves was observed 5 minutes after application. The antagonist attached effectively to the woolly base of the caps and survived throughout the season.

### 3.1 INTRODUCTION

Mango (*Mangifera indica* L.) is subject to various leaf, fruit and blossom diseases that affect fruit quality and cause significant loss for the growers (De Villiers & Korsten, 1994; Dodd & Jeffries, 1989; Jacobs, 2002). Limited control of mango fruit diseases has thus far been achieved with various pre- and post-harvest chemical applications (Korsten *et al.*, 1993; De Villiers & Korsten, 1994). Additionally, an increasing number of fungicides are no longer acceptable for use on fruit destined to certain export markets. Global concern of pesticides on human health, build up of pathogen resistance to certain fungicides and accumulation of chemicals in the environment, particularly in soils have added to the negative perception of modern crop protectants. Growing international environmental awareness necessitate that agricultural industries seek natural alternative methods of disease control (Korsten *et al.*, 1991; De Villiers & Korsten, 1994).

Integrated control that includes the use of biocontrol agents is a viable alternative method to reduce excessive use of chemicals (Korsten *et al.*, 1993). Biological control on its own or as part of an integrated programme has proven to be effective to control several diseases (Guetsky *et al.*, 2001). *Bacillus* spp. have been used to control a number of leaf spot and post-harvest diseases because of its capacity to form endospores that facilitate long-term storage and ensure survival under adverse field conditions (Collins & Jacobsen, 2003). Integrating *B. subtilis* with flutolanil fungicides could effectively control damping-off caused by *Rhizoctonia solani* on tomatoes (Kondoh *et al.*, 2001). *Bacillus subtilis* was also effective in the control of *Cercospora* spot on avocado used either on its own or integrated with copper oxychloride (Korsten *et al.*, 1997). Biological control of mango fruit diseases using *Bacillus licheniformis* applied on its own or integrated with copper fungicides has also previously been shown to be effective (Korsten *et al.*, 1992; Korsten *et al.*, 1993). A new alternative approach of adding the biocontrol product *B. licheniformis* to a woolly base of a plastic cap made from polyethylene foam was also recently tested with reasonable success (Silimela & Korsten, 2001).

In order to initiate a successful biological control programme, fundamental information concerning the relationship between biological control agents and the pathogen, and how the environment affects this relationship, is required. In addition, the effects of commercial fungicides or additives on the viability and performance potential of the biological agent need

to be determined (Korsten *et al.*, 1992), since different additives (stickers, spreaders and wetting agents) are commercially applied with fungicides to enhance its adherence to the leaf surface (Hassal, 1990; Harvey, 1992). When spraying in orchards, it is important that spray droplets adequately wet the foliage and fruit surface and effectively cover the complete surface and stick to it and ensure adequate protection. Different stickers and spreaders including Biofilm, Nufilm-P and Agral 90 were previously evaluated and found not to have any negative effect on the attachment and survival of *B. licheniformis* (Korsten *et al.*, 1992). The ability of bacteria to attach effectively to the plant surface for a prolonged period of time is of importance because it ensures the ability of the antagonist to colonise and survive (Marques *et al.*, 2002). In order to enhance biocontrol product performance, nutrient additives such as a biostimulant, Bioboost, are often added during applications to ensure effective antagonist growth and ultimate colonisation.

The purpose of this study was to evaluate the effect of different additives (stickers, wetters and spreaders), Biofilm, Superfilm, Nufilm-P and Agral 90 for their effect to enhance attachment, colonisation and the growth potential of *B. licheniformis*. The fungicide copper oxychloride was also evaluated for its ability to influence the growth of the antagonists when used in integrated applications. In addition, a biostimulant, Bioboost, was evaluated for its potential growth stimulatory activity on the antagonists. Antagonists were also evaluated for their ability to attach to the woolly base of plastic caps and to determine if they could survive for prolonged periods of time under natural environmental conditions.

## 3.2 MATERIAL AND METHODS

### 3.2.1 Effect of additives on *Bacillus licheniformis*

#### 3.2.1.1 Disk diffusion test

Six compounds were tested for its effect on the antagonist's growth, namely a fungicide copper oxychloride (UCP. Universal; 2 550 ppm active ingredient (a.i./ℓ), a biostimulant Bioboost (Impro. Crop. Ltd; 200 ppm. a.i./ℓ) and four different adjuvants namely, Biofilm (spreader-sticker; Plaaskem; 2 500 ppm), Superfilm (sticker-spreader; Plaaskem; 2 500 ppm a.i./ℓ), Nufilm-P (wetter-sticker; Hygrotech; 2 600 ppm a.i./ℓ) and Agral 90 (wetter-spreader; Kynoch. Agrochem; 1 800 ppm a.i./ℓ). Standard 1 nutrient agar (STD 1) (Biolab) was

prepared according to standard procedures and allowed to cool down to  $\pm 40^{\circ}\text{C}$  before adding 5 ml of commercial *B. licheniformis* (Mangogreen) (Stimuplant, Ltd.,  $10^9$  cells/ml) to each litre of medium. The flasks were gently mixed before pouring the medium into standard Petri dishes and allowing it to solidify for 24 hours before use. Nine Petri dishes were selected for each test compound. Each Petri dish with medium was divided into two halves. In the middle of each side, one hole was made using a sterile cockborer (6-mm). The hole on the one side of the Petri dish was filled with 10  $\mu\text{l}$  of sterile water as control and the other with 10  $\mu\text{l}$  of test compound. Petri dishes were stored in the incubator ( $37^{\circ}\text{C}$ ) for 24 hours. Evaluation was done after 24 hours taking three measurements of the inhibition zone if present in diameter (mm). The experiment was repeated three times. Data was statistically analysed using the statistical program GenStat (2000). One-way analysis of variance (ANOVA) was used to test for differences in average mean between test compounds. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at a 5 % level of significance.

#### **3.2.1.2 Dilution plate counts**

The same six compounds used in 3.2.1.1 were tested for their effect on the growth of the antagonists. Three test tubes were prepared for each of the six test compounds. Test tubes were filled with 9 ml sterile nutrient broth (NB) (Biolab) and 1 ml of each test compound at the recommended rate. At first, this experiment was done using Ringer's solution (Merck), but was found to be inadequate for the purpose of this experiment since cells could not survive in the solution for the duration of the experiment of more than 24 hours. It was subsequently decided to repeat the experiment using NB as described. The control test tube contained only 9 ml of NB media. All test tubes, including the control, were inoculated with one ml commercial *B. licheniformis* liquid at  $10^9$  cells/ml (Stimuplant, cc). Test tubes were stored at room temperature ( $\pm 25^{\circ}\text{C}$ ), and aliquots from each tube (0.1 ml) were subsequently plated onto STD 1 media immediately after preparation and after 1, 2, 4, 8 and 24 hours. Plating was done by spreading the bacterial suspension evenly over the entire surface of the STD 1 agar plate. Plates were incubated at  $37^{\circ}\text{C}$  and evaluation for growth was done by counting *B. licheniformis* colonies after 24 hours. Data was analysed statistically as described in 3.2.1.1 for different time intervals.

### 3.2.2 Attachment and colonisation of *Bacillus licheniformis* on mango leaves

#### 3.2.2.1 Effect of additives on the ability of *Bacillus licheniformis* to effectively attach and colonise mango leaves

Three-year-old 'Kent' mango trees, maintained in the greenhouse at the University of Pretoria experimental farm, were selected for this trial. Four different test compounds namely: Biofilm, Superfilm, Nufilm-P and Agral 90 were selected for enhancing antagonist attachment and colonisation.

Three trees were randomly selected per test compound. Trees were sprayed with a hand held one litre spray bottle containing a suspension of commercial *B. licheniformis* ( $10^9$  cells/ml) at 5 ml/l of water mixed with either one of the test compounds prepared at recommended rate. The control trees were sprayed with a suspension of commercial *B. licheniformis* and water only. Eight leaves per tree were picked, two leaves on each side of the tree (northern, southern, western and eastern side). These leaves were picked 1, 5, 15, 30, 60 and 120 minutes after spraying. Five discs were cut from each leaf using a sterile cork borer and were pooled. Thirty of these discs were selected for enumeration. The discs ( $\pm 1$  g) were placed in test tubes with nine ml sterile Ringer's solution and mixed with a vortex shaker before making a dilution series. For enumeration, the spread plate technique on Petri dishes containing STD 1 agar was used as described in 3.2.1.2. After plating, Petri dishes were incubated at 37°C for 24 hours. Evaluation to determine the survival of *B. licheniformis* was done by counting the number of *B. licheniformis* colonies on the Petri dishes after 24 hours incubation. Data were statistically analysed as described before.

#### 3.2.2.2 Attachment and colonisation of *Bacillus licheniformis* on mango leaves using scanning electron microscopy

Three-year-old 'Kent' mango trees similar to those used 3.2.2.1 were selected. Three trees and five leaves per tree were selected for this trial. The leaves were sprayed with 70 % ethanol and wiped with a sterilized cloth. Ten minutes after disinfection, five blocks (5 x 5 mm) were marked with a permanent marker pen on each leaf. Each block was inoculated with 10  $\mu$ l of commercial *B. licheniformis* ( $10^9$  cells/ml) suspension. As a control, tap water was used. The inoculated leaves on the trees were maintained in the greenhouse at  $\pm 28^\circ\text{C}$ . After 1, 5, 15, 30

minutes and 1, 2, 8 and 24 hours, leaf samples were taken for scanning electron microscopy (SEM) to determine attachment and colonisation of the antagonist. Discs were fixed with 2.5% Gluteraldehyde (Electron microscopy sciences, Washington) in 0.075 Molar of phosphate buffer for one hour at pH 7.4. Discs were rinsed three times with the buffer at 15 minutes intervals. Discs were dehydrated with a dilution series of ethanol at 50, 70, 90 and 100 % for 15 minutes at each dilution. In 100 % ethanol, dehydration steps were repeated three times at 15 minutes intervals. After dehydration, discs were critically point dried with liquid carbon dioxide Biorad (Polaron Equipment Limited, Watford, England). Squares were then mounted on aluminium stubs and sputocoated with gold ( $\pm$  15 nm thick) using a SEM Autocoating unit E5200 (Polaron Equipment Limited). Finally, the samples were analysed using a JSM-840 SEM (JEOL, Tokyo, Japan) and viewed at 5 kV. Since this method was ineffective in terms of illustrating successful bacterial attachment and colonisation, the following method was adopted.

Three replicates, of ten discs from unmarked leaves were made using a sterile cork borer and placed in 9 ml NB (NB was used instead of Ringer's solution since the experiment was done over a 24 hour period). Tubes containing the leaves were inoculated with 1 ml commercial *B. licheniformis* ( $10^9$  cells/ml), shaken gently and incubated at 37°C. For the control, disks were placed only in nutrient broth without *B. licheniformis*. After 24 hours, five discs per replicate were removed for SEM studies to determine antagonist attachment and colonisation on leaves. Discs were first washed three times for 15 minutes each with phosphate buffer prior to SEM sample preparations to ensure removal of non-adhered cells. Discs were prepared for the SEM as described for the leaf blocks.

### **3.2.3 Evaluation of *Bacillus licheniformis* attachment and survival on plastic caps with inner wool linings**

Plastic woolly caps (caps were made from polyethylene foam with added wool attached to it) were obtained from Airshield Pty, Ltd. (Johannesburg) and were cut into 15 x 15 cm squares for experimental purposes. Woolly caps were turned upside down so that the woolly part faced upwards for applying antagonist or copper suspensions. Copper oxychloride (UCP, Universal) at 2 550 ppm, a.i./ℓ and commercial *B. licheniformis* ( $10^9$  cells/ml) mixed at 5 m/ℓ of tap water and were prepared in 5 ℓ volumes and applied to upturned woolly caps using a hand sprayer. As for the control, woolly caps were sprayed with clean tap water only. After

spraying, caps were left to air dry at room temperature ( $\pm 25$  °C) for two days in a closed room. Ten *B. licheniformis* sprayed woolly caps were selected for antagonist survival studies. From each cap, three 10 x 10 mm squares were cut and placed in a sterile test tube containing 9 ml Ringers. A dilution series (as described in 3.2.2.1) was subsequently prepared from each tube and plated on STD 1 agar (as described in 3.2.1.2). Plates were incubated at 37°C for 24 hours before colony forming units (c.f.u) were counted and data analysed as described in 3.2.2.1. Five woolly caps sprayed with *B. licheniformis* were selected and five pieces (10 x 10 mm) were cut from each cap to evaluate *B. licheniformis* attachment to the woolly base using the SEM. Each piece was prepared as described in 3.2.2.2 and viewed at 5 kV. The remaining sprayed woolly caps were used in field trials at Bavaria Estate (Hoedspruit) and Ryfontein (Letsitele Valley) respectively as described in chapter 4 (4.2.1). Caps were attached to the stem end of mango fruits ('Kent') ensuring that the sides overlap. The caps were secured into their position by stapling the overlapping sides together, giving an overall protective hood effect. The woolly caps remained attached to the fruits until harvest (late January to early February). A similar procedure for evaluation of antagonists' survival on the woolly caps was followed three months after harvest of fruits as previously described within this paragraph using the dilution series and SEM methods.

### 3.3 RESULTS

#### 3.3.1 Effect of additives on *Bacillus licheniformis*

##### 3.3.1.1 Disk diffusion test

Bioboost, Biofilm and Nufilm-P had no significant inhibitory effect on the growth of *B. licheniformis* as compared to Superfilm, Agral-90 and Copper (Table 3.1). Superfilm and Agral-90 showed a significant inhibitory effect towards the growth of *B. licheniformis*. Copper oxychloride was the most inhibitory towards *B. licheniformis* when evaluated using this method (Table 3.1).

##### 3.3.1.2 Dilution plate counts

Of the test products, Bioboost, Biofilm, Nufilm-P and Agral 90 enhanced *B. licheniformis* viability and growth for even up to 24 hours (Fig. 3.1). Nufilm-P reduced *B. licheniformis*

growth from two hours, but after four hours the growth started to increase. However, Superfilm and copper oxychloride negatively reduce *B. licheniformis* growth after eight hours.

### **3.3.2 Attachment and colonisation of *Bacillus licheniformis* on mango leaves**

#### ***3.3.2.1 Effect of additives on the ability of Bacillus licheniformis to effectively attach and colonise mango leaves***

All the additives used enhanced *B. licheniformis* attachment and colonisation on mango leaves. There was no significant difference between stickers and spreaders for enhancing antagonist attachment and survival on mango leaves (Fig. 3.2). All test compounds evaluated showed a significant increase in viable cells as indicated by c.f.u attached on mango leaves from 5 minutes onwards.

#### ***3.3.2.2 Attachment and colonisation of Bacillus licheniformis on mango leaves using scanning electron microscopy***

No attachment structures were observed under the SEM study using leaves with antagonists applied leaves. However, effective attachment structures and subsequent colonisation were observed with the nutrient broth method (Figs 3.3, 3.4, 3.5). No bacterial cells were observed with the control sample. In most cases, fibrillar strands were observed emanating from the bacteria, which were attached to the surface (Fig. 3.4). Multiplication of *B. licheniformis* cells were also observed on leaves 24 hours after sampling (Fig. 3.5).

### **3.3.3 Evaluation of *Bacillus licheniformis* attachment and survival on plastic caps with inner wool linings**

There was an initial decrease in the number of viable cells on the woolly caps four days after cap preparation (Fig. 3.6). After 91 days in the field, *B. licheniformis* could still be retrieved from the caps although at a significantly lower level compared to the initial counts (Fig. 3.6). Scanning Electron Microscopy studies showed some biofilm formation with the antagonists attaching to the threads of the woolly base of the caps after four days (Fig. 3.7).

### 3.4 DISCUSSION

The *in vitro* study showed that the biostimulant Bioboost, the sticker-spreader Biofilm, wetter-sticker Nufilm-P and wetter-spreader Agral 90, had no growth inhibitory effect on *B. licheniformis*. However, the sticker-spreader Superfilm and Agral 90 showed some inhibitory effect on the growth of the antagonists. Copper oxychloride inhibited the growth of *B. licheniformis*, which is of importance in integrated control if growers apply the two simultaneously.

This study found that *B. licheniformis* attached, colonised and grew effectively on mango leaf surfaces. These results correspond with that of Towsen (1996), who studied attachment, colonisation and survival of *B. subtilis* on avocado leaf and fruit surfaces. Once bacterial cells are exposed to a suitable location, the ability to attach to the plant surface is important since it is the first step in terms of survival and colonisation (Romantschuk, 1992). Irrespective of *B. licheniformis* being mixed with stickers or spreaders, or whether it was used on its own, the minimum time required for effective attachment to the leaf surface was found to be 5 minutes. Different attachment times were previously reported for different surfaces and bacterial combinations. According to Towsen (1996), Leben & Whitmoyer (1979) reported that *Pseudomonas lachrymans* adhered effectively to young cucumber leaves after 10 minutes and Lawrence *et al.* (1987) found *Ruminococcus flavefaciens* to adhere to rye grass after 30 minutes. Differences in attachment times could be explained by various attachment and subsequent colonisation phases, which include reversible attachment, irreversible attachment, reversible adherence, irreversible adherence, growth and colonisation (Towsen, 1996).

None of the stickers or spreaders used in this trial had a negative effect on the ability of the antagonist to attach and colonise on mango leaves, which supports the work done by Korsten *et al.* (1992). Previous commercial integrated practices included spraying *B. subtilis* mixed in or applied simultaneously with copper oxychloride to control *Cercospora* spot on avocado (Korsten *et al.*, 1997). Compatibility of the antagonist used in combinations with copper is therefore critical if consistent effective control is to be attained. Data obtained in this study, therefore, suggest that it is not advisable to combine *B. licheniformis* and copper oxychloride in combined spraying programs but rather to apply them in an alternating programme as is currently done with avocado.

Environmental conditions play an important role in the attachment, survival and colonisation of biological control agents (Guetsky *et al.*, 2001). Under commercial or semi-commercial conditions, plants are subjected to fluctuating temperatures, relative humidity, surface wetness periods, gases and air movement. These conditions can affect the biological control agent directly and indirectly. The field temperatures at which commercial spraying were done (Hoedspruit and Letsitele valley at Limpopo Province) during the summer can be as high as 42°C. Such high temperatures could adversely affect the biocontrol agents' ability to survive. Although an initial decrease in viable counts was recorded on woolly caps within four days after application, *B. licheniformis* could still be retrieved from woolly caps after three months in the field although at significant lower numbers.

This study proved that *B. licheniformis* could effectively attach to woolly caps. Of special interest was the observed biofilm formation on the inert woolly base thread four days after *B. licheniformis* application. This is the first study of its kind that showed the effective attachment of *B. licheniformis* to woolly caps even for a period of up to 91 days under field conditions. These findings highlight the potential use of this method to provide additional disease control through prolonged exposure to the fruit. This aspect will be evaluated in the subsequent chapter.

Table 3.1 Effect of different additives and copper oxychloride on the growth of *Bacillus licheniformis* after 24 hours

Treatment (Test compounds)	Inhibition zone in Diameter (mm) <sup>a</sup>
Control	0c <sup>b</sup>
Bioboost	0c
Biofilm	0c
Nufilm-P	0c
Superfilm	1.56b
Agral 90	3.17b
Copper	8.22a

<sup>a</sup> Inhibition zone values represent an average value of nine replicates.

<sup>b</sup> Different letters in the table indicate significant differences between the test compounds according to Fisher's t-test least significant difference (LSD) at a 5 % level of significance (Pr = 0.001).

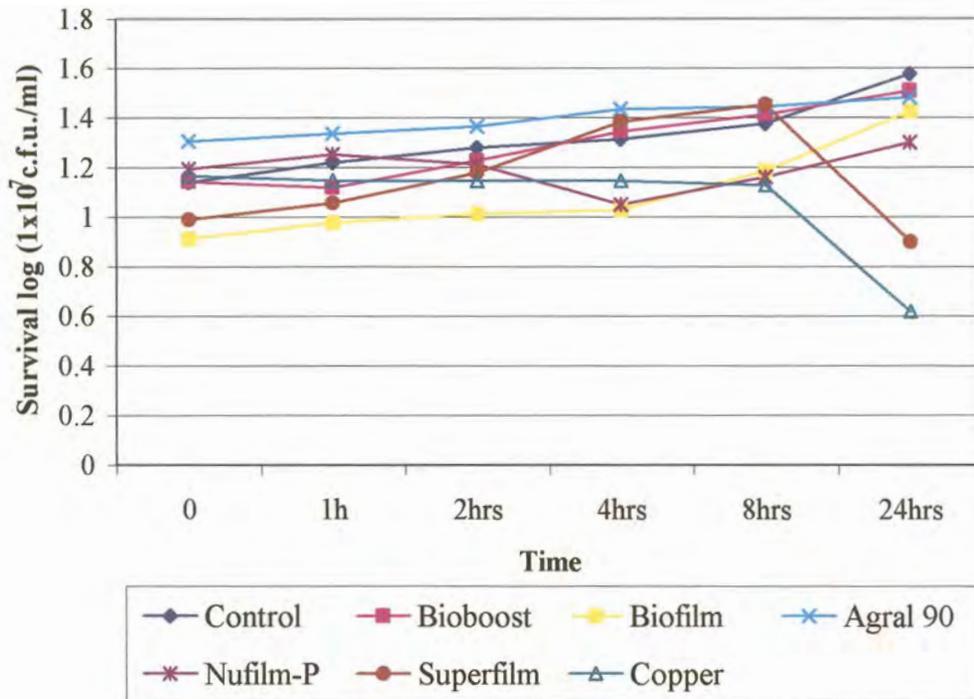


Fig. 3.1 Effect of additives on the growth of *Bacillus licheniformis* over a period of 24 hours (Pr-value = 0. 001).

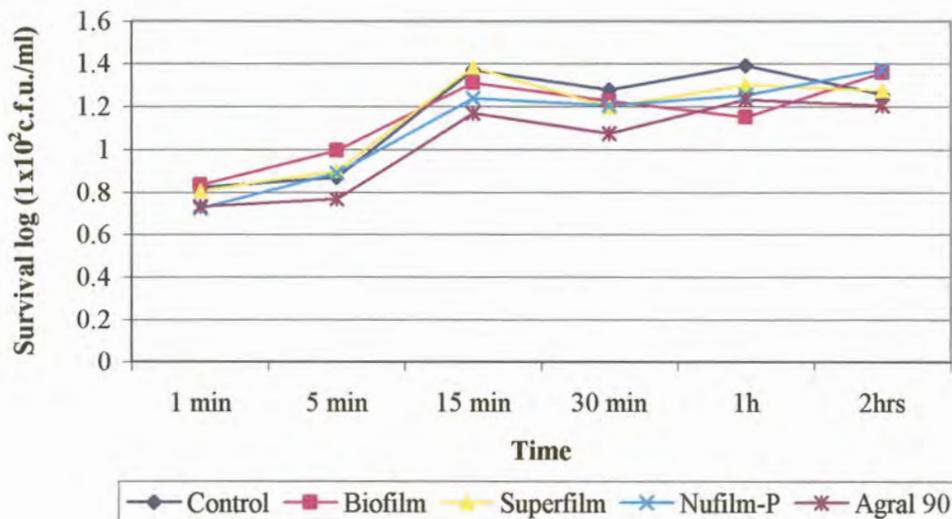


Fig. 3.2 Effect of different additives on the successful attachment and growth of *Bacillus licheniformis* on mango leaves (Pr-value = 0. 008).

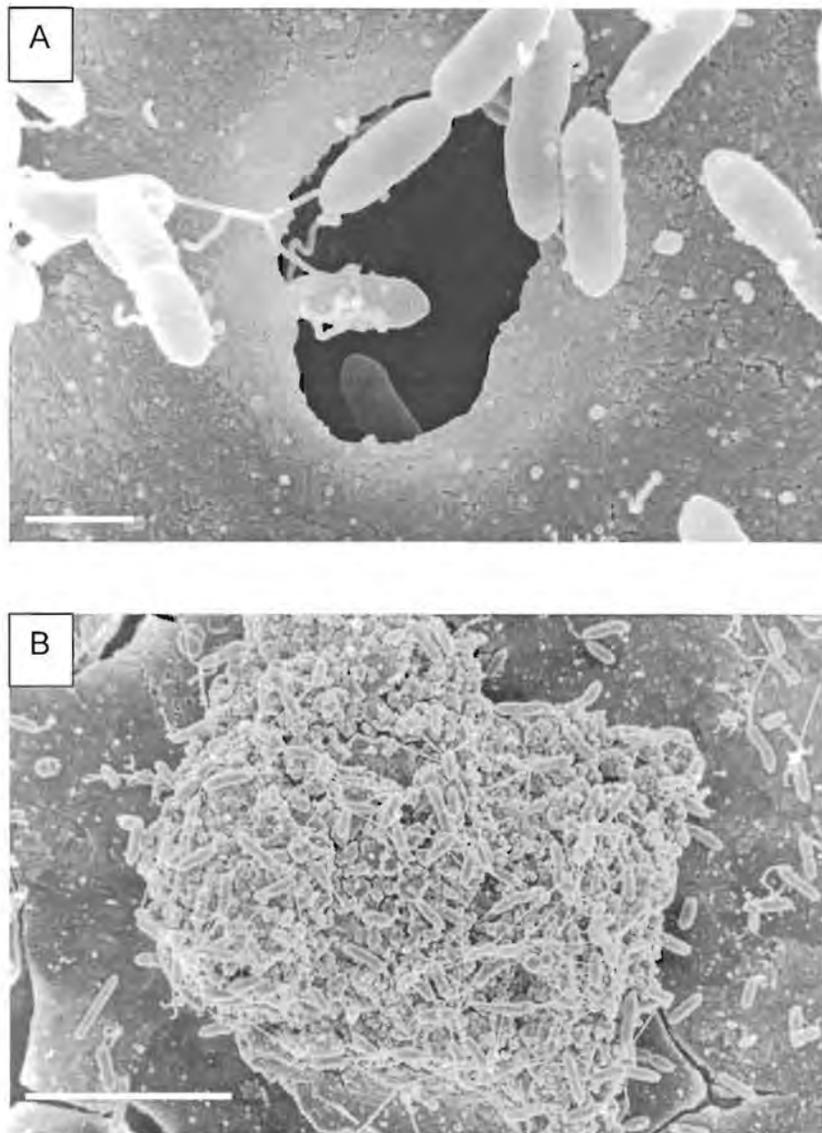


Fig. 3.3 Scanning electron micrographs of *Bacillus licheniformis* showing different attachment structures as observed 24 hours after application of the antagonist to the mango leaf. A) Attachment of *B. licheniformis* cells around and inside the stomatal opening; B) Micro-colony formation of *B. licheniformis* on the mango leaf surface (Bar: A = 1  $\mu\text{m}$ ; B = 10  $\mu\text{m}$ ).

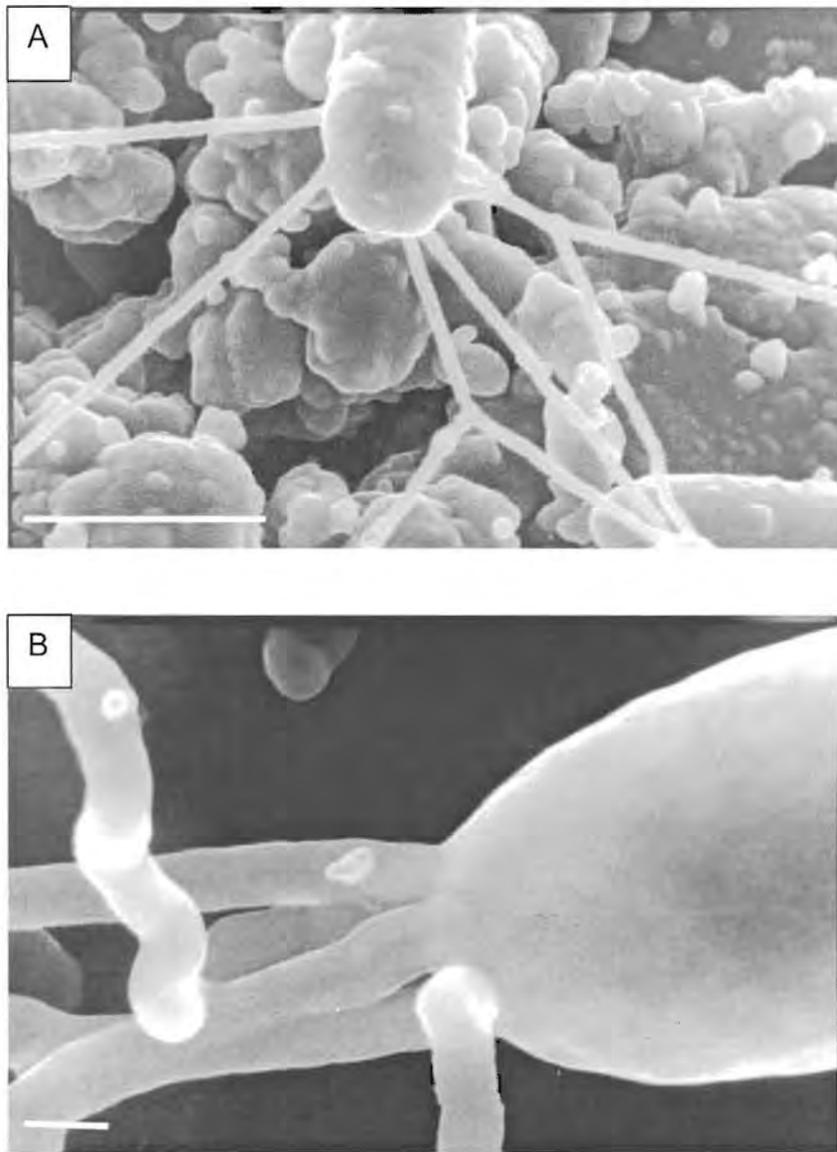


Fig. 3.4 Scanning electron micrographs of *Bacillus licheniformis* indicating attachment structures as observed 24 hours after application of the antagonist to the mango leaf. A-B) Fibril-like strand formations of *B. licheniformis* on a mango leaf (Bar: A = 1 $\mu$ m; B= 100nm).

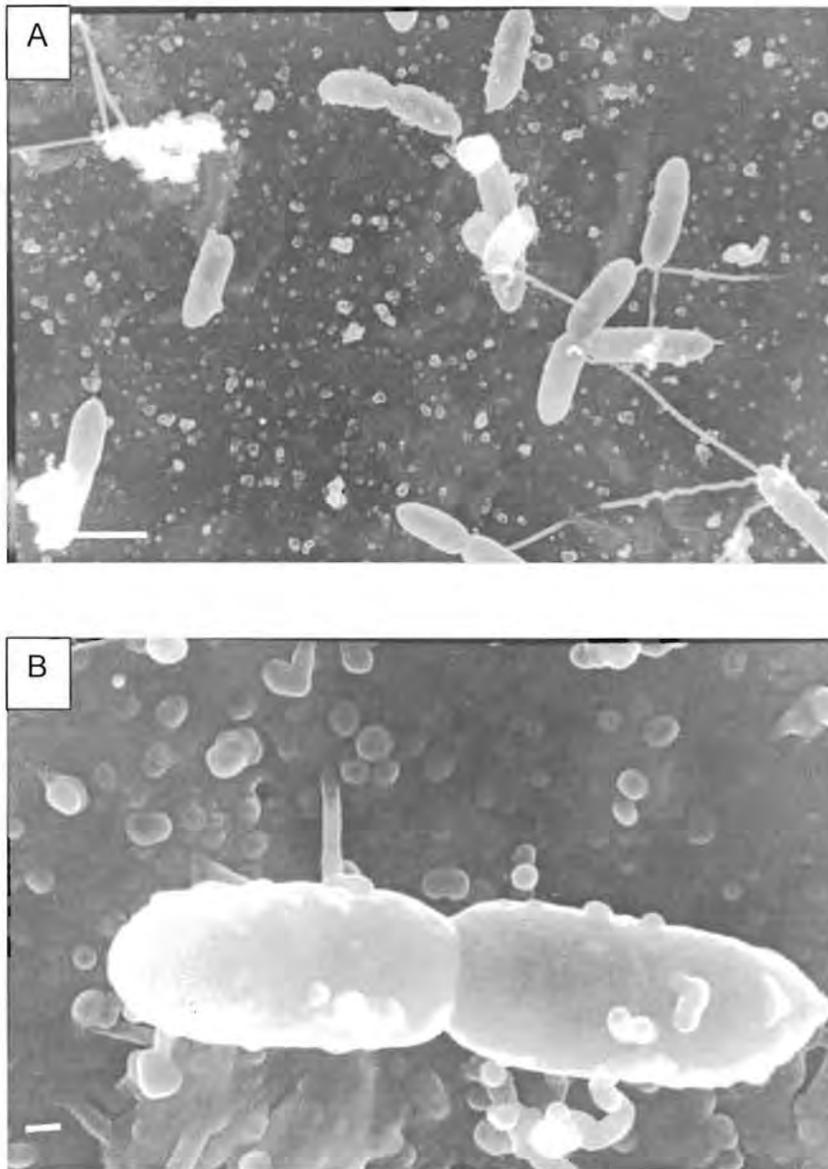


Fig. 3.5 Scanning electron micrographs of *Bacillus licheniformis* to indicate attachment and multiplication of bacterial cells as observed 24 hours after application of antagonist to the mango leaf. A) Attachment and division of bacterial cells; B) *B. licheniformis* fibril-like strand formations and cell division of bacteria (Bar: A = 10 $\mu$ m; B = 100nm).

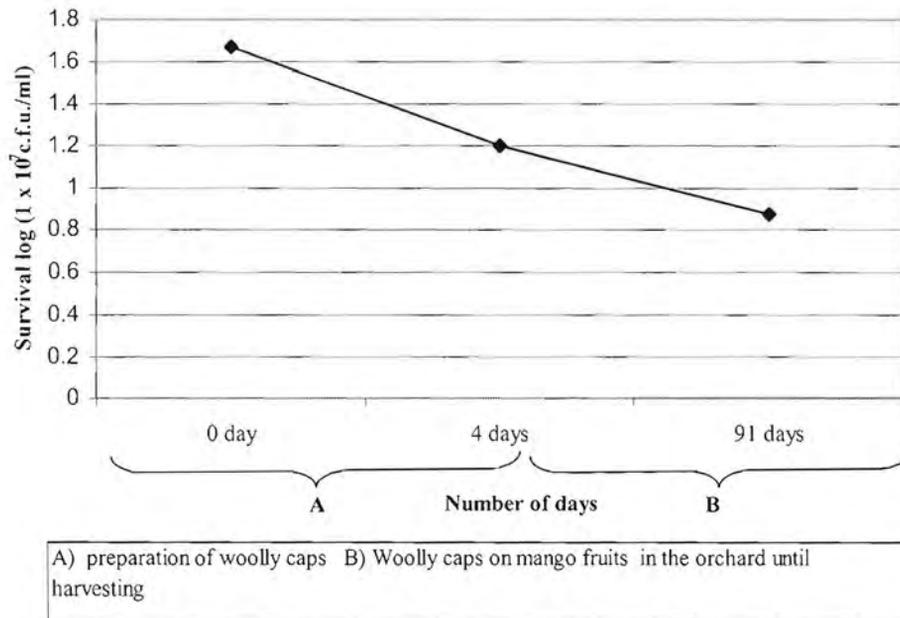


Fig. 3.6 *Bacillus licheniformis* survival on woolly caps after application and after 91 days in the field when used on fruit in a semi commercial trial (Pr-value = 0.001).

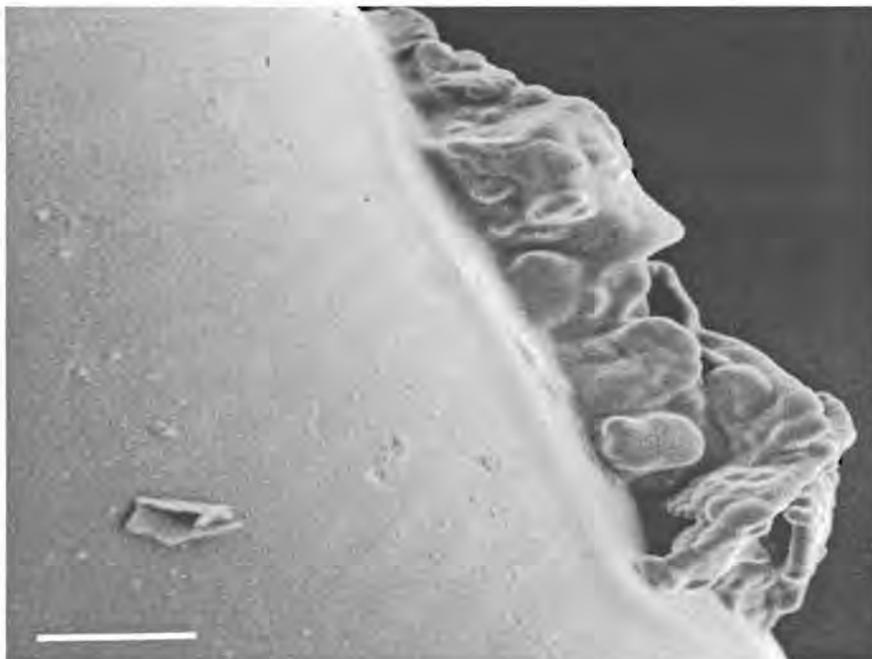


Fig. 3.7 Scanning electron micrographs of *Bacillus licheniformis* attachment to threads of the woolly caps indicating biofilm formation (Bar = 10 $\mu$ m).

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## CHAPTER 4

### FIELD TRIALS WITH ALTERNATIVE APPROACHES TO IMPROVE MANGO FRUIT QUALITY

#### ABSTRACT

Mangoes are an economically important crop in South Africa, but profit margins are often eroded due to pre- and post-harvest diseases as well as physiological disorders and abiotic factors such as sunburn. An innovative new approach to apply fungicide or biocontrol agents to modified plastic caps (made from polyethylene foam) was evaluated. Plastic caps with inner wool linings were treated with *B. licheniformis* or copper oxychloride and placed on fruits after initial fruit drop. Current control of both pre- and post-harvest diseases relies on extensive pre-harvest spraying with mostly copper fungicides. However, continuous monthly sprays are costly and can result in visible residues on fruit that have to be manually cleaned in the packhouse. In addition, copper residues build up in soils due to continuous spraying can significantly contribute to environmental pollution. In this study, *B. licheniformis* (in commercial powder or liquid formulation) was evaluated on its own or alternated with copper fungicides to control fruit diseases. Pre-harvest *B. licheniformis* or copper oxychloride sprays on ‘Kent’ and ‘Tommy Atkins’ trees were applied from flowering until harvesting at three weekly intervals at two geographically distinct farms over two seasons. The results showed that plastic caps with inner wool linings more effectively reduced sunburn compared to currently used commercial plastic caps. Caps impregnated with copper or *B. licheniformis* showed little effect on disease control. Pre-harvest sprays of *B. licheniformis* controlled bacterial black spot and soft brown rot. Antagonist on its own or alternated with commercial copper was also effective in controlling anthracnose on ‘Tommy Atkins’ and ‘Kent’.

## 4.1 INTRODUCTION

The mango (*Mangifera indica*. L) is a highly prized tropical fruit on the European markets. Worldwide, it is rapidly becoming one of the leading trade crops from the tropical and subtropical regions (Nakasone & Paull, 1998). South Africa exports the bulk of its annual mango crop to mainly Europe (Finnemore, 1999), competing with Mexico, Brazil, Peru, Venezuela, Jamaica, Ivory Coast, and Mali (Nakasone & Paull, 1998). In order to remain competitive, the South African mango industry has to ensure that export consignments adhere to consistent high levels of quality fruit that are free from diseases, insect damage, sunburn damage and chemical residues.

Sunburn damage can result in significant losses particularly on fruit produced in the southern hemisphere and when trees are stressed having reduced foliage to properly cover developing fruit. More recently, growers have evaluated the use of paper bags to protect fruit from sunburn and diseases. Individual fruit were covered with paper bags during fruit development (Bugante *et al.*, 1997). This method proved ineffective, since heavy rains damaged bags and caused an increase in disease infection. Currently, commercial practices of covering individual fruits with plastic caps have proved effective in reducing sunburn (Lonsdale, personal communication, November, 2001).

One of the most important pre-harvest diseases of mango is bacterial black spot (BBS), caused by *Xanthomonas campestris*. pv *mangiferaeindicae*. Yield losses of more than 80 % on mango fruits can occur on chemically untreated young orchards especially with susceptible cultivars (Boshoff *et al.*, 1998). Post-harvest diseases of subtropical crops, particularly mango, cause major economic losses in South Africa and worldwide. Anthracnose caused by *Colletotrichum gloeosporioides* Penz & Sacc. is one of the most important post-harvest diseases. Additionally, infection by this pathogen may result in pre-harvest symptoms rendering fruit unmarketable. Soft brown rot (SBR) and stem-end rot (SER) caused by anamorph of *Botryosphaeria* are similarly important and have also been responsible for significant losses in the post-harvest arena (Johnson *et al.*, 1991; Bugante *et al.*, 1997).

Current control measures for both pre- and post-harvest diseases include extensive pre-harvest spraying with copper fungicides. However, extensive copper sprays are providing only limited control (Korsten *et al.*, 1995). Build up of copper levels in soils have reached alarming

proportions, and recent restrictions by the European Union in terms of allowable copper levels in soils has forced the industry to reduce the number of sprays. Biological control has been evaluated successfully on various crops including mango (Korsten *et al.*, 1991; De Villiers & Korsten, 1994). Spraying mangoes pre-harvestly with the antagonist *Bacillus licheniformis* proved effective in controlling both pre- and post-harvest diseases (Korsten *et al.*, 1991; De Villiers & Korsten, 1994). Furthermore, integrating chemical and biological control has been shown to be a viable alternative for control of mango fruit diseases (De Villiers & Korsten, 1996).

The purpose of this study was therefore, to evaluate alternative disease control options combining several approaches and using an innovative new plastic caps approach. The objective of this trial was to adapt plastic caps (made from polyethylene foam) with inner wool linings and impregnate them with either copper fungicide or *B. licheniformis* to control pre- and post harvest diseases in addition to reducing sunburn damage. In addition, the spraying of mango trees with sun-protector and sun-protector used as a carrier for the antagonist was also evaluated under semi-commercial conditions. Another objective of this trial was to evaluate pre-harvest *B. licheniformis* sprays applied on its own or alternated with copper oxychloride on mango trees at three weekly intervals from flowering until harvest for the potential control of fruit diseases.

## 4.2 MATERIAL AND METHODS

### 4.2.1 Effect of plastic woolly caps impregnated with *Bacillus licheniformis* or copper oxychloride and the application of sun-protector to control mango fruit diseases and prevent sunburn

Commercial plastic caps (made from polyethylene foam) and plastic caps with inner woolly linings (Fig. 4.1) were obtained from Airshield Pty, Ltd. (Johannesburg) and were prepared using the same application as in chapter 3 (3.2.3). Caps were cut from the middle of one side towards the centre before being treated. Two different *B. licheniformis* ( $10^9$  cells/ml) formulations (powder and liquid) obtained from Stimuplant, cc. (Pretoria) was sprayed to the woolly caps. *Bacillus licheniformis* liquid formulation (5 ml/l of tap water) or powder formulation (1 g/l of water) were sprayed onto the woolly caps using the same hand held sprayer, which was first thoroughly washed and rinsed with clean water as described for

copper. Treated plastic caps were left to air dry at room temperature for two days. Seven-year-old mango trees cv. Kent was randomly selected at block AR4 at Bavaria Estate (Hoedspruit) and five-year-old 'Kent' trees were selected at Ryfontein farm (Letsitele valley). A complete randomised design was used and three mango trees were selected per treatment, replicated four times. Treatments for the two farms are set out in Appendix 1, 2 and 3, and treated caps were attached to the stem end of the fruit ensuring that the sides overlap. The cap was secured into its position by stapling the overlapping sides together, giving an overall effect of a protective hood. The caps were placed on fruit early November after initial fruit drop on both Bavaria Estate and Ryfontein for the 2000/01 and 2001/02 season.

Sun-protector in powder formulation (200g/200ℓ) (Stimuplant, CC) prepared with *B. licheniformis* as carrier was sprayed (200g/200ℓ) at three weekly intervals until harvesting started at the day when caps were placed on fruits, using high volume sprayers with hand lances. Evaluation for sunburn and BBS was done prior to harvesting. For sunburn and BBS, evaluations were done by separately rating fruit from zero to three. Evaluation criteria used were zero for healthy, one for slightly, two for moderately and three for severely infected. After harvesting, fruits were stored at 10°C for three weeks and evaluation of post-harvest diseases were done at a ready to eat ripeness level. For anthracnose, SBR and SER, evaluations were done by rating fruit from zero to five. Criteria used were zero for healthy, one for slightly, two for moderately, three for quarter of the fruit infected, four for half of the fruit infected and five for three quarters to the whole fruit infected. After statistical analysis, data were determined as percentage of healthy fruits for each treatment. Data were statistically analysed using the statistical program GenStat (2000). One-way analysis of variance (ANOVA) was used to test for differences in percentage healthy fruit between the treatments. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at a 5 % and 10 % level of significance.

#### **4.2.2 Effect of pre-harvest *Bacillus licheniformis* and copper oxychloride sprays to control mango fruit diseases**

Mango trees were sprayed with different biological integrated treatments compared to commercial copper sprays to determine the efficacy of these alternative approaches. For the 2000/2001 season, a trial was done only at Bavaria Estate using seven-year-old mango trees cv. Tommy Atkins (block AR4) and Kent (block AR4). For the 2001/2002 season, a trial was

done at Bavaria Estate on the same cv. Kent trees & Ryfontein farm on six-year-old Kent trees. A complete randomised design was used as described in 4.2.1. Treatments for the two farms are set out in Appendix 4 and 5. Field applications using *B. licheniformis* in liquid or powder formulations, and copper oxychloride on its own or alternated with antagonists, commenced from flowering (early September) until harvesting (January or February) depending on the production regions. Applications were done using high volume sprayers with hand lances at two-weekly intervals for the first four sprays and then at three-weekly intervals until harvesting. Evaluation of fruit and statistical analysis was done for post-harvest diseases as described in 4.2.1.

### 4.3 RESULTS

#### 4.3.1 Effect of plastic woolly caps impregnated with *Bacillus licheniformis* or copper oxychloride and the application of sun-protector to control mango fruit diseases and prevent sunburn

All treatments using plastic caps significantly reduced sunburn damage for both seasons at Bavaria Estate and Ryfontein farms when compared to the control (Figs 4.2, 4.3, 4.4). Of the various treatments, the woolly caps were consistently the most effective to prevent sunburn except for the 2001/02 seasons at Bavaria Estate where there was no significant difference between commercial caps and woolly caps (Fig. 4.4). In the case of sun-protector, it significantly reduced sunburn damage to fruit as effectively as commercial caps except for the 2001/02 season at Bavaria Estate where it actually resulted in an increased sunburn incidence (Figs 4.3, 4.4).

Furthermore, there were no significant differences between treatments for control of BBS at Bavaria Estate and Ryfontein for both the 2000/2001 (Pr = 0.443 (Bavaria); Pr = 0.578 (Ryfontein) and 2001/2002 seasons (Pr = 0.066 (Bavaria)). There was also no effective control of the post-harvest diseases, anthracnose and SER, for both seasons at both farms.

Although the disease incidence was low on both farms for both seasons, woolly caps, commercial caps and sun-protector significantly controlled SBR at Bavaria Estate for the 2000/01 season on 'Kent' (Fig. 4.5). At Ryfontein, there was no significant difference between treatments for the 2000/01 season for control of SBR (Pr-value was 0,781). There

was also no difference between treatments for the 2001/02 season on both farms for SBR control (Pr-value for Bavaria = 0.739; Ryfontein = 0.457).

#### **4.3.2 Effect of pre-harvest *Bacillus licheniformis* and copper oxychloride sprays to control mango fruit diseases**

Antagonist liquid and powder formulations and integration of commercial copper with liquid antagonist formulations were the only treatments that effectively controlled BBS at Bavaria Estate for the 2000/01 season when compared to the control (Fig. 4.6). However, these treatments were not significantly better than the commercial copper or integrated biocontrol powder formulation (Fig. 4.6). For the 2001/02 season there were no significant differences between the treatments on both farms to control BBS (Pr-value: Bavaria = 0.833, Ryfontein = 0.451).

For the 2000/2001 season at Bavaria Estate, commercial copper sprays, powder and liquid antagonist formulations significantly reduced anthracnose infection on 'Tommy Atkins' (Fig. 4.7). Although not as effective as the commercial copper and the biocontrol treatments, the integrated treatments with either liquid or powder formulations also significantly controlled anthracnose. Reduced copper concentration sprays were the least effective (Fig. 4.7). For the 2001/2002 season, commercial copper sprays and the integrated copper with liquid antagonist sprays were the best treatments for effectively reduced anthracnose infection on 'Kent' at Ryfontein and Bavaria Estate (Fig. 4.8). At Bavaria Estate, the best two treatments were not significantly better than the reduced copper or antagonist on its own treatments. At Ryfontein, these two best treatments were also not significantly better than the integrated treatment with the antagonist and reduced copper concentration treatment (Fig. 4.8).

For the control of SBR, the commercial copper, liquid antagonist formulation and both integrated treatments were the most effective on cv. Kent at Bavaria Estate for the 2000/01 season (Fig. 4.9). Although not as effective, the reduced copper concentration treatments could also significantly control SBR on cv. Kent. However, on the cv. Tommy Atkins trial, only the two integrated and the powder antagonist treatment could control SBR. However, the level of control achieved was not significantly better than the liquid antagonist treatment (Fig. 4.9). For the 2001/02 season, commercial copper sprays significantly reduced SBR incidence at Ryfontein (Fig. 4.10). Antagonist on its own, and integration with commercial

copper or reduced copper also significantly reduced SBR infection but not as effectively as commercial copper. There were no significant differences for the control of SER on both farms for both seasons.

#### 4.4 DISCUSSION

The main aim of this study was to evaluate alternative methods to reduce sunburn damage and control pre-and post-harvest diseases on mango fruits. Plastic caps with inner wool linings significantly reduced sunburn damage at both Bavaria Estate and Ryfontein for both seasons. The addition of a woolly base to caps significantly enhanced their efficacy to reduce sunburn when compared to currently used commercial plastic caps without inner wool linings. During heavy winds or windy rains, commercial caps curled and moved out of position exposing fruits to sunburn, whereas woolly plastic caps remained stable during these conditions (Silimela & Korsten, 2001).

The plastic cap approach in general gave better results compared to previously used brown paper bags. Bugante *et al.* (1997) showed that covering fruits with brown paper bags were ineffective since paper bags became translucent and clung to the fruit when wet. By adding a woolly base to the plastic caps, an improved level of sun protection could be obtained. This was also found to be the most effective treatment in this study.

However, from a cost effective point of view, woolly caps are more expensive. Currently woolly caps cost 12 cents per cap compared to four cent for the currently used commercial caps. Woolly caps will therefore cost roughly R 1 920/ha while commercial caps will cost R 670/ha (Appendix 6). Labour cost incurred when placing caps on fruits is currently calculated at R 352/ha and picking them up during and at the end of the season at R 160/ha. Keeping in mind that woolly caps can be re-used; it can reduce the initial input cost over time. When woolly caps were used there was an average of 30 % increase in marketable fruit compared to 22 % with the use of current commercial caps. From a cost benefit point of view, commercial plastic caps are almost a third of the price of woolly caps but are less effective in providing protection (Appendix 6). In contrast, sun-protector sprays cost R 90/ha for a single application and can provide on average 10 % more healthy fruit when three applications are applied per season. It might be even more cost effective to get a 10 % improvement on quality per hectare when compared to the currently used commercial caps. Although this is

only a rough comparison of costs vs benefits, it is obvious that growers in areas with high sunburn damage could consider the more expensive alternative depending on the expected profit margins for the season.

There was no significant difference between the woolly cap treatments for the control of BBS. This may be due to the low disease incidence on both farms for the seasons under investigation. Using woolly caps can be an effective alternative for BBS controls in places where heavy winds or windy rains prevail. Caps may provide protection against sand particles blowing against the fruit which can cause wounding. These openings can potentially serve as infection sites for pathogens (Silimela & Korsten, 2001). Plastic caps protect fruits during heavy winds and windy rains and thus prevent fruit abrasions from occurring when fruit rub against each other. In addition, caps can prevent droplets from rain or dew washing down onto fruits, which can infect fruit if inoculated with bacterial or fungal spores, thereby forming the characteristic tear drops lesions. Woolly caps did not provide the expected control of post-harvest diseases, although in one trial it showed promise in controlling SBR.

From a production point of view, woolly caps can contribute to increased yields, but from an economic point of view it seems unprofitable for growers to utilise adaptive woolly caps unless trees are planted in an open area where the wind factor is critical. Caps with a woolly base were less likely to swirl around and damage the stem as the current commercial caps can do resulting in increased fruit drop and less effective protection against the sun. The sun-protector products proved less effective than anticipated in reducing sunburn as compared to the commercial or woolly caps. Using sun-protector as a carrier base for the antagonist also failed to give enhanced disease control benefits.

Despite the low disease incidence, some positive BBS control was observed at Bavaria Estate on 'Kent' for the 2000/01 season using *B. licheniformis* sprays. Similar promising results were previously reported for BBS control using the same *B. licheniformis* antagonist but in a non-commercial formulation (Visser *et al.*, 1990). However, integrated programmes with fungicides and *B. subtilis* sprays provided most consistent control for pre-harvest *Cercospora* spot over several seasons and locations (Korsten *et al.*, 1997). Although *B. licheniformis* showed some potential against BBS, consistent control still lacks and it would therefore require a more in depth study in terms of improving product formulation, application intervals or timing of application.

Copper used at the registered rate remains the most effective method to consistently control anthracnose and SBR on mango fruits. However, the global move towards food safety and pressure to reduce the use of copper sprays due to its build up in soils makes integrated control a viable alternative. Taking this into account, the approach of alternating copper and *B. licheniformis* sprays could reduce reliance on copper and proved equally effective in anthracnose control when compared to commercial copper sprays only.

This study confirms previous reports that an integrated approach can effectively control anthracnose (Korsten *et al.*, 1992; Korsten *et al.*, 1993; Silimela & Korsten, 2002). Antagonist on its own or alternated with commercial copper showed similar effective control of anthracnose and SBR. Disappointing results were, however, obtained with the control of SER. In previous years, *B. licheniformis* were found to be effective for the control of anthracnose, SBR and SER applied on its own, as a pre-harvest spray or as an integrated treatment with copper oxychloride (Korsten *et al.*, 1992). Pre-harvest *B. subtilis* sprays in semi-commercial trials on avocado were found more effective in controlling SER and anthracnose compared to commercial copper sprays (Korsten *et al.*, 1989).

Taking the endophytic nature of the *Botryosphaeria* pathogen into account, it is to be expected that non-systemic products will not be able to provide effective SBR and SER control. The semi-commercial trials in this study were done for two consecutive years indicating consistency of product performance. However, the product was evaluated in previous studies in an unformulated form compared to this study, where a commercial formulation was tested. Once the product is formulated for commercial use, less effective or inconsistent results are often reported. It is known that biocontrol product performances are often affected by large-scale production and requires optimisation to ensure consistent efficacy levels (Emmert & Handelsman, 1999).

The same biocontrol product *B. licheniformis* has been successfully evaluated for post-harvest disease control (anthracnose, SBR and SER) (Korsten *et al.*, 1991; Korsten *et al.*, 1993; De Villiers & Korsten, 1994; Govender & Korsten, 2001). Using biocontrol on its own can be more expensive than using copper only as calculated per hectare (Appendix 7). Biocontrol is furthermore more acceptable in organic farming and provides an alternative for disease control. The current price for organic mangoes (4 kg box) on the export market is estimated to be R 20 vs the R 13 for non-organic fruits. In addition, there was no difference in the

percentage increase of healthy fruits when copper, antagonist or integrated control was compared for BBS. Due to previous reports describing the efficacy of *B. licheniformis* in controlling mango post-harvest diseases and its potential in using a commercial formulation pre-harvestly as was found in this study, it might be an effective alternative approach to combine the two approaches in future studies. In such a case, special attention should be focused on the potential build up of pathogen resistance.

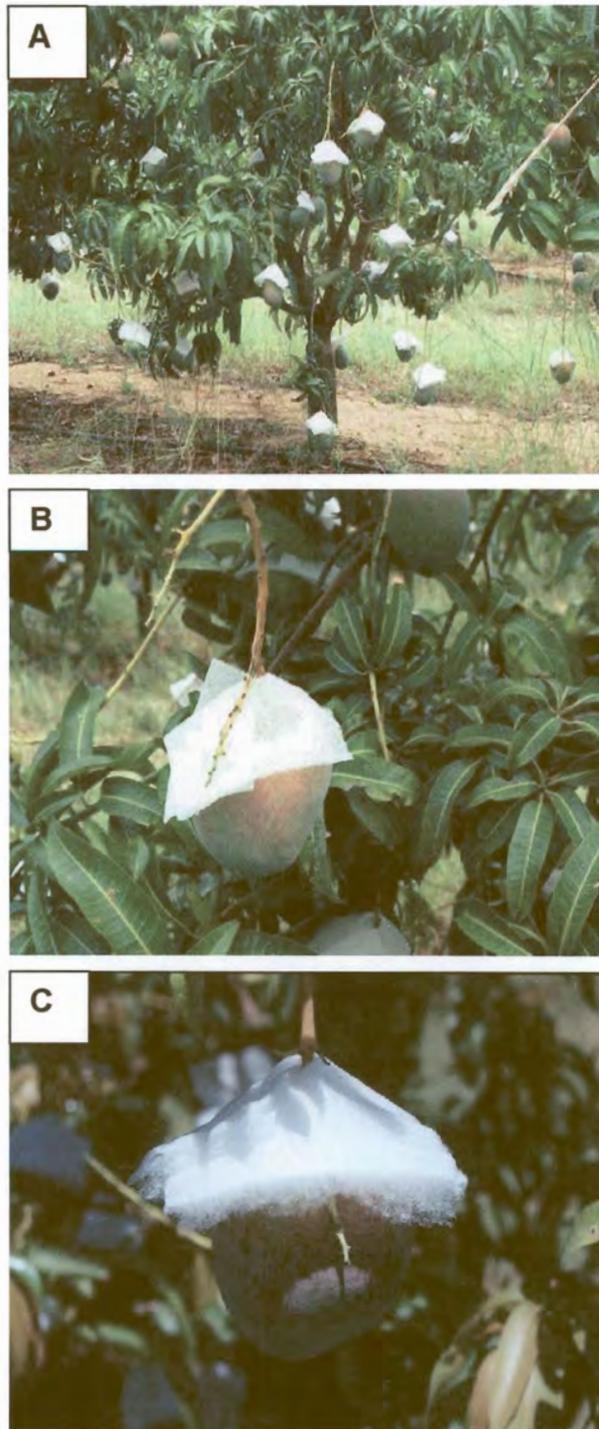
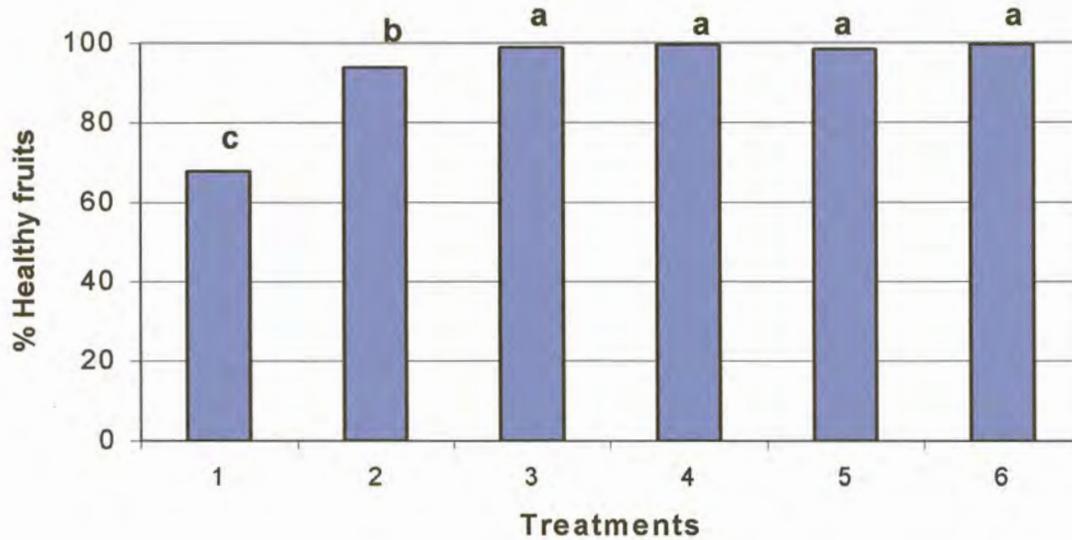
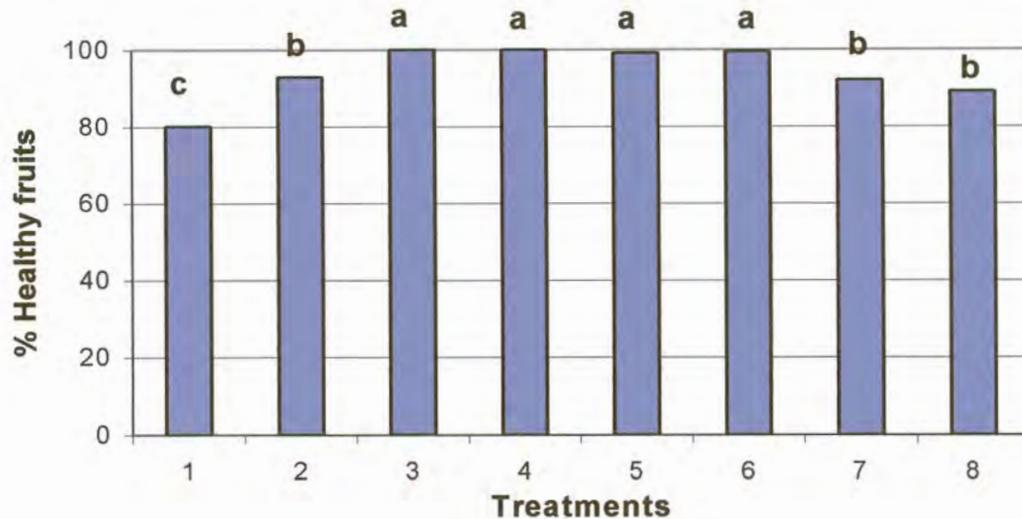


Fig. 4.1 Commercial orchard showing A) a mango tree with fruits covered with plastic caps with inner wool linings; B) a mango fruit covered with a commercial plastic cap without inner wool lining; C) a mango fruit covered with a plastic cap with an inner wool lining.



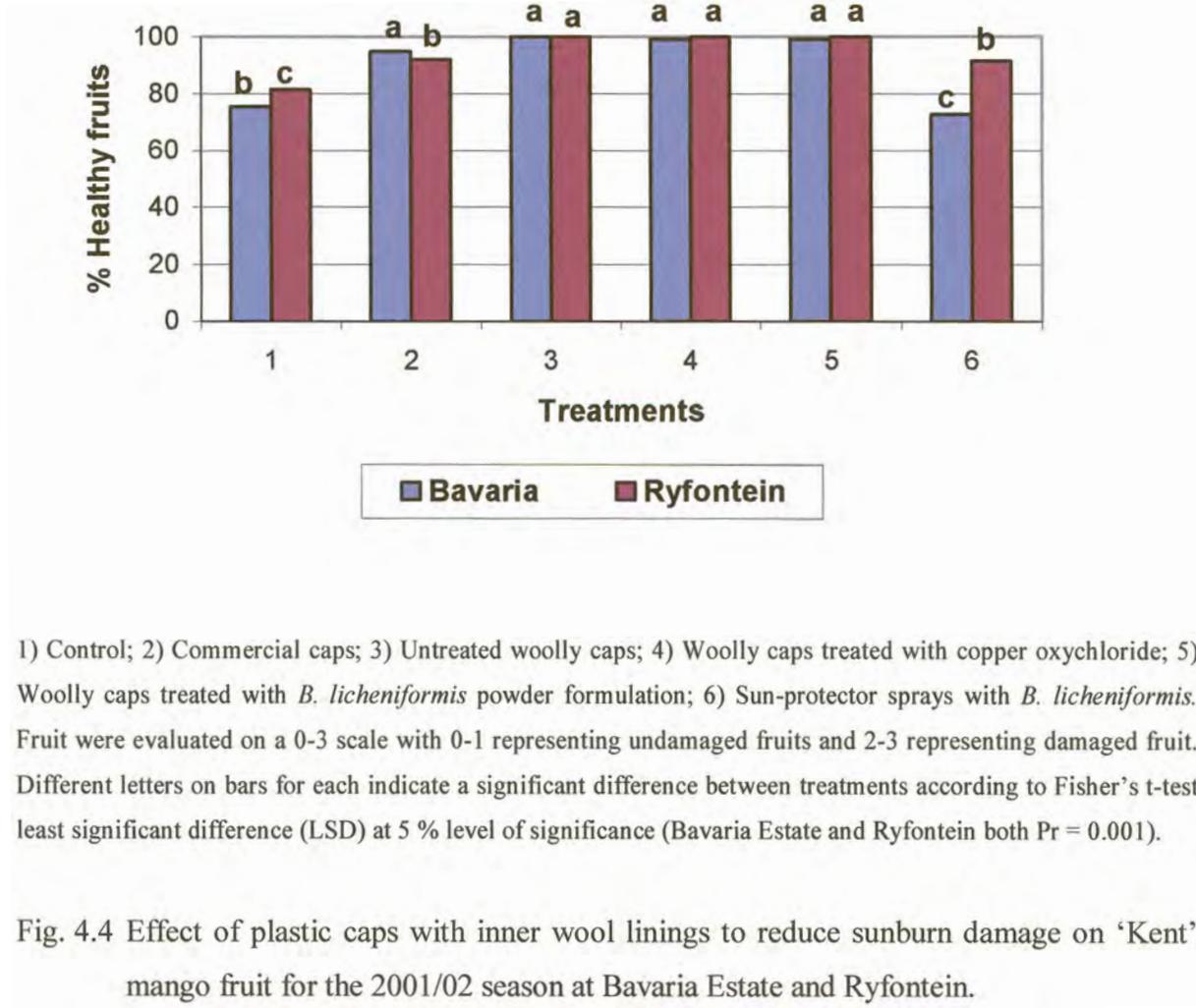
1) Control; 2) Commercial caps; 3) Untreated woolly caps; 4) Woolly caps treated with copper oxychloride; 5) Woolly caps treated with the *B. licheniformis* powder formulation; 6) Woolly caps treated with the *B. licheniformis* liquid formulation. Fruit were evaluated on a 0-3 scale with 0-1 representing undamaged fruits and 2-3 representing damaged fruit. Different letters on bars for each indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at 5 % level of significance (Sunburn Pr = 0.001).

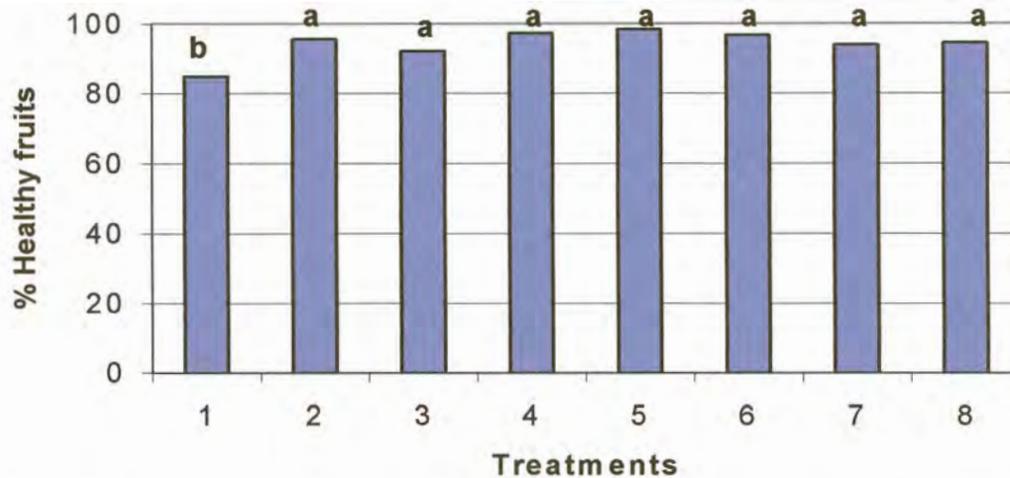
Fig. 4.2 Effect of plastic caps with inner wool linings in reducing sunburn damage on 'Kent' mango fruit for the 2000/01 season at Ryfontein farm.



1) Control; 2) Commercial caps; 3) Untreated woolly caps; 4) Woolly caps treated with copper oxychloride; 5) Woolly caps treated with the *B. licheniformis* powder formulation; 6) Woolly caps treated with the *B. licheniformis* liquid formulation; 7) Trees sprayed with sun-protector; 8) Trees sprayed with sun-protector and *B. licheniformis*. Fruit were evaluated on a 0-3 scale with 0-1 representing undamaged fruits and 2-3 representing damaged fruit. Different letters on bars for each indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at 5 % level significance (Sunburn Pr = 0.001).

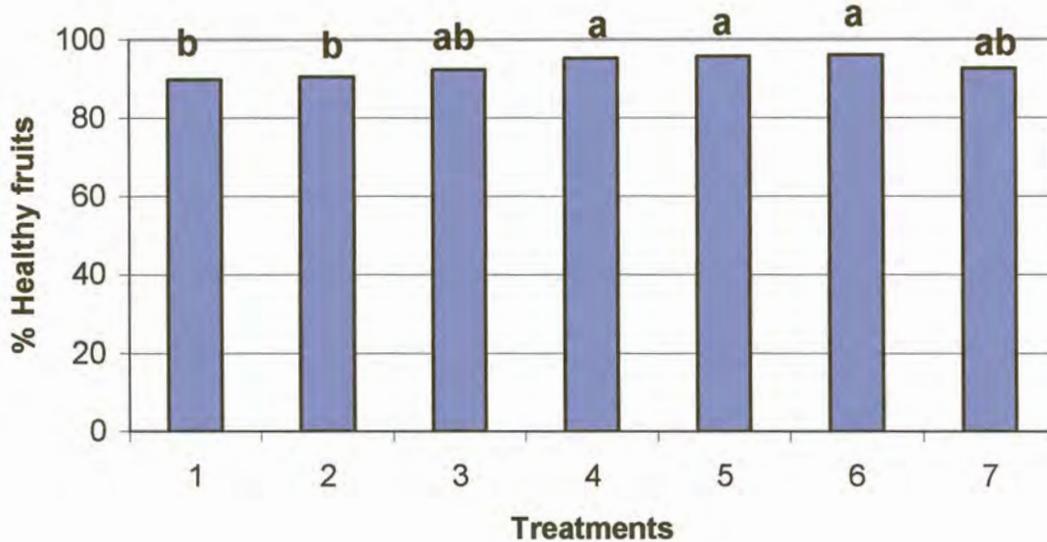
Fig. 4.3 Effect of plastic caps with inner wool linings in reducing sunburn damage on 'Kent' mango fruit for the 2000/01 season at Bavaria Estate.





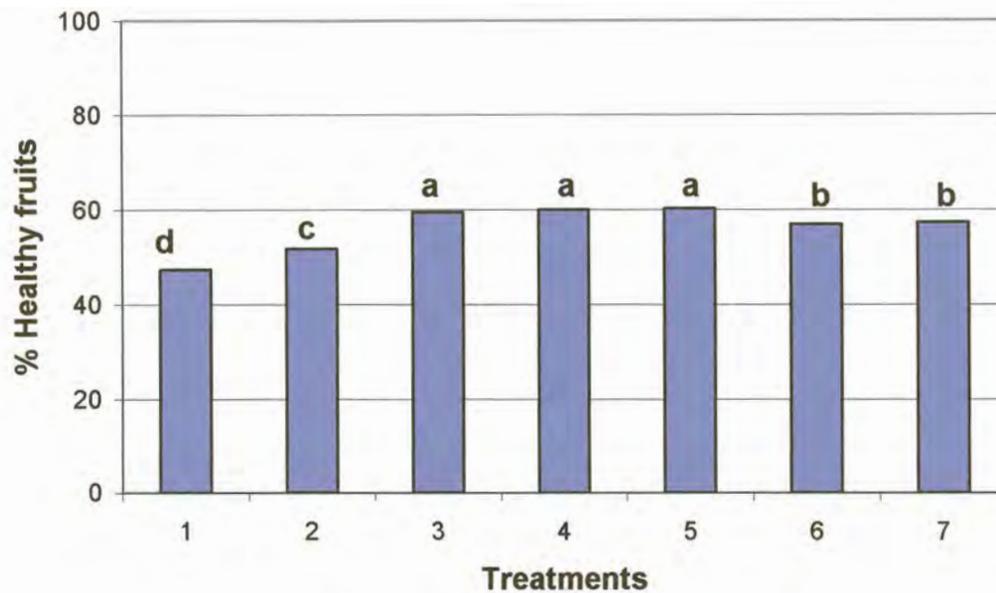
1) Control; 2) Commercial caps; 3) Untreated woolly caps; 4) Woolly caps treated with copper oxychloride; 5) Woolly caps treated with the *B. licheniformis* powder formulation; 6) Woolly caps treated with the *B. licheniformis* liquid formulation; 7) Trees sprayed with sun-protector; 8) Trees sprayed with sun-protector and *B. licheniformis*. Fruit were evaluated on a 0-5 scale with 0-1 representing healthy fruits and 2-5 representing infected fruit. Different letters on bars for each indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at 5 % level of significance (SBR Pr = 0.016).

Fig. 4.5 Effect of plastic caps with inner wool linings impregnated with either *Bacillus licheniformis* or copper oxychloride for the control of SBR on 'Kent' mango fruit for the 2000/01 season at Bavaria Estate.



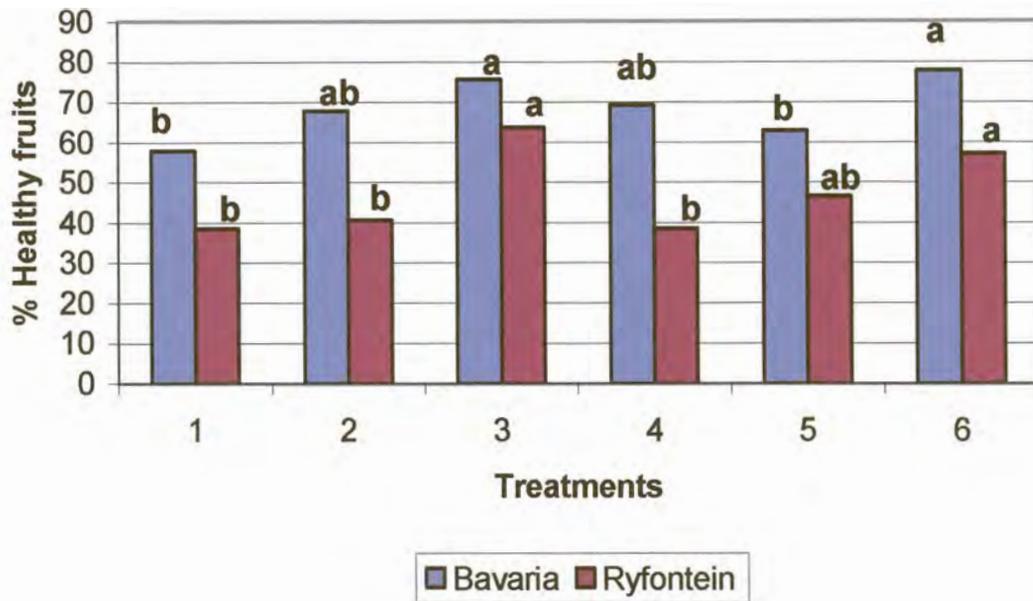
1) Control; 2) Copper sprays at 200g/200ℓ of water; 3) Commercial copper sprays at 600g/200ℓ of water; 4) *B. licheniformis* liquid formulation at 1ℓ/200ℓ of water; 5) *B. licheniformis* powder formulation at 200g/200ℓ of water; 6) Integration of *B. licheniformis* liquid formulation (1ℓ) and commercial copper (600g) per 200ℓ of water; 7) Integration of *B. licheniformis* powder formulation (200g) and commercial copper (600g) per 200ℓ of water. Fruit were evaluated on a 0-3 scale with 0-1 representing healthy fruits and 2-3 representing infected fruit. Different letters on bars for each BBS indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at a 5 % level of significance (BBS Pr = 0.037).

Fig. 4.6 Effect of *Bacillus licheniformis* and copper oxychloride sprays for the control of bacterial black spot of 'Kent' mango fruit for the 2000/01 season at Bavaria Estate.



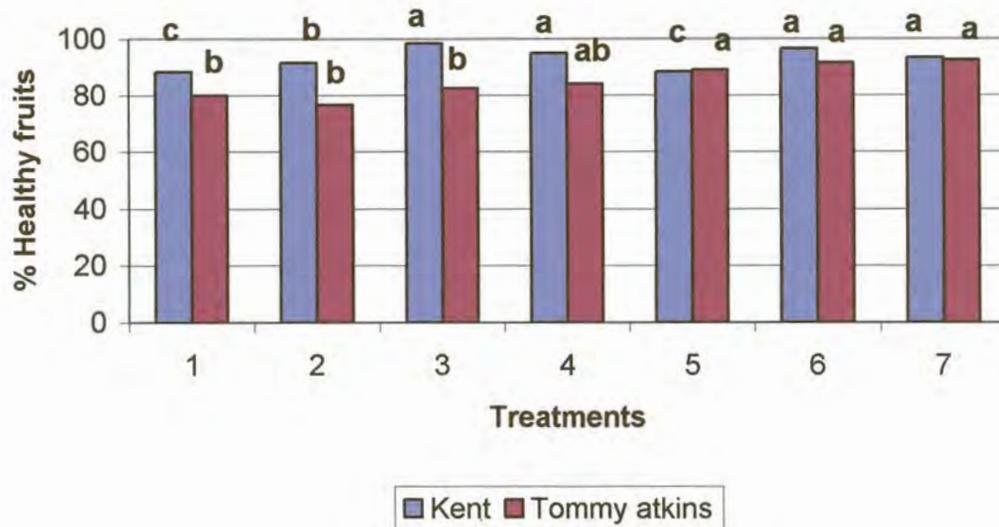
1) Control; 2) Copper sprays at 200g/200ℓ of water; 3) Commercial copper sprays at 600g/200ℓ of water; 4) *B. licheniformis* liquid formulation at 1ℓ/200ℓ of water; 5) *B. licheniformis* powder formulation at 200g/200ℓ of water; 6) Integration of *B. licheniformis* liquid formulation (1ℓ) and commercial copper (600g) per 200ℓ of water; 7) Integration of *B. licheniformis* powder formulation (200g) and commercial copper (600g) per 200ℓ of water. Fruit were evaluated on a 0-5 scale with 0-1 representing healthy fruits and 2-5 representing infected fruit. Different letters on bars for each indicate a significant difference between treatments on anthracnose control according to Fisher's t-test least significant difference (LSD) at a 5 % level of significance ('Tommy Atkins' Pr = 0.006).

Fig. 4.7 Effect of *Bacillus licheniformis* and copper oxychloride sprays for control of anthracnose on 'Tommy Atkins' mango fruit for the 2000/01 seasons at Bavaria Estate.



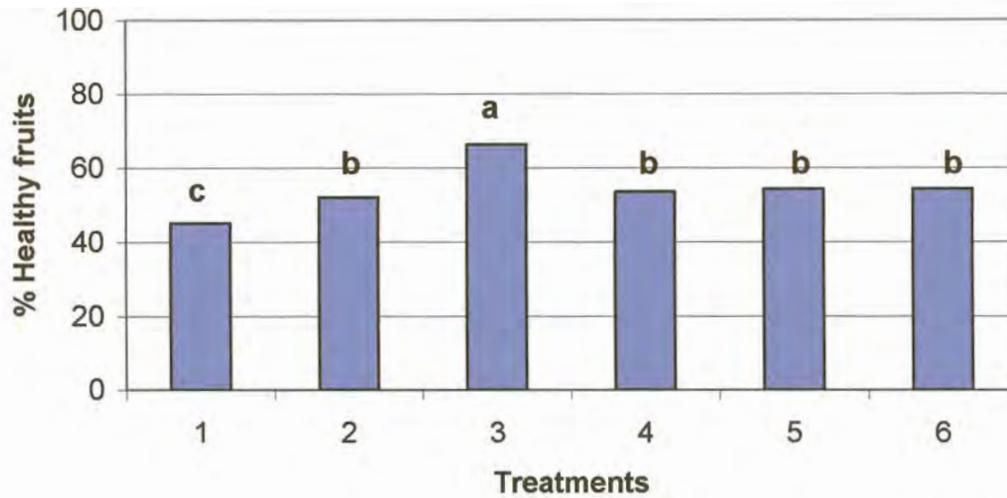
1) Control; 2) Copper sprays at 200g/200ℓ of water; 3) Commercial copper sprays at 600g/200ℓ of water; 4) *B. licheniformis* liquid formulation at 1ℓ/200ℓ of water; 5) Integration of *B. licheniformis* (1ℓ) and copper (200g) per 200ℓ; 6) Integration of *B. licheniformis* (1ℓ) and commercial copper (600g) per 200ℓ of water. Fruit were evaluated on a 0-5 scale with 0-1 representing healthy fruits and 2-5 representing infected fruit. Different letters on bars for each farm indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at a 5 % level of significance for Bavaria Estate (ANT Pr = 0.02) and at a 10 % level at Ryfontein (Pr = 0.056).

Fig. 4.8 Effect of *Bacillus licheniformis* and copper oxychloride sprays for control of anthracnose on 'Kent' mango fruit for the 2001/02 season at Bavaria Estate and Ryfontein.



1) Control; 2) Copper sprays at 200g/200l of water; 3) Commercial copper sprays at 600g/200l of water; 4) *B. licheniformis* liquid formulation at 1l/200l of water; 5) *B. licheniformis* powder formulation at one 200g/200l of water; 6) Integration of *B. licheniformis* liquid formulation (1l) and commercial copper (600g) per 200l of water; 7) Integration of *B. licheniformis* powder formulation (200g) and copper (600g) per 200l of water. Fruit were evaluated at a 0-5 scale with 0-1 representing healthy fruits and 2-5 representing infected fruit. Different letters on bars for each cultivar indicate a significant difference between treatments on anthracnose control according to Fisher's t-test least significant difference (LSD) at a 10 % level of significance for 'Kent' (Pr = 0.06) and at a 5 % for 'Tommy Atkins' Pr = 0.032).

Fig. 4.9 Effect of *Bacillus licheniformis* and copper oxychloride sprays for control of soft brown rot on mango fruit ('Kent' and 'Tommy Atkins') for the 2000/01 seasons at Bavaria Estate.



1) Control; 2) Copper sprays at 200g/200ℓ of water; 3) Commercial copper sprays at 600g/200ℓ of water; 4) *B. licheniformis* liquid formulation at 1ℓ/200ℓ of water; 5) Integration of *B. licheniformis* (1ℓ) and copper (200g/200ℓ); 6) Integration of *B. licheniformis* (1ℓ) and commercial copper (600g) per 200ℓ of water. Fruit were evaluated on a 0-5 scale with 0-1 representing healthy fruits and 2-5 representing infected fruit. Different letters on bars for each farm indicate a significant difference between treatments according to Fisher's t-test least significant difference (LSD) at a 10 % level of significance at (Ryfontein Pr = 0.075).

Fig. 4.10 Effect of *Bacillus licheniformis* and copper oxychloride sprays for control of soft brown rot on 'Kent' mango fruit for the 2001/02 season at Ryfontein.

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## CHAPTER 5

### GENERAL DISCUSSION

Since its global movement away from the tropic epicentre, mango has been exposed to different growing conditions, which are not always adequate for optimal yields. Extensive cultivation and breeding has resulted in improved cultivars ideally suited for the regions and with characteristics according to the export market requirements. Introductions of new cultivars to varied environmental conditions increased exposure to pests and pathogens, directly affecting the quality of the fruits.

Mango diseases and physiological disorders affect the quality of the fruit resulting in its rejection at the packhouse (Ridgway, 1989; De Villiers & Korsten, 1994). Limited control for both pre- and post-harvest diseases can be achieved with copper fungicides. To an extent, these treatments can reduce the inoculum load in the orchards and ensure fruit quality and shelf life during export (De Jager, 1999; Gerhardson, 2002). However, extensive fungicide sprays are becoming uneconomical and can result in build up of copper in soils (Korsten *et al.*, 1995; Boshoff *et al.*, 1998; Gerhardson, 2002). Pesticide residues on fresh produce are furthermore a major concern, as food safety becomes a minimum requirement in global trade. This has resulted in growing international concern and pressure to reduce the number of chemical sprays (Hall, 1995).

The development of biological control programmes has been shown to provide an effective alternative method to control fruit diseases (Ippolito & Nigro, 2000; Saxena *et al.*, 2000; Gerhardson, 2002; Collins & Jacobsen, 2003). *Bacillus licheniformis* for instance, has previously controlled mango fruit diseases when using the antagonist on its own or integrating it with copper (Korsten *et al.*, 1992; Korsten *et al.*, 1993). However, neither commercial formulations in semi-commercial applications nor the efficacy of the product when used in different geographical regions and on different cultivars have been tested before. In order to enhance biocontrol product performance, additives such as biostimulants can be added to ensure effective growth of the antagonists, while stickers and spreaders can be used to enhance product performance through better distribution and adherence. Stickers increase the adhesion or stickiness of the product that otherwise might easily be dislodged from the leaf surface during field spraying. It also reduces evaporation and provides a waterproof covering

(Hassal, 1990; Harvey, 1992). Spreaders improve the contact between fungicides and plant surfaces (Hassal, 1990; Harvey, 1992).

The first objective of this study was to evaluate alternative disease control options using innovative new approach. These include the use of plastic caps with inner wool linings (woolly caps) impregnated with either copper oxychloride or *B. licheniformis*, to prevent sunburn or control fruit diseases. Prior to evaluation of these innovative approaches, the attachment and survival potential of *B. licheniformis* on the woolly caps over an extended period of time had to be determined.

This study showed that *B. licheniformis* could successfully attach to the woolly base of plastic caps and could survive for up to three months even under harsh environmental conditions. The use of plastic woolly caps could significantly reduce sunburn damage on mango fruits over a two year period when compared to current commercially used plastic caps or caps without inner wool linings and sun-protector. This was found at both Bavaria Estate and Ryfontein (Silimela & Korsten, 2001). The more effective performance of the woolly caps compared to the current commercial caps can be related to a more effective fit and subsequent protection. During heavy rains or heavy winds, commercial caps curled and moved out of position exposing fruits to sunburn, whereas woolly plastic caps remained stable during harsh environmental conditions (Silimela & Korsten, 2001). Sun-protector was effective in reducing sunburn on mango fruits but not as effective as woolly caps.

Woolly caps impregnated with copper or antagonists did not significantly prevent BBS. However, it is perhaps important to note that the incidence of this disease was low on both farms during the period when the trials were conducted. It is therefore not an accurate reflection of the potential of *B. licheniformis* impregnated woolly caps to control BBS. With further studies and perhaps an improved product formulation of *B. licheniformis*, impregnated caps may provide the desired control. Furthermore, the use of plastic caps can provide additional protection of fruit to prevent wounding due to sand carried by heavy winds or windy rains. Caps can also prevent abrasion from forming, when adjacent fruits rub against each other during heavy winds. Wounds on fruit serve as sites of entry for many pathogens especially bacteria. The infection potential of BBS can be reduced by protecting the fruit from wounding (Boshoff *et al.*, 1998). Caps can also protect fruit during rain or early

morning dew, preventing the development of typical teardrop symptoms (Silimela & Korsten, 2001).

Disappointing results were obtained for the control of post-harvest diseases anthracnose, SBR and SER when using plastic caps. Interestingly, in the case of SBR, some protection was noted. In contrast to SER, SBR is a body rot resulting from secondary infections, while SER is an endophytic infection emanating from *Botryosphaeria* moving down into the fruits through the stem end (Jacobs, 2002). It is highly unlikely that impregnated caps will protect fruits against a systemic infection, but may provide protection against aerial infections. The biggest advantage of using impregnated woolly caps is to its potential to provide sustained protection particularly during periods of heavy rains when growers cannot access their orchards. However, from a cost effective point of view, woolly caps can be more expensive. Currently, the cost of 12 cent per woolly cap compared to 4 cent for the commercial cap result in an input cost of R 1 920/ha vs R 670/ha. Keeping in mind that woolly caps can be re-used for at least another season, it can potentially reduce some of the initial costs. However, the 30 % increase in marketable fruit obtained when woolly caps were used compared to the average 22 % for commercial caps and 10 % for sun-protector clearly illustrates the potential of this approach. In cases where new orchards are established in open areas with a critical wind factor, it could be more advantageous to use the more expensive woolly caps.

The second objective of this study was to evaluate pre-harvest spraying of mango trees with copper oxychloride or *B. licheniformis* or both by alternating the products at three-weekly intervals from flowering until harvesting to control pre- and post-harvest diseases on a semi-commercial scale. Prior to evaluating the pre-harvest trials, the *in vitro* effect of different additives (stickers, spreaders, wetters and biostimulants) and copper on the antagonists' growth, and *in vivo* ability to attach and colonise the leaf surface was evaluated.

Bioboost, Nufilm-P, Biofilm, and Agral 90 did not negatively affect antagonist growth *in vitro*. However, copper oxychloride and Superfilm inhibited growth of *B. licheniformis* on agar and reduced growth after eight hours. Integrating copper with the antagonists and applying it simultaneously can potentially have a negative effect on the survival of the antagonist. The most appropriate application would therefore be to alternate copper and *B. licheniformis* sprays on a monthly basis, to prevent a negative inhibitory effect on the

antagonists' growth. The *in vivo* study showed that stickers, spreaders or wetters could not improve the ability of *B. licheniformis* to attach and subsequently colonise the leaf surface.

Scanning electron microscopy studies showed that the antagonist had the ability to effectively attach and colonise the mango leaf surface. For effective growth and colonisation, attachment of bacterial cells to plant surfaces is the first significant step (Romantschuk, 1992) prior to establishing antagonist populations in biocontrol programs. Similar studies were previously reported by Towsen (1996), who showed effective attachment of *B. subtilis* on avocado leaves. The minimum time required for *B. licheniformis* to effectively attach to mango leaves was 15 minutes. Different attachment times of bacteria were previously reported from different plant surfaces. According to Towsen (1996), Leben & Whitmoyer (1979) reported that *Pseudomonas lachrymans* effectively adhered to young cucumber leaves after 10 minutes, while Lawrence *et al.* (1987) found *Ruminococcus flavefaciens* adhered to rye grass after 30 minutes. Multiplication of *B. licheniformis* was observed on mango leaves 24 hours after application.

Pre-harvest sprays with copper at registered rates from flowering until harvesting remains the most effective and consistent method for the control of anthracnose and SBR on mango fruits. Integrating copper sprays alternated with antagonist applications provided equally effective control of anthracnose when compared to the commercial copper sprays. The potential of integrated control may provide growers with an effective alternative method, which can lead to the reduction in the number of copper sprays and the resultant, lower chemical residues on harvested fruits. This is especially true if the last sprays of the season were to be replaced with the biocontrol products (Silimela & Korsten, 2002). Using *B. licheniformis* on its own proved as effective as commercial copper at Bavaria Estate for the first year of spraying to control anthracnose. It was also the only treatment that could control BBS. Although the biocontrol programme could not achieve the same level of consistent control compared to the copper sprays, the potential option of marketing this fruit under the organic label will ensure increased profit margins since such products currently sell at premium prices on export markets. Effective control of fruit diseases using the biocontrol products were also reported by Korsten *et al.* (1989), where pre-harvest sprays using *Bacillus subtilis* were more effective compared to standard copper oxychloride sprays in reducing anthracnose and SER on avocado. Similarly, the integrated programme of copper and *B. subtilis* sprays were the most

consistent to control *Cercospora* spot on avocado compared to the biocontrol product on its own (Korsten *et al.*, 1997).

To a certain extent, promising results were obtained with the control of SBR when using antagonist on its own or alternated with copper, which worked effectively on both 'Kent' and 'Tommy Atkins' at Bavaria Estate. These results confirmed previous reports by Korsten *et al.* (1992) using the same isolates but as an unformulated product, to control SBR with pre-harvest sprays. Disappointing results were however obtained for the control of SER. Taking the endophytic nature of the pathogen into account, it is understandable that non-systemic products will not be able to provide effective disease control of SBR and SER.

In conclusion, this study shows that woolly caps can provide a suitable method to reduce sunburn on mango fruits. Using caps for the control of sunburn is labour intensive, but can ensure improved quality and reduce fruit rejections at the packhouse due to sunburn damage. Use of the antagonist *B. licheniformis* or an integrated approach with copper oxychloride might be an effective way of controlling anthracnose and SBR. However, more studies are required to evaluate *B. licheniformis* on a commercial scale in order to obtain more consistent results and to ensure product registration. Combining a successful integrated pre-harvest and post-harvest biocontrol program could in future be investigated taking into account the possibility of build up of pathogen resistance.

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**Appendix 1** Treatments and concentrations used during experimental trials at Bavaria Estate, where plastic woolly caps and sun-protector were evaluated for sunburn protection and disease control, during the 2000/2001 season on cv. Kent

Treatment number	Treatments	Concentration used
1	Control	-
2	Commercial caps	-
3	Woolly caps	-
4	Woolly caps impregnated with copper oxychloride	3 g/l of water
5	Woolly caps impregnated with <i>Bacillus licheniformis</i> (powder formulation) at $10^9$ cells/ml	1 g/l of water
6	Woolly caps impregnated with <i>B. licheniformis</i> (liquid formulation) at $10^9$ cells/ml	1 l
7	Sun-protector sprays in the orchard	1 g/l of water
8	Sun-protector & <i>B. licheniformis</i> (powder formulation) at $10^9$ cells/ml sprays	1 g and 1 g/l of water

Caps were placed on fruits on the 10<sup>th</sup> of November 2000 on cv. Kent at Bavaria Estate. Fruit were harvested on the 6<sup>th</sup> of February 2001.

**Appendix 2** Treatments and concentrations used during experimental trials at Ryfontein farm, where plastic woolly caps were evaluated for sunburn protection and disease control, during the 2000/2001 season on cv. Kent

Treatment number	Treatments	Concentration used
1	Control	-
2	Commercial caps	-
3	Woolly caps	-
4	Woolly caps impregnated with copper oxychloride	3 g/l of water
5	Woolly caps impregnated with <i>Bacillus licheniformis</i> (powder formulation) at $10^9$ cells/ml	1 g/l of water
6	Woolly caps impregnated with <i>B. licheniformis</i> (liquid formulation) at $10^9$ cells/ml	1 l

Caps were placed on fruit on the 13<sup>th</sup> of December 2000 on cv. Kent at Ryfontein. Fruit were harvested on the 30<sup>th</sup> of January 2001.

**Appendix 3** Treatments and concentrations used during experimental trials at Bavaria Estate and Ryfontein, where plastic woolly caps were evaluated for sunburn protection and disease control, during the 2001/2002 season on cv. Kent

Treatment number	Treatments	Concentration used
1	Control	-
2	Commercial caps	-
3	Woolly caps	-
4	Woolly caps impregnated with copper oxychloride	3 g/l of water
5	Woolly caps impregnated with <i>Bacillus licheniformis</i> (powder formulation) sprays at $10^9$ cells/ml	1 g/l of water
6	Sun-protector & <i>B. licheniformis</i> (powder formulation) at $10^9$ cells/ml sprays	200 g/l

Caps were placed on fruit on the 26-27<sup>th</sup> of November 2001 on cv. Kent at Bavaria Estate & Ryfontein farm. Fruit were harvested at Ryfontein on the 30<sup>th</sup> of January 2002 and at Bavaria Estate on the 6<sup>th</sup> of February 2002.

**Appendix 4** Treatments and concentrations used during experimental trials at Bavaria Estate, where spray trials were evaluated for disease control, during the 2000/2001 season on cv. Kent and Tommy Atkins

Treatment number	Treatments	Concentration
1	Control	-
2	Copper oxychloride sprays	200 g/200 ℓ litre of water
3	Commercial copper oxychloride sprays	600 g/200 ℓ of water
4	Commercial <i>Bacillus licheniformis</i> (liquid formulation) at $10^9$ cells/ml	1 ℓ/200 ℓ of water
5	<i>B. licheniformis</i> (powder formulation) at $10^9$ cells/ml	200 g/200 ℓ of water
6	<i>B. licheniformis</i> (liquid formulation) at $10^9$ cells/ml & commercial copper oxychloride	1 ℓ (antagonist) & 600 g (commercial copper)/200 ℓ of water
7	<i>B. licheniformis</i> (powder formulation) at $10^9$ cells/ml & commercial copper oxychloride	200 g (antagonist) & 600 g (commercial copper)/200 ℓ of water

Sprays were only done at Bavaria Estate on 'Kent' and 'Tommy Atkins'. Spraying commenced on the 31<sup>st</sup> of August 2000 (on both cultivars) until 14<sup>th</sup> December 2000 on 'Tommy Atkins' and 22<sup>nd</sup> January 2001 on 'Kent' at three-week intervals. Fruit were harvested on 'Tommy Atkins' on the 10<sup>th</sup> of January 2001 and on 'Kent' on the 6<sup>th</sup> of February 2001.

**Appendix 5** Treatments and concentrations used during experimental trials at Bavaria Estate and Ryfontein farm, where spray trials were evaluated for disease control, during the 2001/2002 season on cv. Kent

Treatment number	Treatments	Concentration
1	Control	-
2	Copper oxychloride sprays	200 g/200 ℓ litre of water
3	Commercial copper oxychloride sprays	600 g/200 ℓ of water
4	Commercial <i>Bacillus licheniformis</i> (liquid formulation) at $10^9$ cells/ml	1 ℓ/200 ℓ of water
5	<i>B. licheniformis</i> (liquid formulation) at $10^9$ cells/ml & copper oxychloride sprays	1 ℓ (antagonist) & 200 g (copper)/200 ℓ of water
6	<i>B. licheniformis</i> (liquid formulation) at $10^9$ cells/ml & commercial copper oxychloride	1 ℓ (antagonist) & 600 g (commercial copper)/200 ℓ of water

Spraying commenced on the 6<sup>th</sup> of September 2001 until the 9<sup>th</sup> of January 2002 at three-weekly intervals on both farms. Fruit were harvested at Ryfontein on the 30<sup>th</sup> of January 2002 and at Bavaria Estate on the 6<sup>th</sup> of February 2002.

**Appendix 6** Comparative cost structure for the 2001/2002 season using commercial and woolly caps as well as sun-protector to prevent sunburn damage on mango fruit

Product	Cost/ha	Cost/ha (placing caps on fruit/spraying orchard)	Cost/ha (picking of caps after harvest/number of sprays)	Total input cost	Average% increase in undamaged fruits/ha
Commercial caps	R 670	R 350	R 160	R 1 180	22 %
Woolly caps	R 1 920	R 350	R 160	R 2 430	30 %
Sun-protector	R 20/200 g	R 90/ha/single spray	3 sprays	R 270	10 %

Woolly caps can be re-used for a subsequent season, which would reduce input cost. Price indicated is estimated, and can differ depending on production season, workers salary and retailer price.

**Appendix 7** Comparative cost structure as determined for the 2001/2002 season for chemical, biological and integrated control spray programs for the control of bacterial black spot on mango

<b>Products</b>	<b>Cost/ha/single spray (in R) (product &amp; labour cost)</b>	<b>No. of sprays</b>	<b>Total input cost/ha/season (R)</b>	<b>% Increase in healthy fruit/ha for BBS</b>
1) Commercial copper oxychloride	R 100	5	R 500	4 %
2) Biocontrol ( <i>Bacillus licheniformis</i> )	R 150	5	R 750	6 %
<b>3) Integrated treatment</b>				
3.1 Commercial copper &	R 100	2	R 200	} R 650 5 %
3.2 Biocontrol	R 150	3	R 450	

Price indicated is estimated, and can differ depending on production season, workers salary and retailer price.