Control strategies for citrus postharvest diseases

by

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Erika E. Auret
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Les grandes penseés viennent du coeur

French Proverb

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GENERAL INTRODUCTION

Although the first orange trees (*Citrus sinensis* (L.) Osbeck) were planted in South Africa shortly after the arrival of Jan van Riebeeck in 1652, citrus was only exported from 1902 (Stanbury, 1996). Since then the South African citrus industry has grown to become the third largest exporter in the world, following Spain and the USA. The total production of citrus fruit increased from 1.26 million tonnes in the 1997/98 season to approximately 1.33 million tonnes in the 1998/99 season (Abstract of Agricultural Statistics, 2000). Due to the growing industry, exports doubled and new markets were developed which included the Far East, Eastern Europe and North America (Branders, 1996; Anonymous, 1998).

With development of new export markets and the subsequent long shipping conditions, shelf life of fruit have to be extended without compensating quality (Johnson & Sangchote, 1993). One of the major constraints to extent shelf life is postharvest diseases, and losses of up to 6% can occur on the export market (A. Heitmann, Capespan (Pty) Ltd., Cape Town, SA, personal communication). These diseases include *Alternaria* rot (*Alternaria citri* Ellis & N.Pierce), *Aspergillus* rot (*Aspergillus niger* Tiegh.), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.), stem-end rot (*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.), sour rot (*Geotrichum citri-aurantii* (Ferraris) E.E. Butler), green mould (*Penicillium digitatum* (Pers.: Fr. Sacc.), blue mould (*Penicillium italicum* Wehmer), *Rhizopus* rot (*Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill), *Trichoderma* rot (*Trichoderma viride* Pers. Fr.) (Brown & Eckert, 1989; Eckert & Brown, 1989) and whisker mould (*Penicillium ulaiense* Hsieh, Su & Tzean) (Holmes *et al.*, 1994). These pathogens infect either pre- (*A. citri*, *C. gloeosporioides*, *L. theobromae*, *T. viride*) or postharvestly (*A. niger*, *G. candidum*, *P. digitatum*, *P. italicum*, *P. ulaiense*, *R. stolonifer*, *T. viride*) through wounds or natural openings (Sommer, 1982; Brown & Eckert, 1989; Eckert & Brown, 1989).

Postharvest applications of fungicides such as guazatine, imazalil and thiabendazole are the primary means of controlling postharvest diseases of citrus (Shachnai & Barash, 1982; Eckert & Ogawa, 1985; Pelser, 1988; Smilanick *et al.*, 1997). However, widespread and intensive use of these fungicides resulted in the development of fungicide resistant strains of the postharvest

pathogens (Bancroft *et al.*, 1984; Bus *et al.*, 1991; Eckert *et al.*, 1994). Furthermore, regulatory restrictions by the US Environmental Protection Agency make registration of new fungicides increasingly difficult (Couey, 1989). This, and the increased public concern about fungicide residue on fruit (Johnson & Sangchote, 1993), created a need for development of alternative decay control strategies (Couey, 1989; Van Staden, 1994).

Several environmentally-friendly approaches are available for the control of postharvest pathogens, including a) biological control, b) natural plant products, surfactants and disinfectants, c) induced resistance and d) harvesting and handling techniques which minimise injury and infection by pathogens (Wisniewski & Wilson, 1992).

Biological control of citrus postharvest pathogens is a widely studied field, with numerous reports referring to the use of bacteria, yeasts and fungi (Table 1). Remarkable successes with biocontrol agents have been obtained on avocado (Korsten *et al.*, 1995), apple (Janisiewicz *et al.*, 1998), peach (Pusey *et al.*, 1988), mango (Koomen & Jeffries, 1993; De Villiers & Korsten, 1996), litchi (Korsten *et al.*, 1993), and pear (Janisiewicz & Criof, 1992, Benbow & Sugar, 1999). However, only a few of these antagonists have been registered and are available as commercial products for the control of postharvest diseases, e.g. Aspire (*Candida oleophila* strain I-182; Ecogen Inc., Langhorne, PA), Bio-Save 110 and 111 (*Pseudomonas syringae* strains ESC 10 and ESC 11, EcoScience Corp., Worcester, MA) (Cook *et al.*, 1996; Teixidó *et al.*, 1998) and Avogreen (*Bacillus subtilis* (B246), Pretoria, SA) (L. Korsten, University of Pretoria, personal communication).

Another approach to disease control is the use of natural plant products. Singh *et al.* (1980) found that certain essential oils exhibited strong antifungal activity against *Bipolaris oryzae* (Breda de Haan) Shoemaker. More recently, Wilson *et al.* (1997) successfully tested plant extracts and essential oils against *Botrytis cinerea* Pers. Fr., a postharvest pathogen of various fruit commodities. This research area has remained largely unexplored in the citrus environment.

For many years, food and dairy industries have made wide use of surfactants and disinfectants for cleaning processing plants and decontamination of raw foodstuffs (Park *et al.*, 1991). This approach to the control of postharvest diseases has been studied in several fruit commodities. The use of surfactants and disinfectants usually forms part of fruit washing, as it reduces the risk of dip tank water as a source of inoculum (Spotts &

Cervantes, 1992; Johnson & Sangchote, 1993). Various surfactants and disinfectants have been tested with success, including calcium chloride, Triton X-100 and ozone on apples (Conway *et al.*, 1992; Ong *et al.*, 1996; Roy *et al.*, 1996) and Agrisan, ethanol, Iodet, KOCl, Terminator, SU 319 and Stericlen on avocado (Boshoff *et al.*, 1995; Van Dyk et al., 1997). Ethanol and Terminator reduced postharvest decay of mango (De Villiers & Korsten, 1996) and peach (Feliciano *et al.*, 1992; Margosan *et al.*, 1997; Zeneca Agrochemicals users pamphlet). Postharvest diseases of pear were controlled using sodium hypochlorite, flotation salts, Ortho X-77, Ag-98, chlorine dioxide and ozone (Spotts & Peters, 1980; Spotts, 1984; Spotts & Cervantes, 1987, 1989, 1992). Effective control of strawberry diseases was obtained using chitosan (El Ghaouth *et al.*, 1992). In the citrus industry, sodium dodecylbenzenesulfonate, ethanol and chlorine dioxide were found effective against green mould by Stange & Eckert (1994), Smilanick *et al.* (1995) and Lesar (1997), respectively, while sodium carbonate and potassium bicarbonate showed fungistatic ability (Smilanick *et al.*, 1999).

Several mechanisms of disease resistance operate simultaneously in fruit. Ben-Yehoshua *et al.* (1992) studied the role of preformed and induced antifungal materials in the resistance of citrus fruit to green mould. Subjecting fruit to fungal challenge and/or abiotic stress (heat or UV illumination) induce production of scoparone. This phytoalexin was more fungicidal to *P. digitatum* than preformed antifungal compounds such as citral. Heat treatment of citrus fruit to induce resistance has been widely studied, with reports as early as 1922 by Fawcett. Heat is usually applied to a fruit commodity via air or water (Barkai-Golan & Phillips, 1991). However, water is a more efficient heat transfer medium than air having a higher transfer coefficient (Jacobi *et al.*, 1993). Therefore, hot-water treatments are quicker and more effective in heat transfer to fruit than hot air treatments (Shellie & Mangan, 1993). Numerous investigations using heated water treatments have shown benefits for citrus postharvest disease control in many countries, including Israel (Rodov *et al.*, 1996), Italy (Dettori *et al.*, 1996), South Africa (De Villiers *et al.*, 1996) and the USA (Brown & Baraka, 1996). Hot water treatment of other fruit commodities such as nectarines and peaches, also substantially reduced postharvest decay (Margosan *et al.*, 1997), with similar results obtained in avocado (Plumbley *et al.*, 1993) and mango (Spalding & Reeder, 1986). In the papaya industry, immersion of fruit in hot water has been the principal postharvest treatment for decay control since 1964 (Akamine, 1967). Fungicides such as imazalil (Smilanick *et al.*, 1997) and biocontrol agents (De Villiers *et al.*, 1996) may be integrated with hot water treatments to enhance their efficacy.

Efficacy of all the above decay control strategies can be enhanced by proper handling and sanitation procedures (Sommer, 1982). It is well-known that careless harvesting and handling practices, along with high inoculum levels of postharvest pathogens in the fruit environment, are the main factors involved in the initiation of disease (Di Martino Aleppo & Lanza, 1996). Spores of pathogenic fungi are produced on decayed fruit and are transferred by air currents, water dip tanks and fruit handling equipment to sound fruit in the packhouse (Gardner *et al.*, 1986). According to Gardner *et al.* (1986), efficacy of a management strategy to reduce fruit contamination depends on several key elements, viz. a) isolation of spore-generating areas, b) careful handling of fruit, c) proper sanitation procedures for decontamination of fruit handling equipment, d) weekly assays to monitor pathogen spore populations, e) judicious use of fungicides and f) modification of existing packhouse operations to prevent dispersal of pathogen inoculum.

In this study, some of the alternative decay control strategies discussed were investigated, viz. introduction of an antagonist, warm water and integrated applications, determination of infection sites in packhouses and disinfestation with non-selective chemicals, all to form part of a total quality management system for citrus fruit which would ensure the required quality and product consistency demanded by consumers (Hilton, 1993).

Table 1 Published reports on biological control of citrus postharvest diseases

Antagonist	Pathogen	References
Aureobasidium pullulans (De Bary) G. Arnaud	*Penicillium digitatum* + *Penicillium italicum*	Wilson & Chalutz, 1989
Bacillus pumilus	*P. digitatum*	Huang *et al.*, 1992
Bacillus subtilis	*Alternaria citri* + *Geotrichum citri-aurantii* + *P. digitatum*	Singh & Deverall, 1984
	A.citri + *Botrytis cinerea* + *Colletotrichum gloeosporioides* + *G. citri-aurantii* + *P. digitatum* + *P. italicum*	Arras, 1993
	B. cinerea + *P. digitatum*	Arras & D'Hallewin, 1994
Candida famata (FC Harrison) SA Meyer & Yarrow	*P. digitatum*	Arras, 1996, 1999
	P. digitatum	D'Hallewin *et al.*, 1999
Candida guilliermondi (Castelani) Langeron & Guerra [teleomorph *Pichia guilliermondii* Wickerham]	*P. digitatum*	McGuire, 1994
	P. digitatum	Droby *et al.*, 1999
Candida oleophila Montrocher	*G. citri-aurantii* + *P. digitatum*	Shachnai *et al.*, 1996
	G. citri-aurantii + *P. digitatum* + *P. italicum*	Droby *et al.*, 1998
	P. digitatum	El-Neshawy & El-Sheikh Aly, 1998
	P. digitatum	Droby *et al.*, 1999
	P. digitautm	McGuire & Dimitroglou, 1999
Candida sake	*P. italicum*	Arras *et al.*, 1997
	P. digitatum	Droby *et al.*, 1999

Debaryomyces hansenii (Zopf) Lodder & Kreger van Rij	*G. citri-aurantii* + *P. digitatum* + *P. italicum*	Chalutz *et al.*, 1988
	P. digitatum	Droby *et al.*, 1989
	P. digitatum + *P. italicum*	Wilson & Chalutz, 1989
	G. citri-aurantii + *P. digitatum* + *P. italicum*	Chalutz & Wilson, 1990
	P. italicum	Chalutz & Wilson, 1992
	P. digitatum + *P. italicum*	Mehrotra *et al.*, 1996
	G. citri-aurantii	Mehrotra *et al.*, 1998
	B. cinerea + *P. digitatum* + *P. italicum*	Arras & Arru, 1999
	P. digitatum	Droby *et al.*, 1999
Kluyveromyces sp.	*P. digitatum*	Cheah & Tran, 1995
Myrothecium roridum Tode	*P. digitatum*	Appel *et al.*, 1988
Myrothecium verrucaria (Alb. & Schwein.) Ditmar	*P. digitatum*	Appel *et al.*, 1988
Paecilomyces lilacinus (Thom) Samson	*P. digitatum* + *P. italicum*	Wang *et al.*, 1996
Pichia guilliermondii Wickerham	*P. digitatum*	Droby *et al.*, 1993, 1997
Pseudomonas cepacia	*P. digitatum* + *P. italicum*	Wilson & Chalutz, 1989
	P. digitatum	Huang *et al.*, 1991
	P. digitatum	Smilanick & Denis-Arrue, 1992
	P. digitatum	Huang *et al.*, 1993a
	P. digitatum + *P. italicum*	Huang *et al.*, 1993b
Pseudomonas corrugate	*P. digitatum*	Smilanick & Denis-Arrue, 1992
Pseudomonas fluorescens	*P. digitatum*	Smilanick & Denis-Arrue, 1992
Pseudomonas glathei	*P. digitatum*	Huang *et al.*, 1995

Pseudomonas syringae	*P. digitatum* + *P. italicum*	Wilson & Chalutz, 1989
	P. digitatum	Smilanick *et al.*, 1996
	P. digitatum + *P. italicum*	Bull *et al.*, 1997
	P. digitatum	Smilanick *et al.*, 1999
Saccharomyces cerevisiae Meyer ex. Hansen	*P. digitatum*	Cheah & Tran, 1995
Trichoderma viride	*G. citri-aurantii* + *P. digitatum*	De Matos, 1983
	P. digitatum	Borrás & Aguilar, 1990
	C. gloeosporioides + *G. citri-aurantii*	Borrás *et al.*, 1993

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Chapter 2

COMPARISON OF POSTHARVEST *BACILLUS* APPLICATIONS, FUNGICIDAL AND INTEGRATED TREATMENTS FOR CONTROL OF CITRUS POSTHARVEST DISEASES

ABSTRACT

Bacillus spp., previously isolated from subtropical plants and fruit, were evaluated *in vitro, in vivo* and in packhouse dip or wax treatments for control of citrus postharvest diseases caused by *Alternaria citri, Colletotrichum gloeosporioides, Lasiodiplodia theobromae, Geotrichum citri-aurantii, Penicillium digitatum, P. italicum* and *Trichoderma viride*. *Bacillus subtilis* (B246) inhibited *in vitro* growth of the citrus postharvest pathogens most effectively, while *Bacillus licheniformis* (B251) and *B. subtilis* (B248) were the most effective antagonists *in vivo* against *P. digitatum*. In packhouse experiments an integrated control, consisting of antagonist combined with application of quarter-strength solutions of the standard concentrations of guazatine, imazalil, thiabendazole, was included along with a standard chemical treatment. Although treatment with antagonists B246 and *B. licheniformis* (B254) reduced the percentage of fruit infected, the reduction was less than that achieved with the standard chemical treatment. Integrated treatments were as effective as the chemical treatments, implicating the integrated approach as an alternative disease control strategy requiring reduced rates of fungicides.

INTRODUCTION

Alternaria citri Ellis & N. Pierce (*Alternaria* rot), *Aspergillus niger* Tiegh. (*Aspergillus* rot), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (anthracnose), *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (stem-end rot), *Geotrichum citri-aurantii* (Ferraris) E.E. Butler (sour rot), *Penicillium digitatum* (Pers.: Fr.) Sacc. (green mould), *Penicillium italicum* Wehmer (blue mould), *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. (*Rhizopus* rot), *Trichoderma viride* Pers.: Fr. (*Trichoderma* rot) (Brown & Eckert, 1989; Eckert & Brown, 1989) and *Penicillium ulaiense* Hsieh, Su & Tzean (whisker mould) (Holmes *et al.*, 1994), are the major pathogens causing citrus postharvest decay. Control of these pathogens relies mainly on treatment with fungicides such as guazatine, imazalil and thiabendazole (Shachnai & Barash, 1982; Eckert & Ogawa, 1985; Pelser, 1988). However, the use of fungicides is becoming increasingly restricted because of their potential detrimental effect on the environment and human health (Norman, 1988; Huang *et al.*, 1995). In addition, development of pathogen resistance is further restricting use of these fungicides (Bancroft *et al.*, 1984; Bus *et al.*, 1991; Eckert *et al.*, 1994). This, and regulatory restrictions by the US Environmental Protection Agency regarding the use of fungicides (Couey, 1989), necessitate development of new technologies for control of postharvest diseases as an alternative to fungicides (Van Staden, 1994).

Biological control using microbial antagonists is considered a desirable and rapidly developing alternative, either on its own or as part of an integrated control strategy to reduce fungicide input (Teixidó *et al.*, 1998). Antagonists have been reported to control postharvest diseases in many fruit commodities e.g. avocado (Korsten *et al.*, 1995), apple (Janisiewicz *et al.*, 1998), peach (Pusey *et al.*, 1988), mango (Koomen & Jeffries, 1993, De Villiers & Korsten, 1996), litchi (Korsten *et al.*, 1993), and pear (Janisiewicz & Criof, 1992; Sugar & Spotts, 1999). Currently, a number of commercial products have been registered and are available for control of postharvest diseases, including Aspire (*Candida oleophila* strain I-182; Ecogen Inc., Langhorne, PA) and Bio-Save 110 and 111 (*Pseudomonas syringae* strains ESC 10 and ESC 11, EcoScience Corp., Worcester, MA) (Cook *et al.*, 1996; Teixidó *et al.*, 1998).

According to Chalutz & Wilson (1990), an early observation on biological control of citrus postharvest diseases was reported by Gutter & Littauer in 1953, who isolated *Bacillus subtilis* from citrus fruit that inhibited the growth of citrus pathogens. More recently, several other antagonists have been reported to control citrus postharvest diseases, including *Candida* spp. (Arras, 1996; Arras *et al.*, 1997, 1999; Droby *et al.*, 1997), *Pichia* spp. (Droby *et al.*, 1997) and *Pseudomonas* spp. (Huang *et al.*, 1995; Bull *et al.*, 1997; Smilanick *et al.*, 1999). This study reports the effect of *Bacillus* spp., alone or integrated with fungicides, on postharvest decay of Valencia oranges.

MATERIALS AND METHODS

In vitro screening of antagonists

Antagonists (obtained from L. Korsten, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, SA) were screened for inhibition of citrus postharvest pathogens *A. citri, C. gloeosporioides, L. theobromae, G. citri-aurantii, P. digitatum, P. italicum* and *T. viride* (obtained from K. Lesar, Capespan Citrus Centre, Nelspruit, SA). Antagonists included *Bacillus subtilis* (isolates B246 and B248 from avocado), *B. licheniformis* (isolates B250 and B251 from mango and isolate B254 from litchi leaf surfaces), *B. stearothermophilus* (isolate B252 from litchi), *B. megaterium* (isolate B253 from litchi) and *B. cereus* (isolates B247 and B249 from avocado). These *Bacillus* antagonists were rpeviously shown to be effective against pathogens associated with the subtropical fruit from which they were isolated (Korsten *et al.*, 1992, 1993; Korsten, 1993).

The dual culture technique (Porter, 1924; Skidmore, 1976) was used to test antagonism on nutrient agar (Biolab, Johannesburg, SA). Antagonists were streaked 30 mm from the 5-mm diameter pathogen disc. Three replicates were included for each antagonist-pathogen combination and controls consisted of fungal discs without antagonist streaks. Mean percentage pathogen inhibition was determined from the formula of Skidmore (1976): (r - C) x 100 / C, where r = the radius from the middle of the pathogen colony, on the side facing the antagonist, and C = growth from the middle to the edge of the pathogen on control plates. Data was statistically analysed using an analysis of variance and Duncan's multiple range test to

separate means (P = 0.05).

In vivo screening of antagonists

Antagonists (B246, B248, B250, B251, B254) evaluated in *in vitro* screening assays were grown in 500 ml Erlenmeyer flasks containing 250 ml Standard 1 nutrient broth (STD 1) (Biolab). After 48 h shake-incubation (rotary shaker, 67 rpm) at 28°C, cells were harvested by centrifugation for 10 min at 8000 g. The resulting pellet was dissolved in quarter-strength Ringer solution (Merck, Johannesburg) to obtain a concentration series of 10^6, 10^7, 10^8 and 10^9 cells ml^{-1} using a Petroff-Hausser counting chamber. Citrus postharvest pathogen, *P. digitatum*, was cultured for 14 days on potato-dextrose agar (Biolab) at 25°C under a near-UV light source. Conidia were harvested in quarter-strength Ringer solution, counted with a haemacytometer and serially diluted to obtain a concentration series ranging from 10^3 to 10^6 conidia ml^{-1}.

Valencia orange fruit were used to determine the most effective antagonist concentration according to the checkerboard-titration technique used by Korsten *et al.* (1995). Fruit were surface-sterilised by dipping in 70 % ethanol and left to air-dry. Squares (5 mm x 5 mm) were drawn on one side of the fruit with waterproof ink to give five vertical and five horizontal rows forming a checkerboard pattern of 25 squares. The surface of the fruit in each square was prick-wounded centrally to a depth of 5 mm with a sterile 1 mm-diameter inoculation needle. Twenty microlitres of each *P. digitatum* concentration was applied to a square resulting in horizontal rows having the same conidial concentration (Fig. 1). Fruit were left to air-dry and after 24 h, 20 µl of the appropriate antagonist was applied to a square using the same concentration in vertical rows. The last row received 20 µl quarter-strength Ringer solution only. Three fruit were used for each antagonist-*P. digitatum* combination. Inoculated fruit were randomly packed in cardboard boxes lined with moistened cotton wool to maintain humidity. Boxes were stored at 25 °C, and disease development was scored after seven days according to an arbitrary scale of 0 and 1, where 0 = no disease development or slight browning around the edge of the inoculation point, and 1 = fungal growth within the wound or necrotic enlargement spreading from the wound.

Compatibility of antagonists with chemicals

Antagonists selected for their strong inhibitory action *in vitro* against the citrus postharvest pathogens were tested for compatibility with fungicides and a wax applied commercially in citrus packhouses, using a modified paper-disc method (Thornberry, 1950). Antagonists were incorporated in STD1 agar . Paper discs (Whatman, 5mm) saturated with one of the following chemicals, guazatine (1000 ppm and 250 ppm) (Decotine) (Waltiernon Dormas (Pty) Ltd., Johannesburg); thiabendazole (1000 ppm and 250 ppm) (Tecto) (Logos Agrowett (Pty) Ltd., Johannesburg) 2,4-D (500 ppm and 125 ppm) (Agrihold, Silverton, Pretoria, SA); imazalil (500 ppm and 125 ppm) (Agrihold) and sodium-orthophenyl-phenol (SOPP) (1000 ppm and 250 ppm) (Agrihold) were placed on the agar. Controls consisted of discs saturated with sterile water. Citrashine wax (Plaaschem, Houghton, SA) was tested similarly to method used to test the chemicals. Plates were incubated at 25 °C for 24 and 48 hours after which inhibition or stimulation zones were recorded using the scale 0 = no growth; 1 = > 5 mm inhibition zone around paper disc; 2 = < 5 mm inhibition zone around paper disc and 3 = paper disc overgrown.

Postharvest experiments

Efficacy of antagonists on their own, integrated with quarter-strength chemicals and commercial chemical treatments were evaluated on Valencia orange fruit, obtained from Letaba Estates (Tzaneen, Northern Province, SA) (Tables 1, 2 and 3). Antagonists were prepared according to Korsten *et al.* (1989).

Fruit used in all treatments were submerged in a commercial chlorine bath (100 ppm) followed by high-pressure water spray (40 bar) before treatment. Fruit were mechanically injured subsequently by rolling them over a strip of drawing pins (Fig. 2d, e). This caused 7-9 pinpricks up to 3 mm deep on each fruit. Wounded fruit were artificially inoculated by dipping the fruit for 3 min in a drum containing 25 l inoculum (Fig. 2c, f). Inoculum was prepared by macerating fruit naturaly infected with postharvest pathogens (*A. citri, C. gloeosporioides, L. theobromae, G. citri-aurantii, P. digitatum, P. italicum* and *T. viride).* The homogenate was filtered through four layers of cheesecloth (Fig. 2a, b). Fungal spores in the filtrate were counted using a haemacytometer and the filtrate was diluted in tap water to a concentration of 10^2, 10^3 or 10^5 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight at 25 °C and treated the

following day.

Fruit were dipped in 50 l containers with tap water containing either the antagonist or chemical (2,4-D, guazatine, imazalil and TBZ in experiment 1; or guazatine and imazalil in experiments 2 and 3) or both (Fig. 2g). After fungicide dip treatment in experiments 2 and 3, fruit were waxed with Citrashine supplemented with TBZ. Integrated treatments were dipped in water containing quarter-strength of the standard fungicide concentrations (imazalil and guazatine) and subsequently waxed with Citrashine supplemented with antagonist and quarter-strength TBZ. In the last two experiments (2 and 3) all antagonists were applied in wax. Each treatment consisted of 800 fruit.

After dip and wax treatments, fruit were left to air-dry, packed in cartons (80 fruit per box) (Fig. 2h) and sealed. Cartons were covered with black plastic and stored in darkness at room temperature. The plastic was removed after four days and the fruit stored for an additional 14 days. Sound fruit per carton were counted and percentages computed. Data were analysed statistically using ANOVA, and Student's t-least significant differences were calculated to determine treatment differences (P = 0.05).

RESULTS

In vitro screening of antagonists

Differences in the degree of fungal inhibition were evident between the nine antagonists evaluated (Table 4). Overall, *B. subtilis* (isolate B246) inhibited growth of the postharvest pathogens the most, although not significantly more than *B. licheniformis* (isolate B250) and *B. megaterium* (isolate B253). Growth of *C. gloeosporioides* was affected less than that of *A. citri*, *G. citri-aurantii*, *P. digitatum* and *P. italicum*.

In vivo screening of antagonists

At pathogen concentrations of 10^4 conidia.ml^{-1} and lower, all antagonists at all concentrations

prevented infection by *P. digitatum* except for isolate B246 which was only effective at a concentration of 10^9 cells ml^{-1} for a 10^4 conidia ml^{-1} inoculum and at 10^7 cells ml^{-1} and higher for a 10^3 conidia ml^{-1} inoculum (Table 5). Concentrations of 10^8 cells ml^{-1} and higher of B248 and B251 were necessary to prevent infection at an inoculum level of 10^5 conidia ml^{-1}, while 10^9 cells ml^{-1} of B250 and B254 were required for effective control. None of the antagonists could prevent infection by *P. digitatum* at an inoculum level of 10^6 conidia ml^{-1}.

Antagonist compatibility with chemicals

None of the chemicals except Citrashine were fully compatible with the antagonists, when tested at commercial concentrations (Table 6). However, when tested at quarter- strength concentrations only SOPP totally inhibited the growth of all antagonists.

Postharvest experiments

Experiment 1: Only the chemical and integrated treatments significantly decreased postharvest decay, although not significantly more so than B248 dip against natural infection and B254 dip against both natural and artificially infected fruit (Fig. 3, 4).

Experiment 2: All treatments significantly increased percentage sound fruit compared to the wax control. The chemical, quarter-strength chemical and integrated treatments were the most effective treatments (Fig. 5). Applying B246, B250 and B254 as a mixture in wax was less effective than wax treatment with B246 and B254 individually, but not than treatment with B250 wax.

Experiment 3: The chemical, integrated and quarter-strength treatments increased percentage sound fruit from less than 10 % to more than 90 % (Fig. 6).

DISCUSSION

Bacillus subtilis is a known antagonist of fruit pathogens, including those of citrus (Korsten, 1993). This, however, is the first report of the antagonistic ability of *B. licheniformis*, *B.*

megaterium and *B. stearotermophilus* against citrus postharvest pathogens.

The most effective antagonist *in vitro* was *B. subtilus* (B246). However, high concentrations of the bacterium were required to control *P. digitatum in vivo*. Control could also be achieved only at lower inoculum concentrations of the pathogen. Corresponding findings were reported by Huang *et al.* (1992), regarding the control of *P. digitatum* with *Bacillus pumilus*.

With the exception of B246, all antagonists tested *in vivo*, were capable of controlling *P.digitaum* inoculated at 10^5 conidia ml^{-1} when applied at concentrations of 10^9 cells ml^{-1}. *Bacillus licheniformis* (B254) and *B. subtilis* (B248), however, achieved control at this pathogen inoculum level using concentrations of only 10^8 cells ml^{-1}. In general, enhanced disease control was associated with increased antagonist concentration or a decrease in the pathogen inoculum level, a phenomenon which is well described in biocontrol (Pusey & Wilson, 1984; Janisiewicz & Roitman, 1988; Korsten *et al.*, 1995). The disparity between *in vitro* and *in vivo* results highlights the importance of using both methods in a screening programme (Utkhede *et al.*, 1986; Chalutz & Wilson, 1990).

According to Chalutz & Wilson (1990), packhouse antagonist treatments must be as effective and provide consistent control comparable to that achieved with chemical treatments. Although some antagonist treatments reduced decay compared to the control in both experiments 1 and 2, they were not as effective or consistent as chemical treatments. Shachnai *et al.* (1996) and Droby et *al.* (1998) experienced a similar phenomenon in packhouse experiments with *Candida oleophila* Montrecher. However, when the yeast was applied in combination with SOPP and low concentrations of TBZ, control equivalent to chemical treatment was achieved. Antagonists used in the present study were tolerant to low concentrations of imazalil, 2.4 D and TBZ. In packhouse experiments, the efficacy of these antagonists was enhanced when used in combination with low concentrations of the above chemicals and disease control achieved was comparable with that of standard chemical treatments.

Application method is another important factor in antagonist treatments of fruit, which has to be compatible with existing packhouse procedures (McGuire, 1994). Pusey *et al.* (1986, 1988) and Korsten (1993) incorporate the antagonist *B. subtilis* into fruit waxes used

commercially in peach and avocado packhouses, repectively. In this study, Citrashine wax provided a viable mean of applying antagonists to fruit. Variability recorded in this study could have resulted from several factors related to the initial quality of the fruit, susceptibility of the fruit to infection, time elapsed between picking and treatment, and initial inoculum density (Droby *et al.*, 1993). Inoculum density in the final experiment was 100 times higher than in the second experiment, thus implicating that inoculum pressure could have been a reason for the ineffectiveness of the antagonists. Applying antagonists in wax had the added advantage of requiring relative small quantities of antagonist (1 l wax for 52 cartons) and being less time- consuming to apply.

As indicated above, control of postharvest diseases depends largely on the inoculum level of the pathogen. Furthermore, effectiveness of antagonist treatments relies on the successful establishment of the antagonist at wound sites prior to pathogen challenge (Janisiewicz & Roitmann, 1988; Huang *et al.*, 1992). In this study, however, the pathogen inoculum was applied prior to antagonist treatment. Artificial infection used in this investigation simulated field conditions, as pathogens usually infect fruit preharvest through wounds, as well as postharvest through wounds and natural openings (Sommer, 1982; Brown & Eckert, 1989; Eckert & Brown, 1989), before antagonist application in the packhouse. According Korsten (1993), using biocontrol agents preharvestly to reduce the incidence of postharvest diseases, leads to a persistent reduction in pathogen inoculum. As antagonists used in this study proved capable of controlling postharvest pathogens *in vitro* and *in vivo*, establishing them preharvest may provide a more effective control option.

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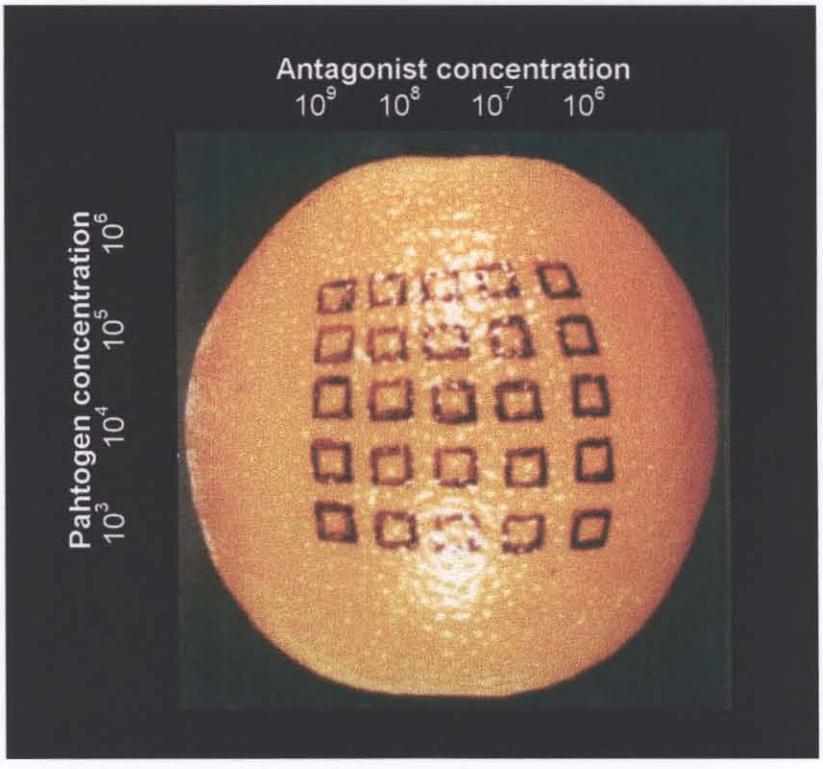


Fig. 1 Checkerboard-titration technique with different antagonist and pathogen concentrations on Valencia orange fruit.

Table 1 Postharvest treatment of Valencia orange fruit for evaluation of biological, integrated and chemical control of citrus postharvest diseases (Experiment 1)

Code	Treatment	Concentration of antagonist (cells ml^{-1}) or chemical (ppm)	Application time (min)
1	Control[y]	-	-
2	B246 dip[y]	1.36×10^7	7
3	B248 dip[y]	7.36×10^7	7
4	B250 dip[y]	6.60×10^7	7
5	B254 dip[y]	1.43×10^7	7
6	B250 wax[y]	1.05×10^7	-
7	Integrated[y]	guazatine (250); 2,4-D (125);	
		thiabendazole (250); imazalil (125);	3
		followed by B254 (1.43×10^7)	7
8	Chemical[z]	guazatine (1000); 2,4-D (500);	
		thiabendazole (1000); imazalil (500)	3

[y] Treatments consisted of fruit both naturally and artificially infected. Fruit were artificially infected with a mixed inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.1×10^2 *Penicillium* conidia ml^{-1}.

[z] Treatment involved only naturally infected fruit.

Table 2 Postharvest treatment of Valencia orange fruit for evaluation of biological, integrated and chemical control of citrus postharvest diseases (Experiment 2)

Code	Treatment [z]	Concentration of antagonist (cells ml^{-1}) or chemical (ppm)	Application time (min)
1	Wax control	-	-
2	B246 wax	7.32×10^7	-
3	B254 wax	6.84×10^7	-
4	B250 wax	7.44×10^7	-
5	Wax antagonist mixture	B246, B250, B254 (1.20×10^7)	-
6	Integrated	guazatine (250) and imazalil (125) dip followed by thiabendazole (250) and B246 (7.32×10^7) wax	3
7	¼ Chemical	guazatine (250) and imazalil (125) dip followed by thiabendazole (250) wax	3
8	Chemical	guazatine (1000) and imazalil (500) dip followed by thiabendazole (1000) wax	3

[z] Fruit of all treatments were mechanically damaged and artificially infected with a mixed inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.2×10^3 *Penicillium* conidia ml^{-1}.

Table 3 Postharvest treatment of Valencia orange fruit for evaluation of biological, integrated and chemical control of citrus postharvest diseases (Experiment 3)

Code	Treatment [z]	Concentration of antagonist (cells ml^{-1}) or chemical (ppm)	Application time (min)
1	Wax control	-	
2	B246 wax	2.53×10^7	
3	B254 wax	5.61×10^7	
4	Wax antagonist mixture	B246, B250, B254 (1.54×10^7)	
5	Integrated	guazatine (250) and imazalil (125) dip followed by thiabendazole(250) and B246 (2.53×10^7) wax	3
6	1/4 Chemical	guazatine (250) and imazalil (125) dip followed by thiabendazole (250) wax	3
7	Chemical	guazatine (1000) and imazalil (500) dip followed by thiabendazole (1000) wax	3

[z] Fruit of all treatments were mechanically damaged and artificially infected with a mixed inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 2.6×10^5 *Penicillium* conidia ml^{-1}.

Table 4 Comparison of *Bacillus* spp. for their inhibitory effect on the *in vitro* growth of seven postharvest citrus pathogens

Antagonist	Code	Percentage inhibition [x]							MEAN[y]	Pr > F
		P1	P2	P3	P4	P5	P6	P7		
Bacillus cereus	B247	49 aA	30 aB	42 aAB	32 bB	12 cC	18 cC	25 bBC	30 c	0.0001
B. cereus	B249	48 aA	24 aC	38 aAB	46 abA	32 bcB	26 bcBC	23 bC	34 bc	0.0006
B. licheniformis	B250	40 abcAB	34 aB	32 abB	42 abAB	52 aA	50 aA	42 aAB	42 ab	0.0056
B. licheniformis	B251	32 eB	24 aC	28 abBC	34 bB	56 aA	54 aA	20 bC	35 bc	0.0001
B. licheniformis	B254	39 bcdAB	28 aB	31 abB	33 bB	40 bA	42 abA	36 abAB	36 bc	0.0053
B. megaterium	B253	36 deB	28 aC	27 abC	36 bB	46 abA	43 abA	42 aA	37 abc	0.0078
B. stearothermophilus	B252	36 deB	30 aBC	34 abB	34 bB	33 bcB	35 bAB	40 aA	35 bc	0.0346
B. subtilis	B246	45 aB	33 aC	31 abC	54 aA	58 aA	56 aA	43 aB	46 a	0.0003
B. subtilis	B248	40 abcAB	28 aC	30 abBC	33 bB	46 abA	43 abAB	22 bC	35 bc	0.0006
MEAN[z]		37 AB	29 C	33 BC	38 AB	42 A	41 A	33 BC		0.0001
Pr > F		0.0021	0.3696	0.0602	0.0395	0.0008	0.0052	0.0463	0.0001	

[x] Determined according to Skidmore (1976); P1 = *Alternaria citri*, P2 = *Colletotrichum gloeosporioides*, P3 = *Lasiodiplodia theobromae*, P4 = *Geotrichum citri-aurantii*, P5 = *Penicillium digitatum*, P6 = *P. italicum* and P7 = *Trichoderma viride*; values within columns (lower case) and rows (upper case) followed by the same letter do not differ significantly according to Duncan's multiple range test (P = 0.05).

[y] Mean percentage inhibition of pathogens P1 – P7.

[z] Mean percentage inhibition of individual pathogens by all *Bacillus* spp.

Table 5 Comparison of the quantitative relationship between different *Bacillus* spp. and *Penicillium digitatum* concentrations according to the checkerboard-titration technique

Antagonist	Code	Concentration (cells ml^{-1})	Disease severity[z]			
			10^3	10^4	10^5	10^6
Bacillus subtilis	B246	10^0	1	1	1	1
		10^6	1	1	1	1
		10^7	0	1	1	1
		10^8	0	1	1	1
		10^9	0	0	1	1
B. subtilis	B248	10^0	1	1	1	1
		10^6	0	0	1	1
		10^7	0	0	1	1
		10^8	0	0	0	1
		10^9	0	0	0	1
B. licheniformis	B250	10^0	1	1	1	1
		10^6	0	0	1	1
		10^7	0	0	1	1
		10^8	0	0	1	1
		10^9	0	0	0	1
B. licheniformis	B251	10^0	1	1	1	1
		10^6	0	0	1	1
		10^7	0	0	1	1
		10^8	0	0	0	1
		10^9	0	0	0	1
B. licheniformis	B254	10^0	1	1	1	1
		10^6	0	0	1	1
		10^7	0	0	1	1
		10^8	0	0	1	1
		10^9	0	0	0	1

[z] Disease development at different inoculum concentrations (conidia ml^{-1}) of *P. digitatum*, where 0 = no disease development or slight browning around the edge of the inoculation point, and 1 = fungal growth within the wound or necrotic enlargement spreading from the wound.

Table 6 Compatibility of *Bacillus* spp. with chemicals commercially used in citrus packhouses

		Antagonist growth[z] at different chemical concentrations (ppm)										
Antagonist	**Code**	2,4-D		guazatine		imazalil		sodium-orthophenyl-phenol		thiabendazole		Citrashine wax
		500	125	1000	250	500	125	1000	250	1000	250	
B. licheniformis	B250	1[z]	2	1	2	0	1	0	0	1	2	3
B. licheniformis	B254	1	2	1	2	0	1	0	0	1	2	3
B. megaterium	B253	1	2	1	2	0	1	0	0	1	2	3
B. subtilis	B246	1	2	1	2	0	1	0	0	1	2	3
B. subtilis	B248	1	2	1	2	0	1	0	0	1	2	3

[z] 0 = no growth; 1 = > 5mm inhibition zone around paper disc; 2 = < 5mm inhibition zone around paper disc; and 3 = antagonist grows over paper disc.

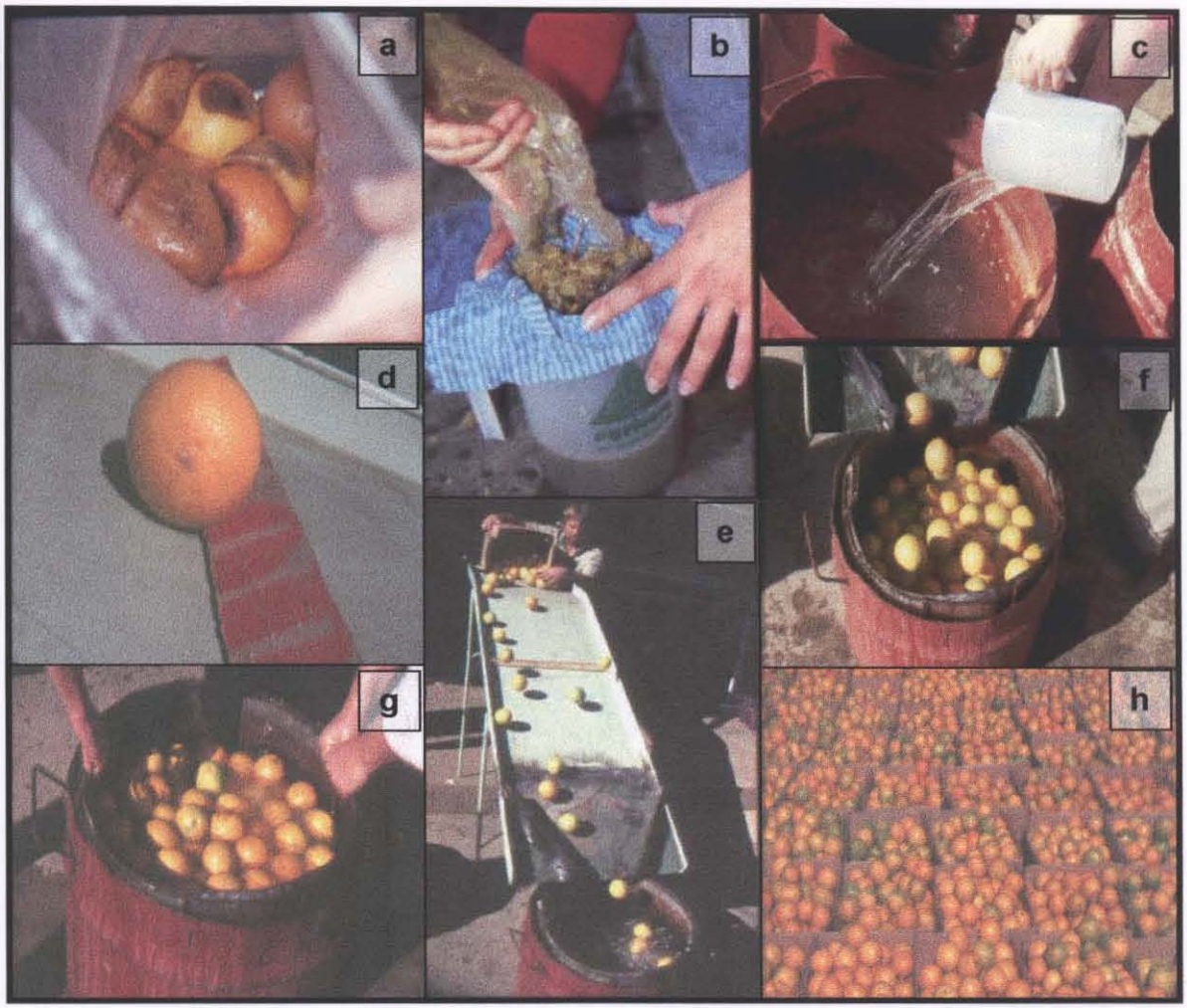


Fig. 2 Artificial inoculation and treatment of Valencia orange fruit.

a Source of inoculum (fruit infected with postharvest pathogens).

b Preparation of inoculum (macerate fruit and strain through cheese cloth).

c Adjust macerated and strained pathogen suspension by adding water to required concentration.

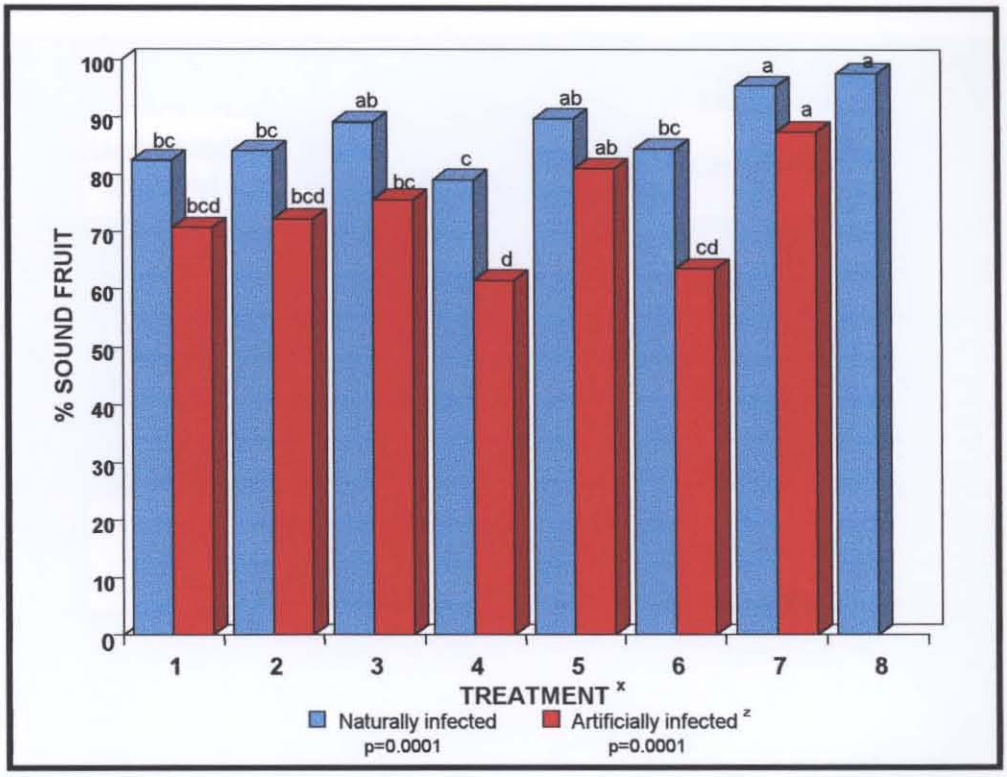
d Close-up of drawing pin strip used to injure fruit (7-9 pinpricks up to 3 mm deep per fruit).

e Process of mechanical injury and artificial inoculation of fruit.

f Close-up of artificial inoculation for 3 min.

g Dip treatment of fruit.

h Packing of treated fruit prior to storage.



Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0.05).

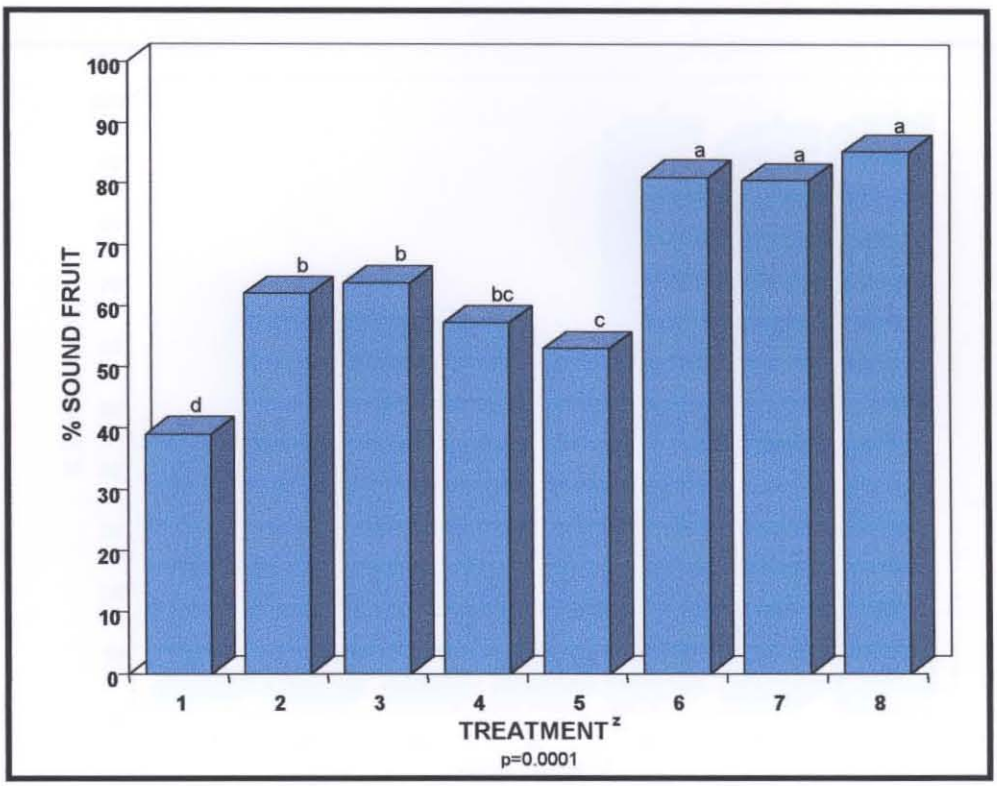
[x] See Table 1 for treatment descriptions: 1 = untreated control; 2 = B246 dip; 3 = B248 dip; 4 = B250 dip; 5 = B254 dip; 6 = B250 wax; 7 = Integrated; 8 = chemical treatment

[z] Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.1×10^2 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day.

Fig. 3 Effect of postharvest *Bacillus* spp., chemical and integrated dip and wax treatments on postharvest deterioration of Valencia orange fruit (Experiment 1).

Fig. 4 Effect of postharvest treatment of Valencia orange fruit on the development of decay (Experiment 1).

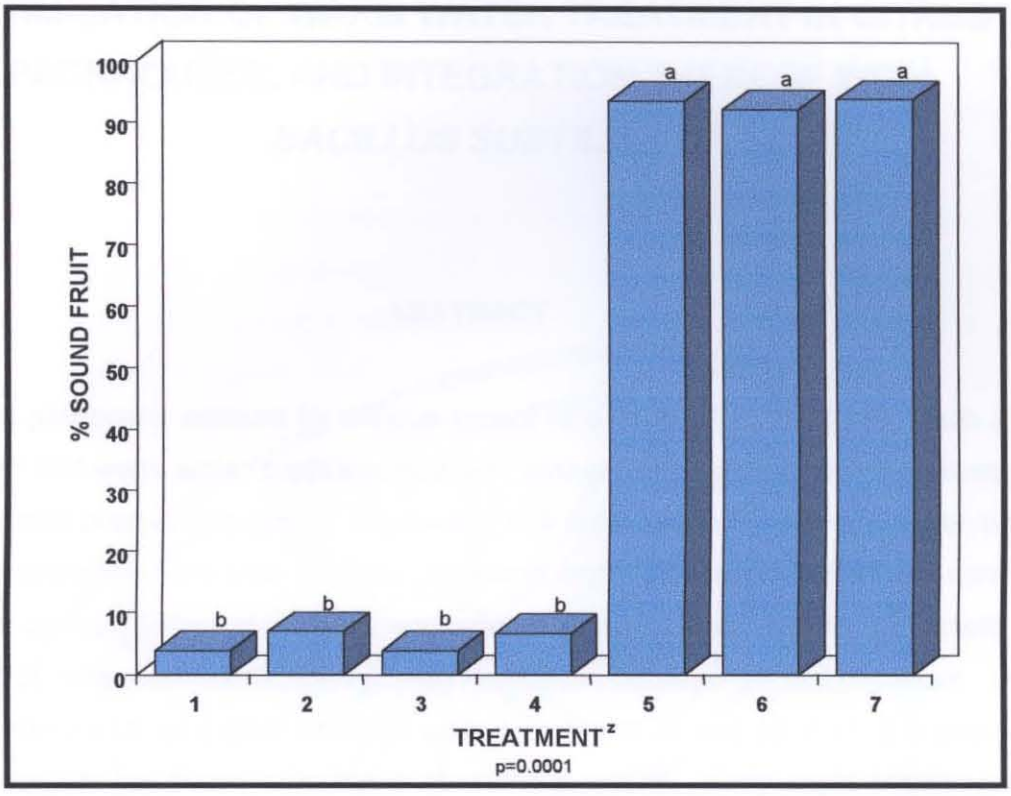
Treatments included: untreated control; chemical control consisting of guazatine (1000ppm); 2,4-D (500ppm); thiabendazole (1000ppm); imazalil (500ppm) 3 min dip; B254 (1.43×10^7 cells ml^{-1}) 7 min dip; and an integrated treatment consisting of guazatine (250ppm); 2,4-D (125ppm); thiabendazole (250ppm); imazalil (125ppm) 3 min dip followed by B254 (1.43×10^7 cells ml^{-1}) 7 min dip.



Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0.05).

[z] See Table 2 for treatment descriptions: 1 = wax control; 2 = B246 wax; 3 = B254 wax; 4 = B250 wax; 5 = wax antagonist mixture; 6 = Integrated; 7 = ¼ strength chemical; 8 = chemical treatment. Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.2 x 10^3 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day.

Fig. 5 Effect of postharvest *Bacillus* spp., chemical and integrated dip and wax treatments on postharvest deterioration of Valenicia orange fruit (Experiment 2).



Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0.05).

[z] See Table 3 for treatment descriptions: 1 = wax control; 2 = B246 wax; 3 = B254 wax; 4 = wax antagonist mixture; 5 = Integrated; 6 = ¼ strength chemical; and 7 = chemical treatment. Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 2.6 x 10^5 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day.

Fig. 6 Effect of postharvest *Bacillus* spp., chemical and integrated dip and wax treatments on postharvest deterioration of Valenicia orange fruit (Experiment 3).

Chapter 3

OPTIMISATION OF WARM WATER TREATMENT IN CITRUS PACKHOUSES, AND INTEGRATION THEREOF WITH *BACILLUS SUBTILIS* *

ABSTRACT

Postharvest pathogens account for serious losses of export citrus fruit from South Africa. Warm water and warm water supplemented with antagonist treatments were evaluated for control of citrus postharvest decay. Inoculated fruit were dipped in a warm water bath at different temperatures and time periods. All warm water treatments effectively controlled postharvest decay. Effect of different warm water treatments on anti-fungal activity was determined by means of TLC bioassay using *Penicillium digitatum* as test organism. Levels of fungal inhibitors causing clear inhibition were detected at 36 and 40 °C (1, 2.5 and 5 min) treatments, which correlated with levels of disease control. Only slight inhibition of *P. digitatum* could be detected from control treatments. Warm water enhanced the efficacy of *Bacillus subtilis* treatment and this integrated treatment was as effective as the chemical treatment in reducing decay although not as consistent.

* DE VILLIERS, E.E., VAN DYK, K., SWART, S.H., SMITH, J.H. & KORSTEN, L. 1997. Potential alternative decay control strategies for South African citrus packhouses. *Proceedings of the 8th Congress of the International Society of Citriculture* 1: 410-414.

INTRODUCTION

Citrus (*Citrus sinensis* (L.) Osbeck) is affected by various postharvest diseases, including *Alternaria* rot (*Alternaria citri* Ellis & N. Pierce), *Aspergillus* rot (*Aspergillus niger* Tiegh.), anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.), stem-end rot (*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.), sour rot (*Geotrichum citri-aurantii* (Ferraris) E.E. Butler), green mould (*Penicillium digitatum* (Pers.: Fr. Sacc.), blue mould (*Penicillium italicum* Wehmer), *Rhizopus* rot (*Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill), *Trichoderma* rot (*Trichoderma viride* Pers.: Fr.) (Brown & Eckert, 1989; Eckert & Brown, 1989) and whisker mould (*Penicillium ulaiense* Hsieh, Su & Tzean) (Holmes *et al.*, 1994). In the past, heat treatment was widely used in citrus packhouses to control postharvest diseases (Fawcett, 1922; Smoot & Melvin, 1963), but has since been replaced by chemical treatment of fruit with fungicide such as guazatine, imazalil and thiabendazole (Shachnai & Barash, 1982; Eckert & Ogawa, 1985; Pelser, 1988). However, the development of resistance by pathogens to fungicides (Bancroft *et al.*, 1984; Bus *et al.*, 1991; Eckert *et al.*, 1994) and the increased demand for fruit free of hazardous chemicals (Brown & Baraka, 1996), have revived interest in heat treatment (Rodov *et al.*, 1996).

Heat is usually applied to a fruit commodity via air or water (Barkai-Golan & Phillips, 1991). However, water is more efficient than air by virtue of its higher heat transfer coefficient (Jacobi *et al.*, 1993; Shellie & Mangan, 1993). Numerous investigations with heated water treatments have shown improved citrus postharvest disease control in many countries, including Israel (Rodov *et al.*, 1996), Italy (Dettori *et al.*, 1996), and the USA (Brown & Baraka, 1996). Hot water treatment of other fruit commodities such as nectarine, peach, avocado and mango substantially reduced postharvest decay (Spalding & Reeder, 1986; Plumbley *et al.*, 1993; Margosan *et al.*, 1997). In the papaya industry, immersion of fruit in hot water has been the principal postharvest treatment for decay control since 1964 (Akamine, 1967). Disinfectants (Smilanick *et al.*, 1995) and fungicides such as imazalil (Smilanick *et al.*, 1997) are often integrated with hot water treatments to enhance the efficacy of postharvest disease control.

Hot water dips reported in the literature for citrus fruit involve temperatures of 50-54 °C and exposure periods of 2-3 min (Dettori *et al.*, 1996), compared to the 40-45 °C of warm water baths in citrus packhouses. This chapter describes the optimisation of warm water treatment in

citrus packhouses for postharvest disease control, and integration of the heat treatment with application of *Bacillus subtilis*.

MATERIALS AND METHODS

Warm water supplemented with antagonist treatments

Preparation of inoculum: Citrus fruit naturally infected with *A. citri, C. gloeosporioides*, *D. natalensis*, *G. citri-aurantii, P. digitatum*, *P. italicum* and *T. viride*, were macerated, filtered through four layers of cheesecloth. The suspension was diluted to obtain a concentration of 10^5 *Penicillium* conidia ml^{-1} in experiment 1 and 10^6 conidia ml^{-1} in experiment 2.

Inoculation procedure: Valencia orange fruit provided by Letaba Estates (Tzaneen, Northern Province, SA) were pretreated, injured and inoculated as described in Chapter 2.

Preparation of antagonist: A mass culture of *B. subtilis* (B246) (obtained from L. Korsten, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, SA), known to inhibit growth of citrus postharvest pathogens (De Villiers *et al.,* 1993), was prepared according to Korsten *et al.* (1995). The antagonist concentration was determined using a Petroff Hauser counting chamber and ajusted to 10^7 cells ml^{-1}.

Treatment of fruit: Each treatment consisted of 800 fruit. The following treatments were applied 24 h after artificial inoculation: 1) untreated control; 2) 10 min dip in water at 40 °C; 3) 3 min dip in water containing the following chemicals: Deccotine (guazatine - 1000 ppm) (Rhône-Poulenc Agrichem SA (Pty) Ltd., Onderstepoort, SA), Fungazil (imazalil - 500 ppm) (Janssens Pharmaceutica, Olifantsfontein, SA) and Tecto (thiabendazole - 1000 ppm) (Logos Pharmaceuticals (Pty) Ltd., Midrand, SA); 4) 10 min dip in 40 °C water supplemented with B246 (10^7 cells ml^{-1}). After treatment fruit were air-dried, packed in cartons (80 fruit/box) and stored at 25 °C for 14 days. Percentage sound fruit was recorded for each box. Data were statistically analysed using ANOVA and Student's t-least significant differences calculated to determine differences between treatments ($P = 0.05$). The experiment was repeated and treatments included tap water dip and tap water supplemented with B246 (10^7 cells ml^{-1}).

Different temperature regimes

Valencia orange fruit obtained from Letaba Estates were artificially inoculated with a postharvest pathogen suspension containing 10^5 ml^{-1} *Penicillium* conidia as described above. Each treatment consisted of 480 fruit and included water dips at 23, 39, 43, 47, 51, 55 and 59 °C for 3, 6 or 9 min. An inoculated control not dipped in water was also included. After treatment fruit were air-dried, packed in boxes (80 fruit/box) and stored at 25 °C for 14 days. Percentage sound fruit was recorded per box for each treatment. Data were analysed using CSS Non-linear Estimations to calculate the relationship between treatments (temperature and time) and response (percentage sound fruit).

***In vivo* determination of optimal warm water temperature**

Pathogen preparation: *Penicillium digitatum* was isolated from decayed citrus fruit and cultured on potato-dextrose agar (PDA) (Biolab, Midrand) at 25 °C until sporulation occurred. A conidial suspension was prepared by washing the cultures with sterile distilled water and adding it to 25 l tap water to a final concentration of 10^5 conidia ml^{-1}.

Inoculation procedure: Valencia orange fruit were rinsed in chlorine (100 ppm) and air-dried. Wounds were made at five random sites on each fruit with an inoculation needle (0.5 mm diameter) to a depth of 2 mm. Fruit were subsequently dipped for 3 min in the conidial suspension of *P. digitatum*, air-dried and incubated overnight at 25 °C.

Warm water treatment: Treatments consisted of three replicates, each comprising five fruit. Inoculated fruit were dipped in a water bath at different temperatures (36, 40, 44, 48, 52 °C) for 1, 2.5, 5, 7.5 or 10 min at each temperature. Uninoculated and inoculated fruit not subjected to warm water treatment were included as controls. After treatment fruit were air-dried, packed in polystyrene containers, and stored at 25 °C for 21 days. The experiment was repeated twice.

Preparation of crude extracts: A crude extract was prepared from fruit of each treatment according to the procedure described by Ben-Yehoshua *et al.* (1987) and Kim *et al.* (1991). Flavedo tissue from mechanically-injured areas was excised with a scalpel. Samples (20 g in total) were extracted for 24 h with petroleum ether (1:4 v/v) (Biolab) at 25°C. The tissue was homogenised in a Waring-blender and the homogenate filtered *in vacuo* through Whatman No 1 filter paper. Residues were rehomogenised with the same volume of

petroleum ether and filtered again. Filtrates were concentrated *in vacuo* in a Buchi Rotovaporator-RE 120, and the extract collected with 6 ml dichloromethane (Biolab). All samples were stored in vials at 7 °C until further use.

Chromatography of crude extracts: Samples of the crude extracts (20 µl in total) were spotted on TLC plates (Kieselgel 60 F_{245}) (Sigma, Johannesburg, SA) and developed with toluene:ethyl acetate (1:1 v/v) as solvent. Scoparone (6,7-dimethoxycoumarin) (Department of Biochemistry, Rand Afrikaans University, Johannesburg) at a concentration of 1 000 ppm was used as standard. Developed plates were air-dried overnight at 25 °C and exposed to UV light. Fluorescent bands were compared to that of scoparone.

Detection of antifungal activity: Antifungal activity in TLC bands was bio-assayed directly on the plates (Kim *et al.*, 1991). TLC plates were sprayed Czapeck-Dox medium (Homans & Fuchs, 1970) containing a conidial suspension of *P. digitatum* (10^6 conidia ml^{-1}) harvested from 14-day-old cultures. Plates were placed in a moist tray and incubated at 25 °C for three days. Antifungal activity was indicated by the presence of inhibition zones around bands.

Optimisation of warm water treatments in the packhouse

Valencia orange fruit provided by Letaba Estates were artificially inoculated as described in Chapter 2 with a postharvest pathogen suspension containing 10^6 *Penicillium* conidia ml^{-1}. Each treatment consisted of 480 fruit and included warm water dips at 36, 38, 40 and 42 °C for 1, 2 or 3 min. A control consisting of mechanically injured fruit artificially inoculated but not subjected to warm water treatement, was also included. The chemical treatment consisted of a 3 min dip in tap water containing the following chemicals: Deccotine (guazatine - 1000 ppm), Fungazil (imazalil - 500 ppm) and Tecto (thiabendazole - 1000 ppm). After treatment, fruit were air-dried, packed (80 fruit/box) and stored at 25 °C. Percentage sound fruit per box was recorded for each treatment after 14 days. Data were analysed statistically using ANOVA and Student's t-least significant differences were calculated to determine differences between treatments (P = 0.05).

RESULTS

Antagonist integrated with warm water treatment

In both experiments, warm water treatment reduced decay significantly in comparison with the control (Fig. 1 & 2). Supplementation with B246 enhanced efficacy of the warm water treatment although not significantly. In the first experiment (Fig. 1) warm water, whether supplemented with antagonist or not, was as effective as the chemical treatment. However, in the second experiment (Fig. 2) the chemical dip yielded significantly more sound fruit than any of the other treatments.

Different temperature regimes

Exposure for 3, 6 or 9 min to temperatures of 39 °C or above increased the percentage sound fruit to more than 75 % compared to the 60 % sound fruit of the non-heat-treated control (Fig. 3).

***In vivo* determination of optimum warm water temperature**

Effect of heat treatment on decay development: Green mould development was delayed for longer than 14 days in fruit exposed for 1, 2.5 or 5 min to 36 and 40 °C in comparison to control fruit not heat-treated. These warm water treatments had no deleterious physiological effects on the fruit. Fruit treated at 52 °C for 2.5 min or longer showed no decay, but became hard and developed off-odours during storage. Fruit of the remaining treatments rotted within 7-14 days of storage.

Presence of induced compounds: Exposure of developed TLC plates to UV light verified the presence of scoparone when bands were compared to that of the scoparone standard (Rf 0.18). Scoparone was detected in the crude extracts of all treatments, including the controls. Crude extracts of the 36 and 40 °C treatments showed more distinct bands (Rf 0.20) than those of other treatments. Another band (Rf 0.13), not corresponding with that of scoparone, was observed on plates prepared from crude extracts of 36 °C for 1, 2.5 and 5 min treatments.

Antifungal activity of induced compounds: Antifungal activity of scoparone varied between treatments (Table 1). Clear inhibition of *P. digitatum* was evident with extracts of fruit treated at 36 or 40 °C for 1, 2.5 and 5 min (Fig. 4). Extracts from fruit of all other heat treatments showed either slight or no inhibition of the pathogen. *Penicillium digitatum* was inhibited only slightly by flavedo extracts of fruit from both control treatments (Fig. 3). The unknown compound from extracts of fruit heat-treated at 36 °C for 1, 2.5 and 5 min also

caused inhibition of the fungus.

Optimisation of warm water treatments in the packhouse

All treatments significantly reduced decay in comparison with the control (Fig. 5). Chemical treatment nevertheless was significantly more effective than the warm water treatments.

DISCUSSION

Amongst the technologies available to extend shelf-life of fruit, pre-storage heat treatment appears to be one of the most promising for postharvest decay control (Couey, 1989). This study demonstrated that warm water treatment of Valencia oranges at 36 or 40 °C for 1, 2.5 or 5 min, can provide effective control of *P. digitatum in vivo*. These temperatures are not high enough to kill conidia of *P. digitatum* and fall in the range supporting growth of the organism (Domsch *et al.*, 1980). From the results obtained it can be deducted that conidia were probably inactivated at high temperatures. Barkai-Golan *et al.* (1969) observed 30% conidial survival after heat treatment for 5 min at 50 °C, while Dettori *et al.* (1996) observed that dip treatment at 50-52 °C for 2 min caused only a delay in conidial germination for a period of up to 48 h. Since neither scoparone nor the unidentified fungistatic compound was produced at temperatures higher than 44 °C, the effect of heat treatment at temperatures of 44 °C and lower therefore seems to be due to respons of the host. It is known that wounding and pathogen infection of the citrus peel can induce resistance (De Lange *et al.*, 1976; Brown & Barmore, 1983). In addition, heat treatment enhances this effect, causing even low pathogen infestation to successfully elicit resistance (Kim *et al.*, 1991). This resistance comprises two mechanisms. Firstly, alterations to the host cell wall or other structures could provide a more efficient barrier against pathogen penetration e.g. lignification of wounded areas (Brown & Barmore, 1983). Secondly, antifungal compounds such as scoparone are produced in the host tissue (Kim *et al.*, 1991; Ben-Yehoshua *et al.*, 1992).

In this study, mechanically injured-inoculated fruit showed an increase in decay and insignificant production of scorparone compared to mechanically injured-inoculated fruit heat-treated at 36 or 40 °C. Ben-Yehoshua *et al.* (1987) and Kim *et al.* (1991) also reported that low concentrations of scoparone accumulated as a result of mechanical injury and inoculation, but substancially increased (20-fold) following heat treatment (36 °C for 3 days). However,

antifungal activity did not increase following heat treatment alone.

Commercially, heat treatment may be most useful when applied in tandem with an additional postharvest decay control measures such as biocontrol (Brown & Baraka, 1996; Shellie & Skaria, 1998). Huang *et al.* (1995) demonstrated that biocontrol of green mould with *Pseudomonas glathei* can be enhanced when heat is applied to retard conidial germination of *P. digitatum* while simultaneously stimulating multiplication of the bacterium. Although heat treatment enhanced the efficacy of *B. subtilis* (B246) in the present study, the enhancement was not significant and furthermore not comparable to decay control achieved with chemical treatment. This is probably due to the high inoculum level (10^5 and 10^6 conidia ml^{-1}) and low antagonist concentration (10^7 cells ml^{-1}) (Chapter 2). Another possibility is that effective biocontrol may dependent on successful establishment of the antagonist at the wound site prior to challenge by the pathogen (Huang *et al.*, 1995). Arras (1996) observed scoparone accumulation 19 times higher than in untreated inoculated fruit when the antagonist *Candida famata* (FC Harrison) SA Meyer & Yarrow was inoculated 24 h prior to *P. digitatum*, and only four times higher if inoculated 24 h after the pathogen.

In conclusion, there is renewed interest in heat treatment for disease control. Advances in understanding the metabolic responses of citrus fruit to heat could lead to more effective and rational use of heat treatment. This study demonstrated that packhouse dipping of fruit in water for 1-5 min at the relatively low temperatures of 36 and 40 °C reduced postharvest decay whilst maintaining fruit quality. Heat treatment has the further advantage of ease of implementation in South African citrus packhouses and the absence of chemical residues.

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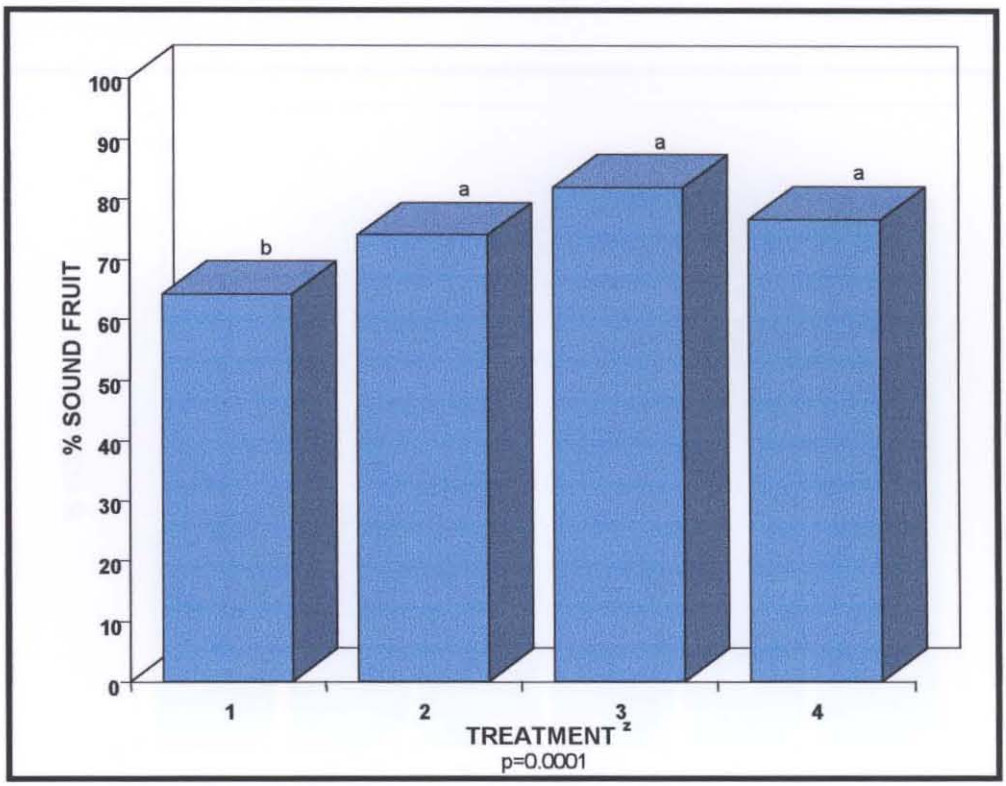
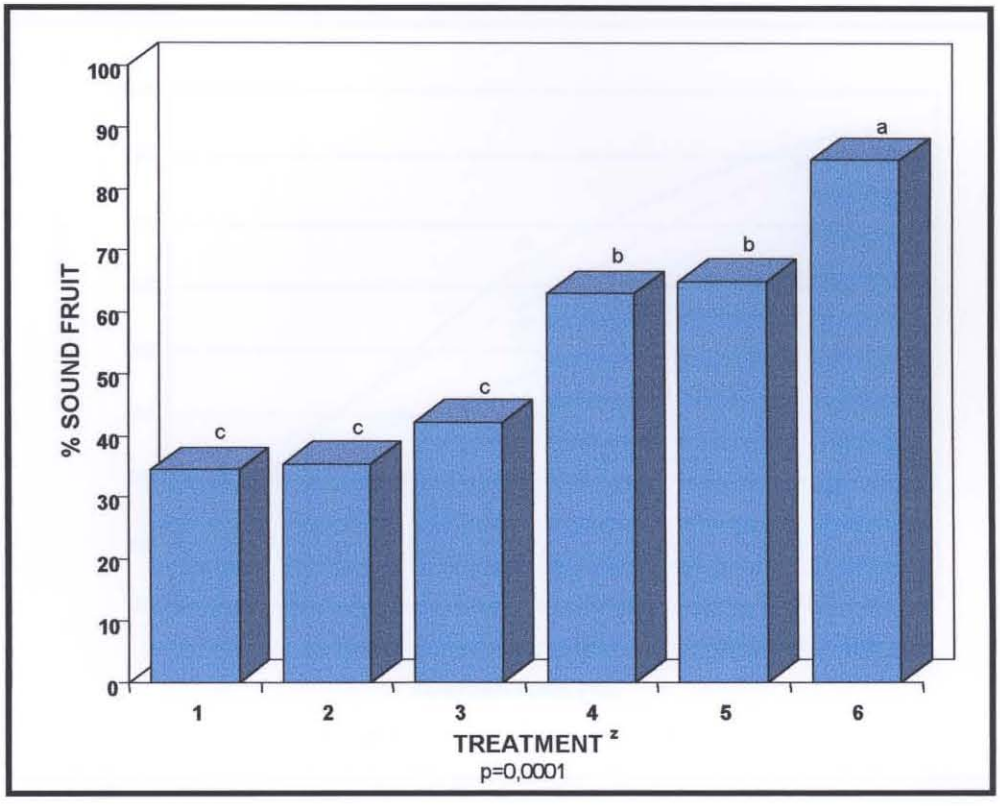


Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0.05).

[z] Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.4 x 10^5 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day. Treatments included 1) Untreated control; 2) Warm water (40 °C) dip for 7 min; 3) Warm water (40 °C) supplemented with *Bacillus subtilis* (B246) (1 x 10^7 cells ml^{-1}) dip for 7 min; 4) Chemical dip (1 000 ppm guazatine, 500 ppm imazalil, 1 000 ppm thiabendazole) for 3 min.

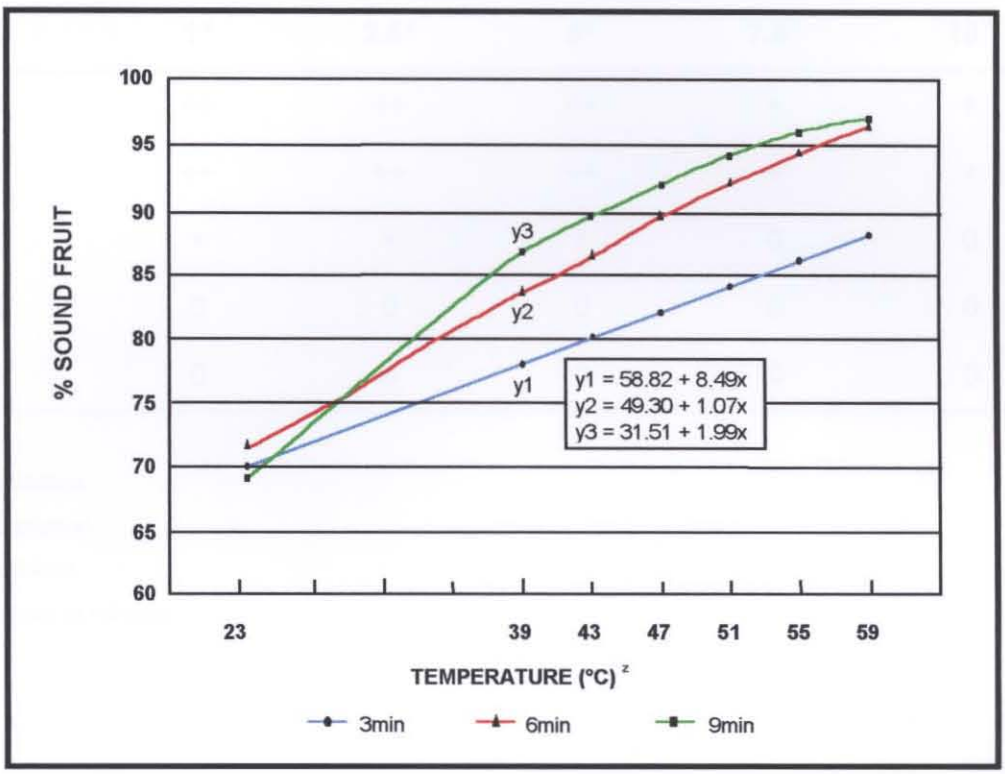
Fig. 1 Effect of chemical, warm water and warm water supplemented with *Bacillus subtilis* dip treatments on postharvest deterioration of Valencia orange fruit (First experiment).



Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0,05).

[z] Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.1 x 10^6 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day. Treatments included 1) Untreated control; 2) Tap water dip for 7 min; 3) Tap water supplemented with *Bacillus subtilis* (B246) (1 x 10^7 cells ml^{-1}) dip for 7 min; 4) Warm water (40 °C) dip for 7 min; 5) Warm water (40 °C) supplemented with B246 (1 x 10^7 cells ml^{-1}) dip for 7 min; 6) Chemical dip (1 000 ppm guazatine, 500 ppm imazalil, 1 000 ppm thiabendazole) for 3 min.

Fig. 2 Effect of chemical, warm water and warm water supplemented with *Bacillus subtilis* (B246) dip treatments on postharvest deterioration of Valencia orange fruit (Second experiment).



[z] Temperature treatments were applied to mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 1.2×10^5 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day.

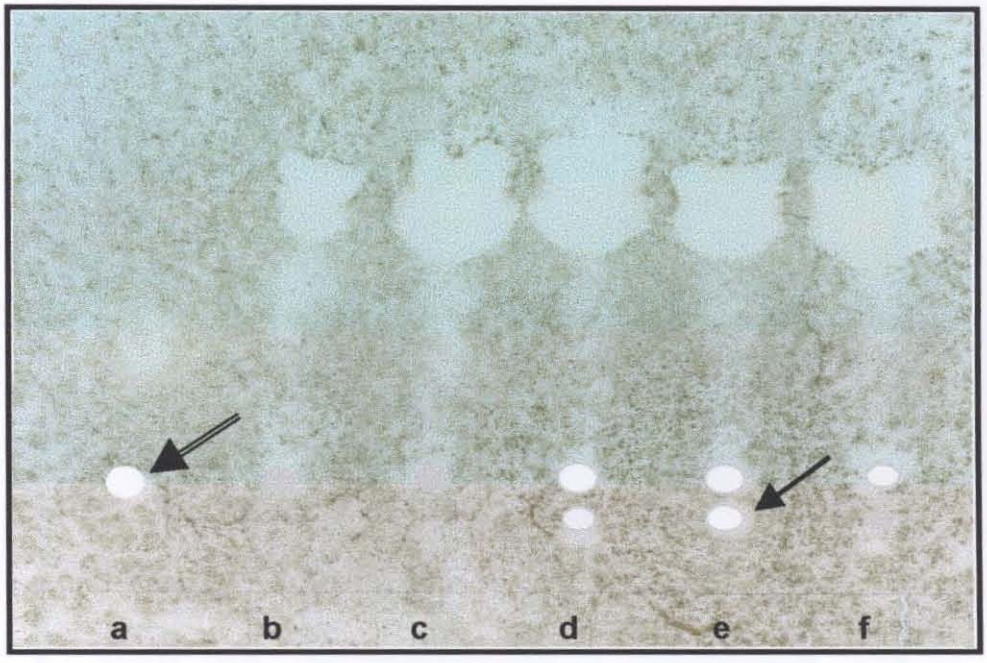
Fig. 3 Effect of temperature and exposure time on postharvest deterioration of Valencia orange fruit.

Table 1 Scoparone activity in Valencia orange fruit exposed for different periods to different temperatures, determined by means of a TLC-bioassay with *Penicillium digitatum*

Temperature	**Inhibition of *P. digitatum* [y]**				
(°C)	**1 [z]**	**2.5 [z]**	**5 [z]**	**7.5 [z]**	**10 [z]**
36	++	++	++	+	+
40	++	++	++	+	+
44	+	+	+	0	0
48	0	0	0	0	0
52	0	0	0	0	0

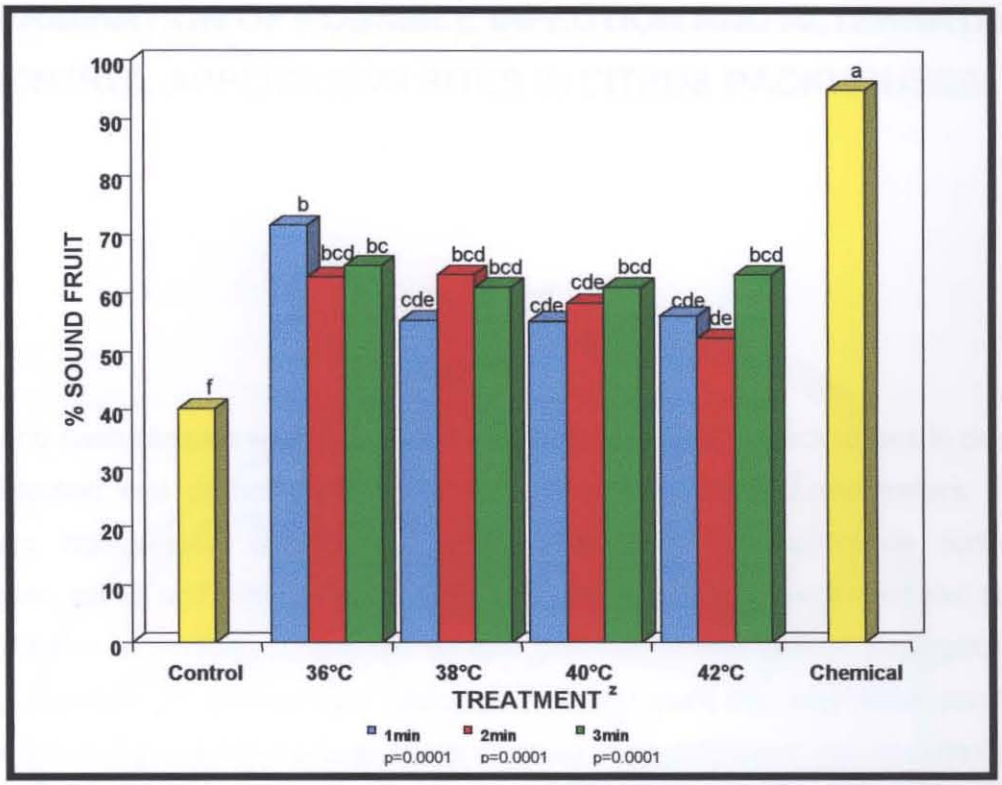
[y] ++ Clear inhibition
\+ Slight inhibition
0 No inhibition

[z] Exposure times in minutes



za	Scoparone
b	Control (mechanically injured)
c	Control (mechanically injured fruit + inoculated with *P. digitatum* (10^6 conidia ml^{-1}))
d	Mechanically injured + inoculated with *P. digitatum* + 36°C warm water dip for 1 min
e	Mechanically injured + inoculated with *P. digitatum* + 36°C warm water dip for 2.5min
f	Mechanically injured + inoculated with *P. digitatum* + 36°C warm water dip for 5 min
←	Scoparone
←	Unknown antifungal compound

Fig. 4 Inhibition of *Penicillium digitatum* on a TLC plate run with petroleum ether extracts of Valencia orange fruit dipped for different time periods in water at 36 °C.



Bars not sharing a common letter differ significantly according to Student's t-LSD (P=0.05).

[y] Control and chemical treatment data are included for comparison.

[z] Treatments consisted of mechanically injured fruit which were artificially inoculated by dipping in 25 l inoculum of *Alternaria citri*, *Colletotrichum gloeosporioides*, *Geotrichum citri-aurantii*, *Lasiodiplodia theobromae*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, containing 2.3 x 10^5 *Penicillium* conidia ml^{-1}. Fruit were incubated overnight and treated the following day. Chemical treatment involved dipping fruit for 3 min in guazatine (1 000 ppm), imazalil (500 ppm) and thiabendazole (1 000 ppm).

Fig. 5 Effect of different warm water regimes on postharvest deterioration of Valencia orange fruit.

Chapter 4

DETERMINATION OF POSSIBLE INFECTION AND ALTERNATIVE CONTROL APPLICATION SITES IN CITRUS PACKHOUSES

ABSTRACT

Air, water and swab samples were collected in six South African citrus packhouses to determine possible infection and control application sites. Sampling sites included trailers, chlorine baths/sprays, high-pressure water sprays, warm water baths, wax application, sorting and packing areas, pallets and train carriages. Varioous surfactants/disinfectants were also tested *in vitro* for inhibition of *Penicillium digitatum* conidial germination and growth. *Aspergillus niger*, *Penicillium digitatum*, *P. italicum* and *Rhizopus stolonifer* were the only citrus postharvest pathogens detected in the packhouses. Large numbers of *Cladosporium* spp. and *Trichoderma* spp. were present in all packhouses. Crates/trailers and dip tank water were identified as the main sites for accumulation of fungi, indicating possible unsanitary conditions. High species densities were also associated with high-pressure water sprays, first sorting, warm water baths, chemical baths, wax application, packing areas and train carriages. Depending on the inoculum level, several surfactants/disinfectants in this study inhibited *in vitro* germination and growth of *P. digitatum* conidia. The most effective products were Multichlor and Tronic for *P. digitatum* inoculum level of 10^3 conidia ml^{-1} and Armoblem, Ecosanitizer (Handwash + Low foam), Frigate, G49, QA5DP, Terminator and Tronic for inoculum level of 10^5 conidia ml^{-1}.

INTRODUCTION

World-wide, citrus is a major agricultural product of considerable economic value. In terms of annual tonnage produced, citrus is the second most important fruit crop produced in South Africa (Abstracts of Agricultural Statistics, 2000). Due to the large number of postharvest diseases (*Alternaria* rot, anthracnose, *Aspergillus* rot, blue mould, green mould, *Rhizopus* rot, sour rot, stem-end rot, *Trichoderma* rot, whisker mould) affecting citrus (Brown & Eckert, 1989; Eckert & Brown, 1989; Holmes *et al.*, 1994), citrus producers rely extensively on fungicides for protecting their crops (Shachnai & Barash, 1982; Eckert & Ogawa, 1985; Pelser, 1988). Notwithstanding, losses still occur and can represent up to 6 % of export consignments (A. Heitmann, Capespan (Pty) Ltd., Cape Town, SA). Losses are particularly severe when occurring in the market place, as they include the cost of sorting, treating, packing, cooling, storing and transportation (Sommer, 1982; Convey *et al.*, 1992). It is well-known that careless harvesting and handling practices, along with inoculum in the fruit environment (soil, debris, plant surface and air), are the main factors involved in the initiation of disease (Stange & Eckert, 1994; Di Martino Aleppo & Lanza, 1996). Spores of postharvest pathogens are produced on perishing fruit as well as on the surface of soil/debris in bins, and are disseminated by air currents, water dips and fruit-handling equipment to sound fruit in the packhouse (Gardner *et al.*, 1986; Spotts & Cervantes, 1989, 1992). Usually, primary infection is initiated through a wound in the pericarp (Sommer, 1982; Brown & Eckert, 1989; Eckert & Brown, 1989).

Several fungicides can been used to control postharvest decay in citrus, including guazatine, imazalil and thiabendazole (Shachnai & Barash, 1982; Eckert & Ogawa, 1985; Pelser, 1988; Stange & Eckert, 1994). However, resistant strains of the postharvest pathogens have emerged under selection pressure of fungicides and reduced the efficacy of these compounds (Bancroft *et al.*, 1984; Bus *et al.*, 1991; Eckert *et al.*, 1994). The build-up of fungicide-resistant strains of pathogenic fungi such as *Penicillium* in packhouses can greatly increase the incidence of decay during storage and transportation (Gardner *et al.*, 1986), with a subsequent reduction in profitability of the industry.

The principle strategies advocated for combating the resistance problem are 1) combining

two or more fungicides with different modes of action, 2) rotating fungicides, 3) use of non-selective compounds, and 4) isolation and destruction of fungal spores through efficient packhouse design and regular packhouse sanitation (Bancroft *et al.*, 1984; Spotts & Cervantes, 1984; Gardner *et al.*, 1986; Stange & Eckert, 1994). Efficacy of the first two strategies is reduced in practice due to the limited number of fungicides available, the frequency with which pathogens develop resistance (Bancroft *et al.*, 1984), and growing public concern over the health and environmental hazards associated with fungicide use (Norman, 1988).

In this study, the occurrence of postharvest pathogens was investigated in various South African citrus packhouses to determine at which sites they are abundant and may cause infection. The disinfecting ability of different surfactants/disinfectants was also evaluated *in vitro* for possible future inclusion in fruit surface disinfestation and packhouse sanitation procedures.

MATERIALS AND METHODS

Packhouse surveys

The presence of fungal populations at each stage of fruit handling was determined in six commercial citrus packhouses in South Africa. Packhouse 1 (Citrusdal) (Fig. 1), packhouse 2 (Patensie) (Fig. 2), packhouse 3 (Addo) (Fig. 3) and packhouse 4 (Fort Beaufort) (Fig. 4) were monitored once in September 1996, and packhouse 5 (Nelspruit) (Fig. 5) in August 1996. Packhouse 6 (Tzaneen) (Fig. 6) was monitored in August and September 1996. Chlorine, fungicide and wax treatments used in all packhouses were according to Capespan regulations (chlorine - 100 ppm; guazatine - 1000 ppm; 2,4-D - 500 ppm; imazalil - 500 ppm; sodium-orthophenyl-phenol (SOPP) - 1000 ppm; thiabendazole (TBZ) - 1000 ppm and wax - Citrashine). Sampling sites and procedures used are described in Table 1. At each site a sample was taken using three replicate petri dishes containing potato-dextrose agar (PDA) (Biolab, Midrand, SA) supplemented with 0.01% chloramphenicol (Chlorcol, Premier Pharmaceutical Co Ltd., Bryanston, SA). Petri dishes were placed in a coolbox, transported

to the laboratory and incubated at 25 °C for 5-10 days. Colonies were counted and species density and diversity calculated for each site and packhouse.

Plates were examined for colonies resembling those of postharvest citrus pathogens. Candidate colonies were sub-cultured on PDA, incubated at 25°C for 10 days under a near-ultraviolet light with a 12-h photoperiod and identified. Fungi that occurred dominantly in each packhouse were also isolated and identified.

Disinfecting qualities of surfactants/disinfectants

Surfactants/disinfectants were evaluated for antifungal acitivity according to the rapid evaluation method used by Wilson *et al.* (1997). Conidial suspensions of *Penicillium digitatum* (Pers. ex St.:am) Sacc. (10^3 and 10^5 conidia ml^{-1}) were prepared by washing PDA cultures of the pathogen with sterile distilled water. Each of the surfactants/disinfectants in Table 2 was added to each conidia suspension to a concentration of 0.5, 1, 5, 10, 25 and 50 ppm of the a.i. One hundred microlitres of each surfactant/conidial suspension was pipetted into a 200 µl well in a 96 multi-well micro-titration plate with lid (Corning, New York). One row in each plate was left blank; one contained only the conidial suspension and one a surfactant/disinfectant concentration series as checks. Each treatment was replicated twice on three different plates. Direct microscopical observations of spore germination were made after one week. After incubating the plates for 24 h and 48 h at 25 °C, the density of fungal growth in each well was measured with a Tutertek Multiskan Plus microplate reader (492 nm filter). Background readings from the checks were subtracted from readings taken of wells with surfactants/disinfectants and *P. digitatum* conidial suspensions. Data were statistically analysed by ANOVA and surfactants/disinfectants compared using Student's t-least significant differences test.

RESULTS

Packhouse surveys

High species densities were detected in samples from crates/trailers (packhouse 1, 2, 3, 5, 6 – 1st and 2nd sampling); chlorine baths (packhouse 1, 2 and 6 – 1st and 2nd sampling); high pressure water sprays (packhouse 1, 2, 3 and 6 – 1st and 2nd sampling); 1st sorting areas (packhouse 3, 5 and 6 – 1st and 2nd sampling); chemical baths (packhouse 1, 2, 3, 4 and 5);

warm water bath/sprays (packhouse 2, 5 and 6 – 1st sampling); conveyor belts (packhouse 5); wax application (packhouse 3, 4, 5 and 6 – 1st and 2nd sampling); steel rollers (packhouse 5); fans (packhouse 3); 2nd sorting areas (packhouse 1 and 3); 3rd sorting areas (packhouse 6 – 1st sampling); fruit sizing (packhouse 3); packing areas (packhouse 1, 2, 4, 5 and 6 – 1st sampling); train carriages (packhouse 6 – 2nd sampling) and air samples 3 and 5 (packhouse 3) (Table 3).

Aspergillus niger Tiegh., *P. digitatum*, *Penicillium italicum* Wehmer and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill were the only postharvest pathogens detected in the citrus packhouses surveyed. Large numbers of *Cladosporium* spp. and *Trichoderma* spp. were present in all packhouses. *Aspergillus niger* was the most dominant pathogen in air samples 1, 2, 3 and 4 (packhouse 1); 1, 2 and 4 (packhouse 2); 1 and 5 (packhouse 3) and 3, 4, 5 and 6 (packhouse 6 – 1st sampling), as well as samples collected from the 1st sorting area (packhouse 3); wax application areas (packhouse 3, 5 and 6 – 1st sampling); fruit sizing (packhouse 3); 3rd sorting area and packing area (packhouse 6 – 1st sampling). *Penicillium digitatum* occurred in samples taken from crates/trailers (packhouse 2, 3 and 6 – 1st and 2nd sampling); high pressure water spray (packhouse 1); 2nd sorting area (packhouse 1); chemical bath (packhouse 2); wax application (packhouse 1 and 6); steel rollers (packhouse 2 and 3); train carriage (packhouse 6 – 2nd sampling) and air samples at points 1, 4 and 6 (packhouse 1); 2 (packhouse 2); 1, 3 and 5 (packhouse 3); 1 (packhouse 6 – 1st sampling) and 6 (packhouse 6 – 2nd sampling). *Rhizopus stolonifer* and *P. italicum* were abundant in air samples 5 and 6 from packhouse 1, respectively, but were detected in low numbers at the other packhouses.

Disinfecting qualities of surfactants/disinfectants

Readings after 24-h showed Multichlor to be the most inibitory product towards germination and growth of the 10^3 conidia ml^{-1} inoculum of *P. digitatum*, albeit not significantly more so than BP Agripon, Ecosanitizer (Hand wash), KOCl, QA5DP and Tronic (Table 4). After 48-h exposure of the same inoculum, Tronic and Multichlor exhibited the lowest absorbency readings.

BP Agripon was the most effective surfactant in limiting germination and growth, of the 10^5 conidia ml^{-1} inoculum after 24 h. After 48 h exposure, Ecosanitizer (Hand wash), Frigate and G49 showed the lowest absorbency readings, although not significantly lower than

Armoblem, Ecosanitizer (Low foam), QA5DP, Terminator and Tronic. After one week, no or little conidial germination or growth were evident at all concentrations of Biotane, Ecosanitizer (Hand wash and Low foam), Formula 10, Frigate, G49, Multichlor, OA5DP and Terminator. Latron and Tronic prevented germination at only the higher concentrations (10, 25 and 50 ppm). All other surfactants/disinfectants supported prolific growth of *P. digitatum*.

DISCUSSION

Green mould, caused by *P. digitatum*, is the main cause of postharvest losses in the citrus industry (Gardner *et al.*, 1986; Stange & Eckert, 1994; Smilanick *et al.*, 1995). In this study, *P. digitatum* was present in all packhouses and at some sites in high numbers. The occurrence of *P. digitatum* in the chemical bath of packhouse 2 implicates a possible build-up of pathogen resistance or that the bath has not been replenished. Bancroft *et al.* (1984), Gardner *et al.* (1986) and Bus *et al.* (1991) reported resistance of *P. digitatum* and *P. italicum* to benomyl, TBZ and imazalil, thus emphasising the need for investigating the existence of fungicide resistance. With the exception of *P. italicum* and *R. stolonifer*, which occurred occasionally and *A. niger*, dominant in all packhouses, no other postharvest pathogens were apparent. Pathogens such as *Alternaria citri* Ellis. & N. Pierce (*Alternaria* rot), *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (anthracnose), *Lasiodiplodia theobromae* (Pat.) Griffon. & Maubl. (stem-end rot) and *Geotrichum citri-aurantii* (Ferraris) E.E. Butler (sour rot) mostly infect in the field and remain latent until fruit ripening (Brown & Eckert, 1989; Eckert & Brown, 1989). Therefore, the possibility exists that these pathogen populations do not build up to detectable levels in the packhouse. Another explanation could be that, due to their low numbers, they are easily overgrown by dominant organisms when enumerated on plates as in this study.

Consistent with results from previous studies (Gardner *et al.*, 1986; Spotts & Cervantes, 1986, 1992, 1993; Van Dyk *et al.*, 1997a) crates/trailers and dip tank water were identified as the main sites for accumulation of fungi at high enough levels to indicate unsanitary conditions. High numbers of fungi were also detected at high-pressure water sprays, 1st sorting areas, warm water baths, chemical baths, wax application, packing areas and train carriages. Low numbers of fungi in the wax of packhouse 2 possibly indicates a recent replacement of wax as a standard packhouse procedure, while increase in numbers from the

train carriage sample of packhouse 6 (1st and 2nd sampling) suggest a build-up of inoculum.

Sampling methods used in this study did not facilitate accurate determination of species diversity and especially species density. Agar plates were often overgrown, making it difficult to distinguish between individual colonies and to accurately calculate species diversities and densities. Similar problems where encountered by Van Dyk *et al.* (1997a) when monitoring avocado packhouses for the presence of postharvest pathogens. Furthermore, low counts obtained with air samples indicate an insufficient sampling size, contrary to Gardner *et al.* (1986) who determined *Penicillium* spore levels in citrus packhouses just by exposing agar plates for 1 min. Values for species diversity and density should therefore be seen as indicative rather than quantitative. A need exists for optimisation of sampling procedure, including evaluation of methods involving moistened swabs, selective media, dilution plating, etc. (Gardner *et al.*, 1986; Robbs *et al.*, 1996). Only then will it be possible to obtain an accurate assessment of the distribution of pathogens within citrus packhouses.

Although all packhouse equipment, floors and walls should be sanitised routinely (Gardner *et al.*, 1986; Beuchat, 1995), more regular sanitation of the problem areas refered to above is advisable. In food processing and dairy industries, surfactants/disinfectants are used on a routine basis to reduce inoculum of spoilage organisms (Park *et al.*, 1991). Apart from evaluation of a quaternary ammonium compound to sanitise bins (Bancroft *et al.*, 1984), chlorine dioxide for water sanitation (Lesar, 1997) and ethanol for postharvest decay control (Smilanick *et al.*, 1995), surfactants/disinfectants were not previously evaluated for their potential to disinfect citrus fruit surfaces and packing equipment.

Depending on the inoculum level, several surfactants/disinfectants in this study inhibited germination and growth of *P. digitatum* conidia *in vitro*. The most effective compounds were Multichlor and Tronic for an inoculum of 10^3 conidia ml^{-1} and Armoblem, Ecosanitizer (Handwash + low foam), Frigate, G49, QA5DP, Terminator and Tronic for an inoculum of 10^5 conidia ml^{-1}. Of these, Terminator has previously been evaluated for its disinfecting properties on avocado (Van Dyk *et al.*, 1997b), mango (De Villiers & Korsten, 1996), as well as on pome and stone fruit (Zeneca Agrochemicals, Users pamphlet). Success was obtained with pome and stone fruit, while anthracnose on avocado decreased slightly, but the compound had no effect on anthracnose and soft brown rot of mango.

Although not one of the most effective compounds in this study, ethanol was evaluated with success on avocado (Van Dyk *et al.*, 1997b), citrus (Smilanick *et al.*, 1995), mango (De Villiers & Korsten, 1996) and stone fruit (Feliciano *et al.*, 1992; Margosan *et al.*, 1997). Boshoff *et al.* (1995) found a reduction in anthracnose incidence using ethanol, but an increase in lenticel damage, probably due to the high concentration used (76% v/v). However, Smilanick *et al.* (1995) reduced green mould on citrus with a heated solution of ethanol (10% v/v at 45°C for 150s) without concomitant lenticel damage. Of the other less effective surfactants/disinfectants used in this study only Tween 80 on apples, and KOCl on avocado (Van Dyk *et al.*, 1997b) and mango (De Villiers & Korsten, 1996) have previously been evaluated for disinfecting abilities, but with no success.

Surfactants/disinfectants selected from the above, particularly Tronic, should be screened under packhouse conditions for disinfection of fruit and packing equipment. Integration with biological and warm water treatments could be included as both these procedures were successful on mango (De Villiers & Korsten, 1996) and citrus (Smilanick *et al.*, 1995).

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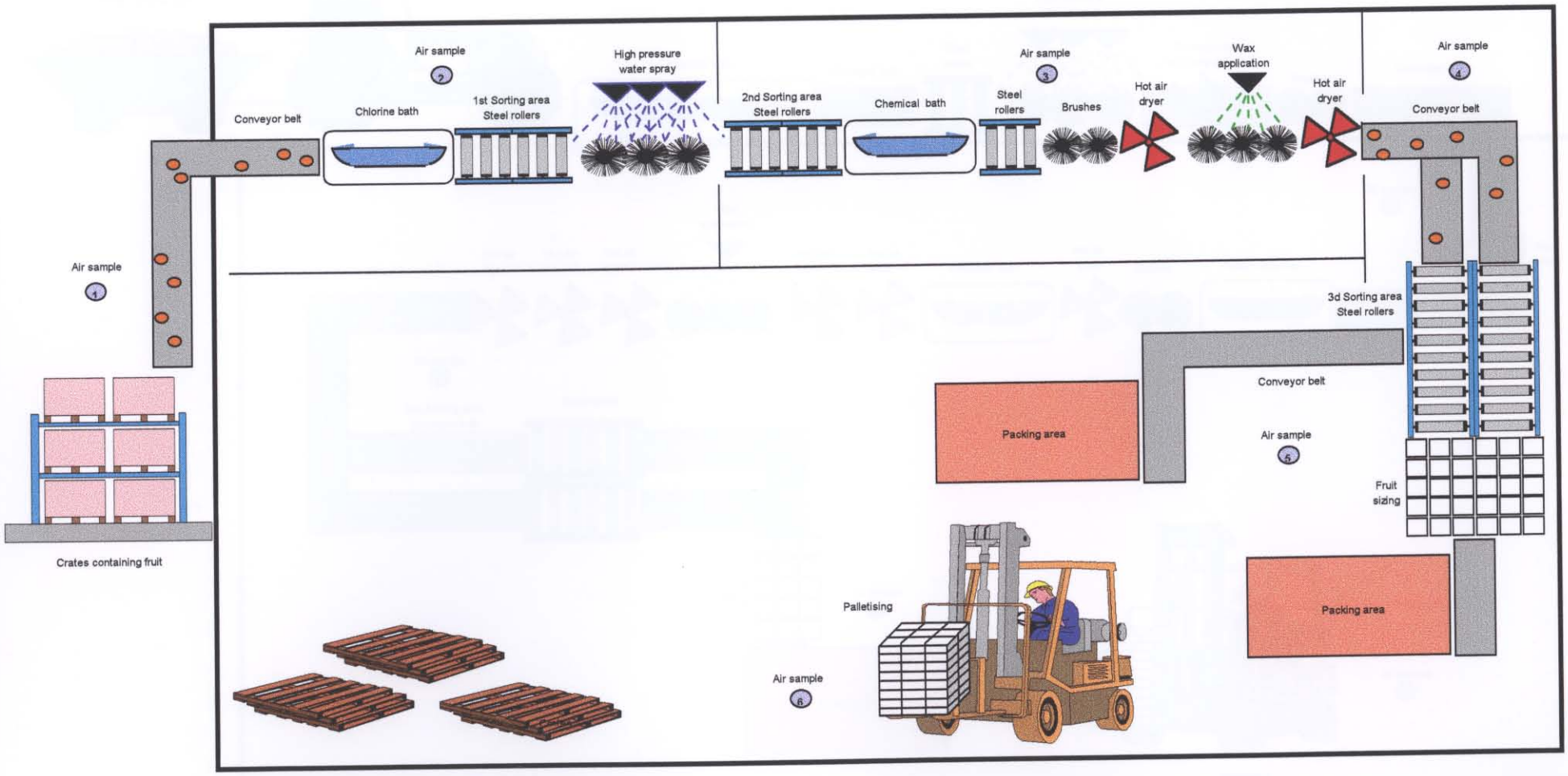

Fig. 1 Schematic representation of Packhouse 1 in Citrusdal.

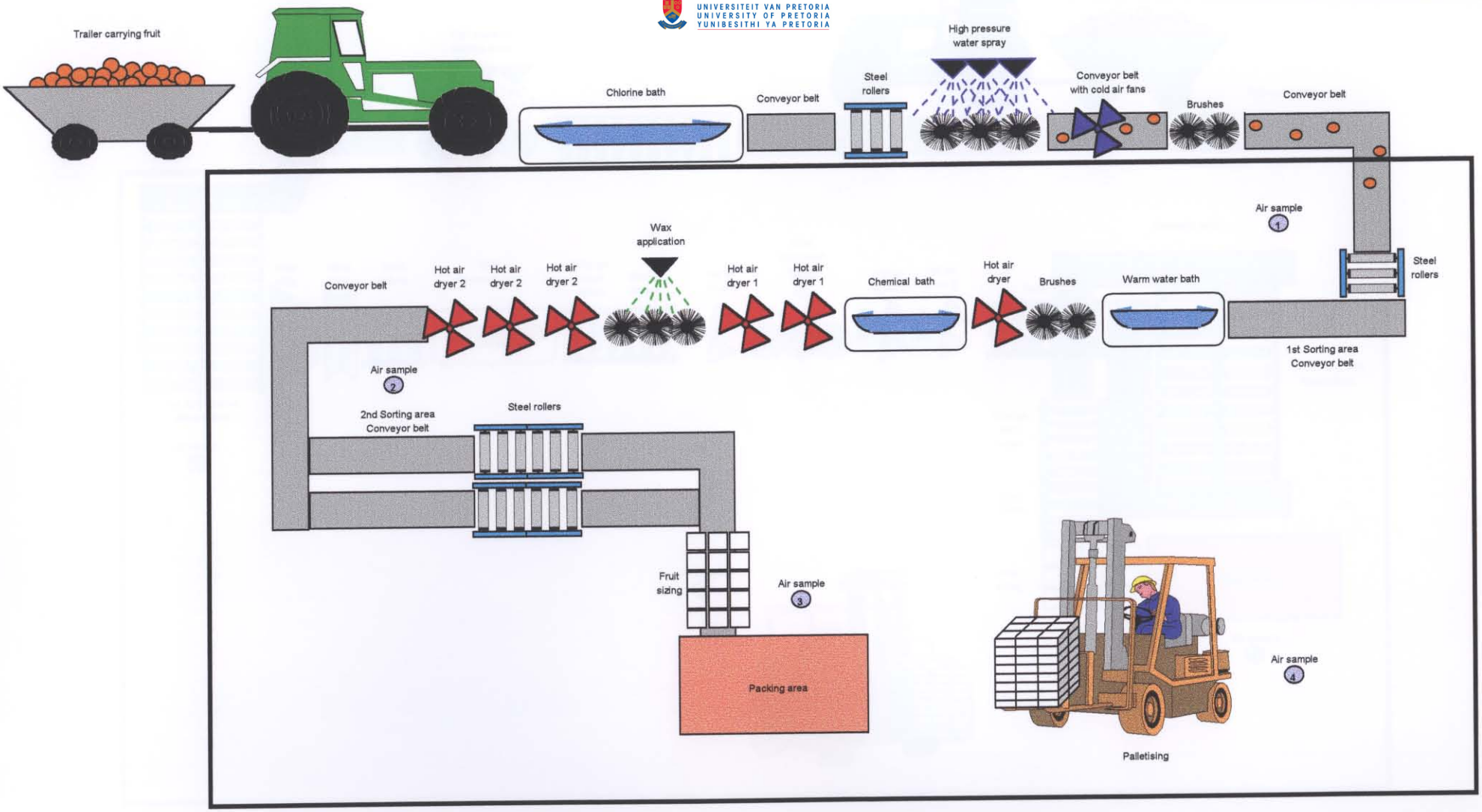

Fig. 2 Schematic representation of Packhouse 2 in Patensie.

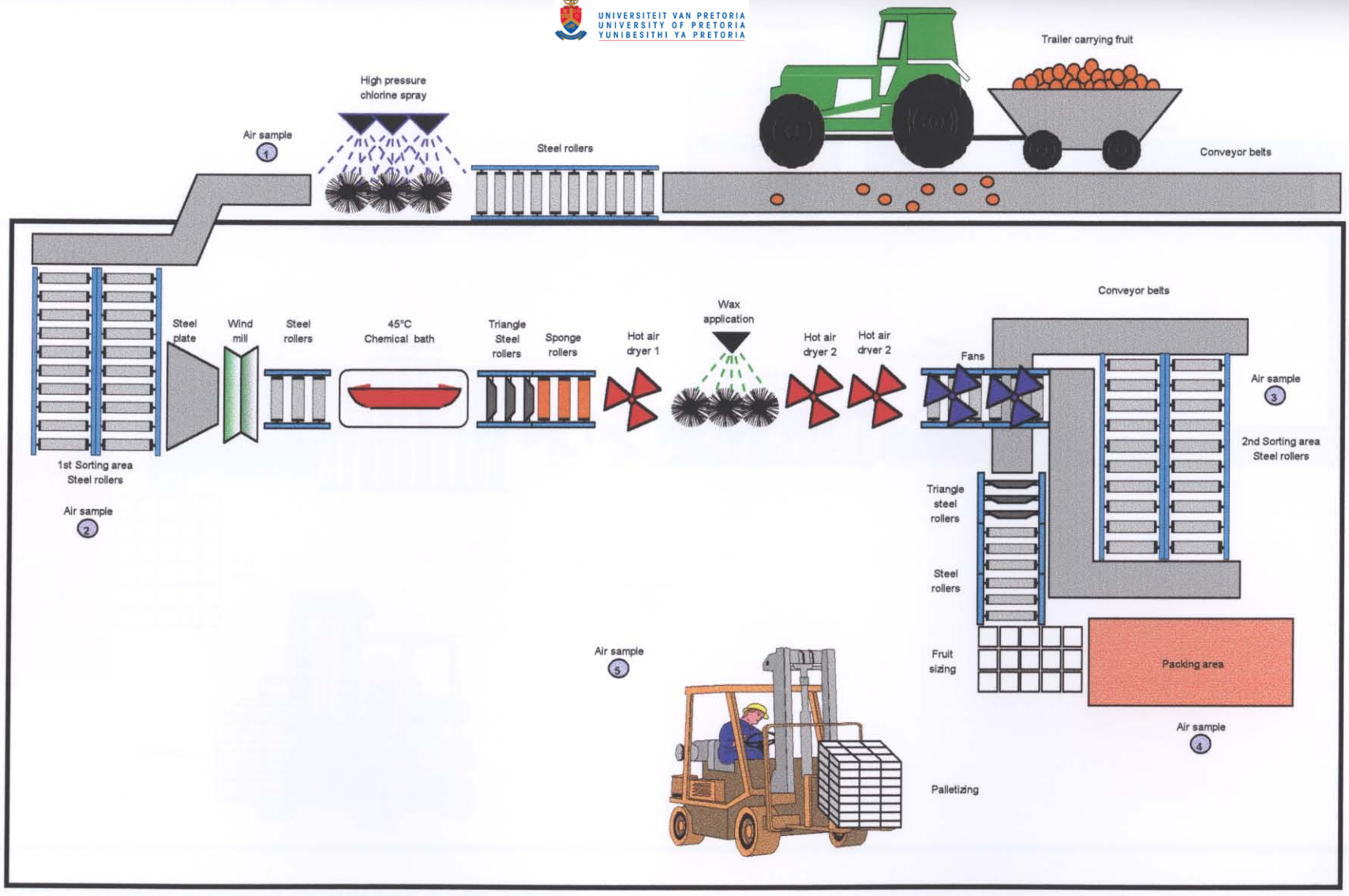

Fig. 3 Schematic representation of Packhouse 3 in Addo.

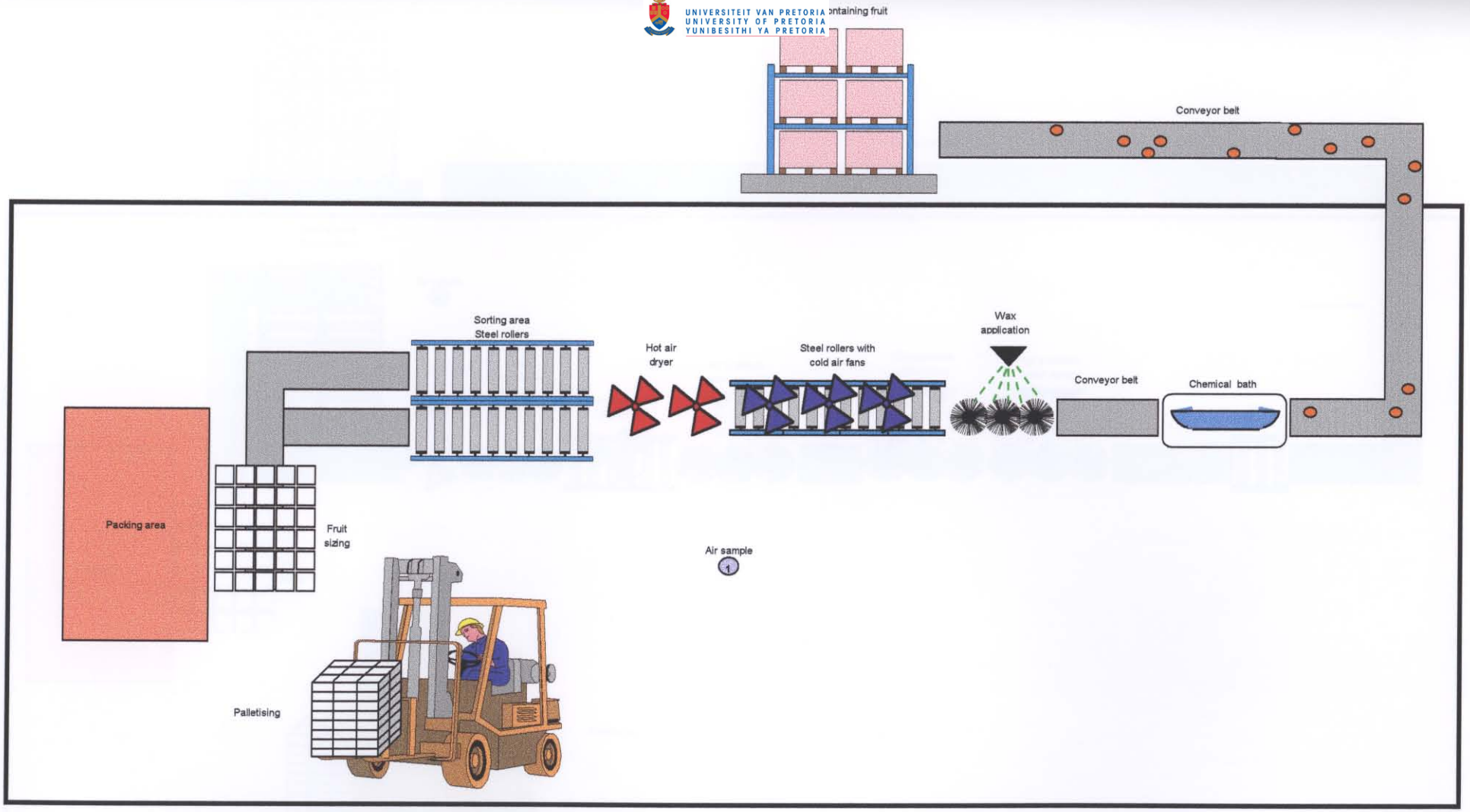

Fig. 4 Schematic representation of Packhouse 4 in Fort Beaufort.

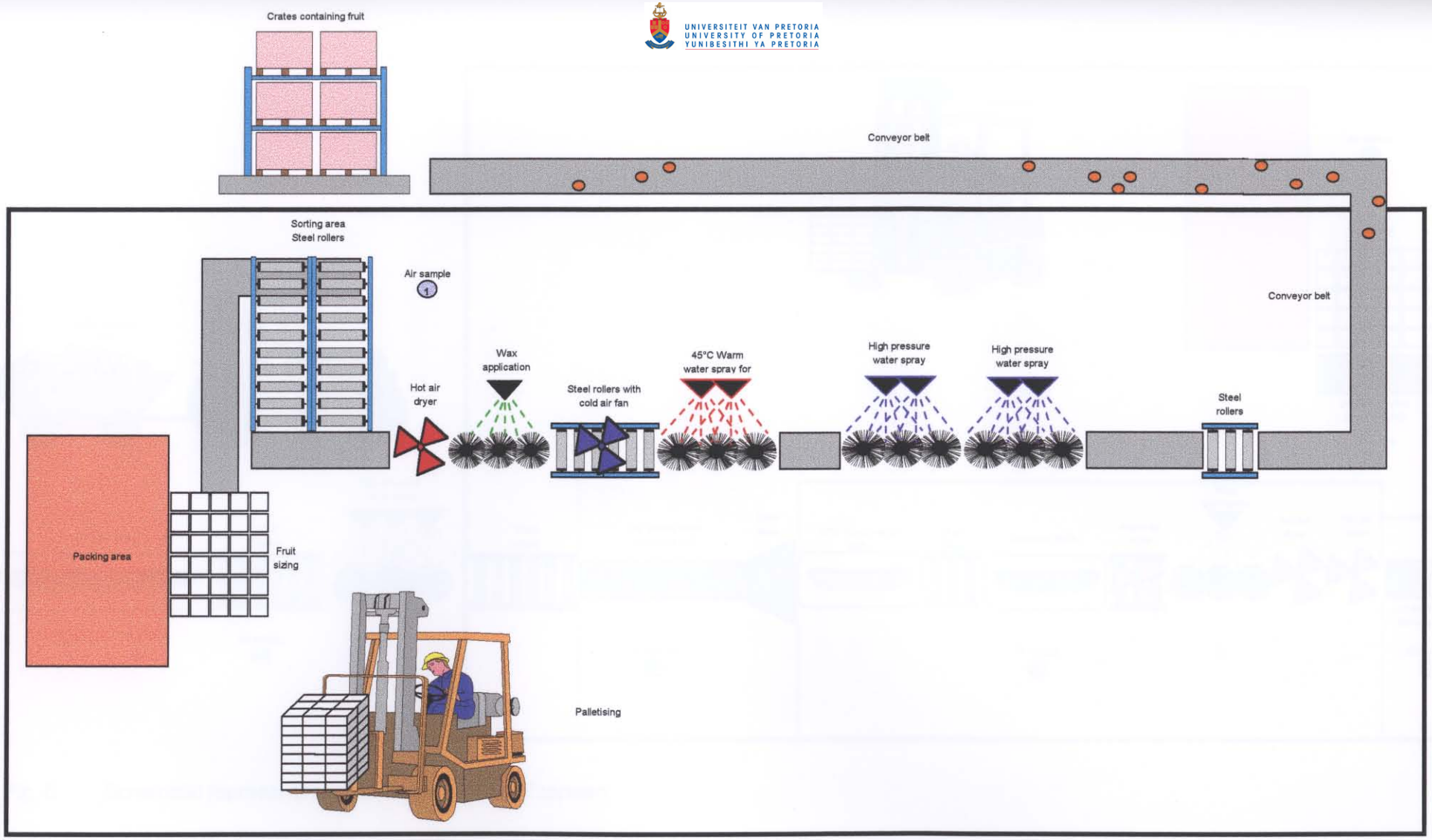

Fig. 5 Schematic representation of Packhouse 5 in Nelspruit.

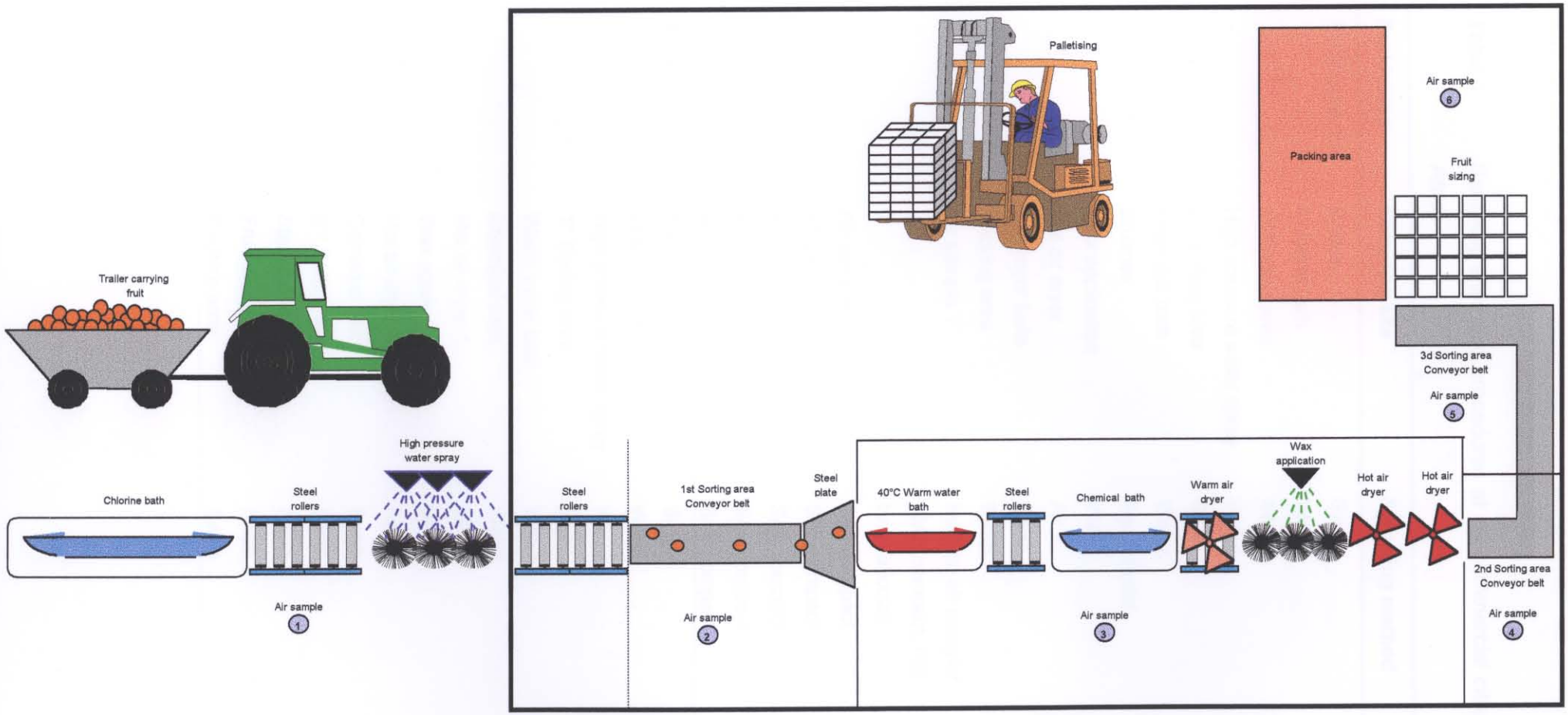

Fig. 6 Schematic representation of Packhouse 6 in Tzaneen.

Table 1 Sampling sites and procedures at six commercial citrus packhouses in South Africa

Packhouse	Sampling site	Sampling method	Sampling size
Packhouse 1	Crates	Swab	100 cm^2
	Chlorine bath	Water	100 µl
	1st Sorting area	Swab	100 cm^2
	High pressure water spray	Water	100 µl
	2nd Sorting area	Swab	100 cm^2
	Chemical bath	Water	100 µl
	Brushes	Agar imprint	64 cm^2
	Wax application	Wax	100 µl
	Hot air dryer	Air	150 l
	Conveyor belts	Swab	100 cm^2
	Packing area	Swab	100 cm^2
	Air sample 1	Surface air sampler (SAS compact, PBI International)	150 l
	Air sample 2	SAS compact	150 l
	Air sample 3	SAS compact	150 l
	Air sample 4	SAS compact	150 l
	Air sample 5	SAS compact	150 l
	Air sample 6	SAS compact	150 l
Packhouse 2	Trailers	Swab	100 cm^2
	Chlorine bath	Water	100 µl
	High pressure water spray	Water	100 µl
	1st Sorting area	Swab	100 cm^2
	Warm water bath	Water	100 µl
	Chemical bath	Water	100 µl
	Hot air dryer 1	Air	150 l
	Wax application	Wax	100 µl
	Hot air dryer 2	Air	150 l
	Conveyor belts	Swab	100 cm^2
	2nd Sorting area	Swab	100 cm^2
	Steel rollers	Swab	100 cm^2
	Fruit sizing	Swab	100 cm^2
	Packing area	Swab	100 cm^2

Table 1 - continued

Packhouse 2 (continued)	Air sample 1	SAS compact	150 l
	Air sample 2	SAS compact	150 l
	Air sample 3	SAS compact	150 l
	Air sample 4	SAS compact	150 l
Packhouse 3	Trailer	Swab	100 cm^2
	High pressure chlorine spray	Water	100 µl
	1st Sorting area	Swab	100 cm^2
	Steel rollers	Swab	100 cm^2
	Chemical bath	Water	100 µl
	Sponge rollers	Agar imprint	64 cm^2
	Wax application	Wax	100 µl
	Hot air dryer 1	Air	150 l
	Wax brushes	Agar imprint	64 cm^2
	Hot air dryer 2	Air	150 l
	Fans	Air	150 l
	Conveyor belt	Swab	100 cm^2
	2nd Sorting area	Swab	100 cm^2
	Fruit sizing	Swab	100 cm^2
	Packing area	Swab	100 cm^2
	Air sample 1	SAS compact	150 l
	Air sample 2	SAS compact	150 l
	Air sample 3	SAS compact	150 l
	Air sample 4	SAS compact	150 l
	Air sample 5	SAS compact	150 l
Packhouse 4	Crates	Swab	100 cm^2
	Chemical bath	Water	100 µl
	Conveyor belts	Swab	100 cm^2
	Wax application	Wax	100 µl
	Fans	Air	150 l
	Steel rollers	Swab	100 cm^2
	Hot air dryer	Air	150 l
	Sorting area	Swab	100 cm^2
	Packing area	Swab	100 cm^2
	Air sample 1	SAS compact	150 l

Table 1 - continued

Packhouse 5	Crates	Swab	100 cm^2
	High pressure water spray	Water	100 µl
	Warm water spray	Water	100 µl
	Wax application	Wax	100 µl
	Hot air dryer	Air	150 l
	Steel rollers	Swab	100 cm^2
	Conveyor belts	Swab	100 cm^2
	1st Sorting area	Swab	100 cm^2
	2nd Sorting area	Swab	100 cm^2
	Packing area	Swab	100 cm^2
	Air sample 1	SAS Compact	150 l
Packhouse 6	Trailers	Swab	100 cm^2
	Chlorine bath	Water	100 µl
	High pressure water spray	Swab	100 cm^2
	1st Sorting area	Water	100 µl
	Warm water bath	Swab	100 cm^2
	Chemical bath	Water	100 µl
	Cold air dryer	Air	150 l
	Wax application	Wax	100 µl
	Hot air dryer	Air	150 l
	2nd Sorting area	Swab	100 cm^2
	3rd Sorting area	Swab	100 cm^2
	Packing area	Swab	100 cm^2
	Train carriage	Swab	100 cm^2
	Air sample 1	SAS compact	150 l
	Air sample 2	SAS compact	150 l
	Air sample 3	SAS compact	150 l
	Air sample 4	SAS compact	150 l
	Air sample 5	SAS compact	150 l
	Air sample 6	SAS compact	150 l

Table 2 Surfactants/disinfectants tested *in vitro* for inhibition of *Penicillium digitatum*

Surfactant/disinfectant	**Chemical character**	**Ionic action**	**Concentration of a.i.**	**Supplier**
Agral 90	90% m/v alkaryl polyglycol ether	Nonionic	940 g l^{-1}	Kynoch Chemicals, Johannesburg
Agrowett	alkaryl polyglycol ether	Nonionic	350 g l^{-1}	Perskor (Pty) Ltd., Durban
Armoblem 650	ethoxylated/propoxylated tallow amine in block polymer mode, mixed with an ethoxylated sorbiton ester	nonionic with some cationic character	550 g l^{-1}	Agricura, Pretoria
Biofilm	alkylaryl polyxyethylene sorbitan mono-oleate (POE), free & combined fatty acids, glycol ethers, dialkyl benzenedicarboxylate	Nonionic	976 g l^{-1}	Plaaschem, Houhgton
BP Agripon	emulsifiable mineral oil plus surfactant	Nonionic	950 g l^{-1}	Agricura
Citowett	alkylaryl POE	Nonionic	1000 g l^{-1}	BASF, Midrand
Ecosanitizer (hand wash)	glutaraldehyde	nonionic	50 ml l^{-1}	Toni Martin cc., Johannesburg
Ecosanitizer (low foam)	glutaraldehyde	nonionic	50 ml l^{-1}	Toni Martin cc.
Ethanol	ethyl alcohol	-	99% v/v	Sigma, Johannesburg

Table 2 – continued

Formula 10 CL	unknown	cationic	20 ml l^{-1}	Health and Hygiene (Pty)Ltd., Sunninghill
Frigate	fatty amine ethoxylate	weakly cationic	800 g l^{-1}	ISK Biotech, Johannesburg
G49	blend of surfactants	nonionic or cationic	370 g l^{-1}	Agricura
KOCl	chlorine	-		Sigma
Latron B-1956	modified tallow glycerol alkyd harpon	nonionic	770 g l^{-1}	Schering, Johannesburg
Multichlor	chlorine	-		Diversey SA (Pty)Ltd., Chloorkop
OA 5 DP	organic tin complex	-	10 ml l^{-1}	Ocean Agriculturals, Boksburg
Sacti-med Biotane	chlorhexidine gluconate	-	50 g l^{-1}	Lever Industrial (Pty) Ltd., Boksburg
Terminator	dimethyl dodecyl ammoniumchloride	nonionic	250 g l^{-1}	UAP Crop Care (Pty) Ltd., Paarl
Tronic	alkylaryl POE glycols, mixed petroleum distillates, alkylamine acetate, alkylaryl sulphonates, polyhydric alcohol	mixture of cationic, anionic and nonionic	900 g l^{-1}	Plaaschem
Tween 80	polyoxyethylenesorbitan	-	99% v/v	Sigma

Table 3 Species diversity and density of fungi at different sites in six South African citrus packhouses

Packhouse	Sampling site and size	Species diversity	Species density	Dominant organism[z]
Packhouse 1	Crates (100 cm^2)	4	80	*Cladosporium* sp. (66)
	Chlorine bath (1 ml)	8	87	*Cladosporium* sp (50). + *Trichoderma* sp. (20)
	1st Sorting area (100 cm^2)	3	5	
	High pressure water spray (1 ml)	4	105	*Penicillium digitatum* (60) + *Cladosporium* sp.(25)
	2nd Sorting area (100 cm^2)	6	48	*P. digitatum* (31)
	Chemical bath (1 ml)	2	60	*Cladosporium* sp. (7) + yeasts
	Brushes (100 cm^2)	5	12	
	Wax application (1 ml)	1	10	*P. digitatum* (10)
	Hot air dryer (150 l)	6	7	
	Conveyor belts (100 cm^2)	4	23	*Trichoderma* sp. (14)
	Packing area (100 cm^2)	4	284	*Cladosporium* sp. (223)
	Air sample 1 (150 l)	3	11	*Aspergillus niger* (6)+ *P. digitatum* (5)
	Air sample 2 (150 l)	3	5	*A. niger* (3)
	Air sample 3 (150 l)	1	6	*A. niger* (6)
	Air sample 4 (150 l)	2	3	*A. niger* (2)+ *P. digitatum* (1)
	Air sample 5 (150 l)	6	17	*Cladosporium* sp. (5) + *Trichoderma* sp.(7) + *Rhizopus stolonifer* (2)
	Air sample 6 (150 l)	4	22	*Cladosporium* (9) + *Penicillium italicum* (9) + *Trichoderma* sp. (3)
Packhouse 2	Trailers (100 cm^2)	6	57	*Cladosporium* sp. (16) + *P. digitatum* (29)
	Chlorine bath (1 ml)	7	72	*Cladosporium* sp. (49)+ *Trichoderma* sp. (10)
	High pressure water spray (1 ml)	6	72	*Cladosporium* sp.(43)
	1st Sorting area (100 cm^2)	4	9	*Trichoderma* sp. (6)
	Warm water bath (1 ml)	3	70	*Trichoderma* sp. (46)
	Chemical bath (1 ml)	2	40	*P. digitatum* (36)
	Hot air dryer 1 (150 l)	3	13	*Trichoderma* sp. (11)
	Wax application (1 ml)	0	0	
	Hot air dryer 2 (150 l)	3	8	*Trichoderma* sp. (5)
	Conveyor belts (100 cm^2)	2	25	*Trichoderma* sp. (18)
	2nd Sorting area (100 cm^2)	2	5	*Trichoderma* sp. (4)
	Steel rollers (100 cm^2)	1	6	*P. digitatum* (6)
	Fruit sizing (100 cm^2)	5	9	
	Packing area (100 cm^2)	1	89	*Trichoderma* sp. (89)
	Air sample 1 (150 l)	2	10	*A. niger* (8)
	Air sample 2 (150 l)	4	13	*A. niger* (7)+ *P. digitatum* (4)
	Air sample 3 (150 l)	2	4	
	Air sample 4 (150 l)	3	4	*Aspergillus* sp. (2)

Table 3 – continued

Packhouse 3	Trailer (100 cm^2)	4	300	*Cladosporium* sp. (172) + *P. digitatum* (109)
	High pressure chlorine spray (1 ml)	5	80	Yeasts (69)
	1st Sorting area (100 cm^2)	4	32	*Aspergillus* sp. (15)+ *Cladosporium* sp. (13)
	Steel rollers (100 cm^2)	3	22	*Cladosporium* sp. (9)+ *P. digitatum* (10)
	Chemical bath (1 ml)	4	40	*Cladosporium* sp. (25) + *Trichoderma* sp. (13)
	Sponge rollers (100 cm^2)	6	8	
	Wax application (1 ml)	5	30	*A. niger* (24)
	Hot air dryer 1 (150 l)	5	8	
	Wax brushes (100 cm^2)	8	58	*Aspergillus* sp. (38)
	Hot air dryer 2 (150 l)	3	8	*Trichoderma* sp. (5)
	Fans (150 l)	5	39	*Cladosporium* sp. (20)
	Conveyor belt (100 cm^2)	4	9	*Trichoderma* sp. (5)
	2nd Sorting area (100 cm^2)	4	40	*Cladosporium* sp. (32)
	Fruit sizing (100 cm^2)	6	39	*A. niger* (21) + *Trichoderma* sp. (6)
	Packing area (100 cm^2)	5	26	*Cladosporium* sp. (15)+ *Trichoderma* sp. (6)
	Air sample 1 (150 l)	5	19	*A. niger* (9) + *P. digitatum* (5)
	Air sample 2 (150 l)	4	29	*Cladosporium* sp. (24)
	Air sample 3 (150 l)	4	41	*Cladosporium* sp. (27) + *P. digitatum* (11)
	Air sample 4 (150 l)	3	5	*P. digitatum* (3)
	Air sample 5 (150 l)	2	65	*A. niger* (34)+ *P. digitatum* (31)
Packhouse 4	Crates (100 cm^2)	4	28	*Cladosporium* sp. (11)
	Chemical bath (1 ml)	1	30	Yeasts (30)
	Conveyor belts (100 cm^2)	5	6	
	Wax application (1 ml)	1	77	*Cladosporium* sp. (77)
	Fans (150 l)	4	10	
	Steel rollers (100 cm^2)	5	18	*Trichoderma* sp. (10)
	Hot air dryer (150 l)	3	7	
	Sorting area (100 cm^2)	6	6	
	Packing area (100 cm^2)	6	43	*Cladosporium* sp. (26)
	Air sample 1 (150 l)	7	7	
Packhouse 5	Crates (100 cm^2)	1	60	*Cladosporium* sp. (60)
	High pressure water spray (1 ml)	3	27	Bacteria (14)
	Warm water spray (1 ml)	2	90	*Cladosporium* sp. (73)
	Wax application (1 ml)	3	47	*A. niger* (32)
	Hot air dryer (150 l)	2	6	
	Steel rollers (100 cm^2)	4	40	*Cladosporium* sp. (21)
	Conveyor belts (100 cm^2)	2	103	*Cladosporium* sp. (101)
	1st Sorting area (100 cm^2)	2	167	*Cladosporium* sp. (154)
	2nd Sorting area (100 cm^2)	2	10	
	Packing area (100 cm^2)	3	31	*P. digitatum* (21) + *Trichoderma* sp. (7)

Table 3 – continued

Packhouse 6 (1st Sampling)	Trailers (100 cm²)	3	140	*Trichoderma* sp. (105) + *P. digitatum* (33)
	Chlorine bath (1 ml)	3	70	*Cladosporium* sp. (60)
	High pressure water spray (1 ml)	5	95	Bacteria (87)
	1st Sorting area (100 cm²)	3	160	*Cladosporium* sp. (149)
	Warm water bath (1 ml)	3	90	*Cladosporium* sp. (43) + *Trichoderma* sp. (40)
	Chemical bath (1 ml)	0	0	
	Cold air dryer (150 l)	4	4	
	Wax application (1 ml)	5	35	*A. niger* (18) + *Cladosporium* sp. (7) + *P. digitatum* (6)
	Hot air dryer (150 l)	5	8	
	2nd Sorting area (100 cm²)	8	11	
	3rd Sorting Area (100 cm²)	5	32	*A. niger* (26)
	Packing area (100 cm²)	6	43	*Cladosporium* sp. (30) + *Trichoderma* sp. (8)
	Train carriage (100 cm²)	1	13	*Trichoderma* sp. (13)
	Air sample 1 (150 l)	3	31	*P. digitatum* (17) + *Cladosporium* sp. (13)
	Air sample 2 (150 l)	3	15	*P. digitatum* (9)
	Air sample 3 (150 l)	3	9	*A. niger* (6)
	Air sample 4 (150 l)	4	14	*A. niger* (7)
	Air sample 5 (150 l)	4	12	*A. niger* (6)
	Air sample 6 (150 l)	1	13	*A. niger* (13)
Packhouse 6 (2nd Sampling)	Trailers (100 cm²)	2	141	*Cladosporium* sp. (95) + *P. digitatum* (46)
	Chlorine bath (1 ml)	3	120	*Cladosporium* sp. (76)
	High pressure water spray (1 ml)	3	150	*Cladosporium* sp. (130)
	1st Sorting area (100 cm²)	2	191	*Cladosporium* sp. (190)
	Warm water bath (1 ml)	2	20	Bacteria (18)
	Chemical bath (1 ml)	1	20	*Trichoderma* sp. (20)
	Cold air dryer (150 l)	5	9	
	Wax application (1 ml)	1	48	*Cladosporium* sp. (48)
	Hot air dryer (150 l)	3	6	
	2nd Sorting area (100 cm²)	6	8	
	3rd Sorting area (100 cm²)	3	5	
	Packing area (100 cm²)	6	23	*Cladosporium* sp. (14)
	Train carriage (100 cm²)	1	300	
	Air sample 1 (150 l)	2	4	*P. digitatum* (2)+ *Trichoderma* sp. (2)
	Air sample 2 (150 l)	2	2	
	Air sample 3 (150 l)	1	1	
	Air sample 4 (150 l)	2	4	
	Air sample 5 (150 l)	1	9	*Cladosporium* sp. (9)
	Air sample 6 (150 l)	3	10	*Trichoderma* sp. (5) + *P. digitatum* (4)

[z] Numbers in brackets indicate the density of the particular organism

Table 4 *In vitro* inhibition of *Penicillium digitatum* by various surfactants and disinfectants

Surfactant/disinfectant	Absorbance (492 nm)[z]			
	P. digitatum at 10^3 conidia ml^{-1}		*P. digitatum* at 10^5 conidia ml^{-1}	
	after 24 h	after 48 h	after 24 h	after 48 h
Agral90	0.016 c	0.104 a	0.085 cd	0.206 b
Agrowett	0.013 c	0.095 ab	0.094 c	0.234 a
Armoblem	0.021 c	0.035 bc	0.027 f	0.050 fg
BPAgripon	-0.010 cd	-0.100 d	0.145 a	0.198 b
Biofilm	0.013 c	0.071 abc	0.005 gh	0.076 e
Biotane	0.041 bc	0.052 abc	0.051 e	0.079 e
Citowett	0.104 ab	0.084 abc	0.123 b	0.171 c
Ecosanitizer (Handwash)	0.003 cd	0.023 c	0.014 fgh	0.048 g
Ecosanitizer (Low foam)	0.012 c	0.030 c	0.019 fgh	0.052 fg
EtOH	0.010 c	0.047 abc	0.073 d	0.152 d
Formula10	0.012 c	0.036 bc	0.024 f	0.058 f
Frigate	0.013 c	0.029 c	0.020 fg	0.044 g
G49	0.014 c	0.031 bc	0.018 fgh	0.044 g
KOCl	0.005 cd	0.049 abc	0.096 c	0.196 b
Latron	0.109 a	0.045 abc	0.166 b	0.075 e
Multichlor	-0.057 d	0.059 abc	-0.045 i	0.169 c
QA5DP	0.000 cd	0.025 c	0.004 h	0.048 fg
Terminator	0.017 c	0.044 bc	0.021 f	0.049 fg
Tronic	0.009 cd	0.028 c	-0.040 i	0.052 fg
Tween80	0.010 c	0.048 abc	0.096 c	0.196 b

[z] Means of surfactant/disinfectant concentration of 0.5, 1, 5, 10, 25 and 50 ppm of the a.i. (Table 2). Values within columns followed by the same letter do not differ significantly according to Student's t-LSD (P=0.05).

GENERAL DISCUSSION

Efficacy of any management strategy to reduce the incidence of postharvest diseases depends on several key elements, viz. isolation of spore-generating areas, careful handling of fruit, proper sanitation procedures for decontamination of fruit handling equipment, weekly assays to monitor pathogen spore populations, judicious use of fungicides and modification of existing packhouse operations to prevent dispersal of pathogen inoculum (Gardner *et al.*, 1986).

It is well documented that careless harvesting and handling practices, along with high inoculum of postharvest pathogens in the packhouse environment, are the main factors involved in postharvest disease development (Sommer, 1982; Di Martino Aleppo & Lanza, 1996). Spores of pathogenic fungi are produced on decayed fruit and transferred by air currents, water dip tanks and fruit handling equipment to sound fruit (Barmore & Brown, 1982; Gardner *et al.*, 1986; Spotts & Cervantes, 1986, 1993). *Aspergillus niger* Thiegh, *Penicillium digitatum* (Pers.: Fr.) Sacc., *P. italicum* Wehmer and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. were present in each of the sampled packhouses, with *A. niger* and *P. digitatum* being the most dominant throughout. The main sources of accumulation of these fungal spores in packhouses were identified as crates/trailers and dip tank water, which corresponds with results from previous studies (Gardner *et al.*, 1986; Spotts & Cervantes, 1986; 1992; 1993; Van Dyk *et al.*, 1997). Strategies aimed at reducing the pathogen inoculum at critical locations in the packhouse are therefore of utmost importance (Gardner *et al.*, 1986).

Sanitation of packhouse equipment, floors and walls with surfactants/disinfectants (Gardner *et al.*, 1986; Beuchat, 1995) has recently been explored as a preventative control measure in the fruit packing industry. Surfactants/disinfectants are routinely used in food and dairy industries to reduce inoculum of spoilage organisms (Park *et al.*, 1991). The usefulness of this technique in other food handling industries warrants further investigation for its use in citrus packhouses. Of the surfactants/disinfectants evaluated in this study to inhibit *in vitro* germination and growth of *P. digitatum*, only Tronic was effective against both 10^3 and 10^5

conidia ml^{-1} of this pathogen. Besides ethanol and chlorine dioxide that have been reported to sanitise bins, control decay and sanitise water (Bancroft *et al.*, 1984; Smilanick *et al.*, 1995; Lesar, 1997), this is the first report of the disinfecting abilities of non-selective chemicals in citrus packhouses. Tronic should be further evaluated in future studies under packhouse conditions for disinfection of both fruit and packing equipment. The usefulness of surfactants/disinfectants in a citrus packhouse cannot be over estimated, and should be part of the total disease management strategy.

To promote the judicious use of fungicides, it is necessary to investigate various alternatives to establish their suitability and usefulness. Such alternatives include biological control and warm water treatments. It is evident from this study that, although antagonists could suppress postharvest diseases *in vitro*, control *in vivo* and in the packhouse is highly dependent on the inoculum levels of the pathogen. While *Bacillus subtilus* was the most effective antagonist *in vitro*, high concentrations (10^9 cells ml^{-1}) of the bacterium were needed to control *P. digitatum* levels of 10^4 conidia ml^{-1} *in vivo*. Packhouse experiments with *B. subtilis* also resulted in no control when the pathogen inoculum was high. Similar results were obtained by various researchers (Janisiewicz & Roitman, 1988; Droby *et al.*, 1989; Huang *et al.*, 1992; Smilanick & Dennis-Arrue, 1992), which emphasises the fact that enhanced control is associated with increasing antagonist concentration, or a decrease in the level of pathogen challenge (Pusey & Wilson, 1984; Janisiewicz & Roitman, 1988; Korsten *et al.*, 1995).

Integration of antagonists with warm water treatment and reduced concentrations of commercially used chemicals, reduced fruit decay. However, control was not as consistent or effective as commercial chemical treatments. This contradicts findings of several studies (Huang *et al.*, 1995; Arras, 1996; Schachnai *et al.*, 1996) which indicated the viability of both these management strategies. The main difference between the conflicting reports is the time of pathogen application. In the present study, pathogen inoculum was applied prior to antagonist treatment to simulate field conditions where infection occurs during harvesting through wound and natural openings (Sommer, 1982; Brown & Eckert, 1989; Eckert & Brown, 1989). In the other studies pathogen inoculum was applied after the antagonist had already been introduced. Effectiveness of antagonist treatments depends on the successful establishment of the antagonist at wound sites prior to pathogen challenge (Janisiewicz &

Roitman, 1988; Huang *et al.*, 1992). Antagonists in this study did not have the advantage of colonising natural openings and wounds before arrival of the pathogen and hence fulfilled a curative rather than preventative function. In formulating an effective postharvest disease management strategy, factors such as timing of antagonist application, reduction of inoculum, prevention or eradication of field infection, suppression of disease development and inactivation of wound infection (Eckert & Ogawa, 1985), should be considered. Preharvest antagonist treatments may therefore provide a feasible alternative to antagonist application in the packhouse for disease management (Jeffries & Jeger, 1990; Korsten, 1993).

Postharvest warm water treatment of artificially inoculated citrus fruit resulted in a significant reduction in fruit decay caused by *P. digitatum* notwithstanding the high inoculum levels of 10^5 and 10^6 conidia ml^{-1}. Control of citrus postharvest pathogens may be attributed to host responses such as production of scoparone (Kim *et al.*, 1991). Packhouse experiments confirmed *in vivo* results, indicating that the most effective warm water treatment is 36 °C for 1 min. Although inhibition of *P. digitatum* by scoparone was also observed when fruit were treated at 36 °C for 2.5 and 5 min, and 40 °C for 1, 2.5 and 5 min, maintaining water temperature at 36 °C and dipping fruit for 1 min is the most cost-effective and less time-consuming treatment. Ben-Yehoshua *et al.* (1992), however, found *Citrus* species to vary considerably in their ability to produce scoparone in response to *Penicillium* and hot air treatments, with consequent variance in decay control. Optimisation of warm water treatment for different citrus cultivars packed in South Africa is therefore needed. Integration of surfactants/disinfectants with biological control and warm water treatments could be proposed, considering the success of these procedures on mango (De Villiers & Korsten, 1996) and citrus (Smilanick *et al.*, 1995), respectively.

Several environmentally friendly approaches are available for managing postharvest diseases, including biological control, surfactants and disinfectants, induced resistance and harvesting and careful handling practices to minimise injury and infection (Wisniewski & Wilson, 1992). This study proved that several of these approaches were viable alternatives to the use of fungicides for control of citrus postharvest diseases. Some aspects require further investigation, but it is nevertheless obvious that integration of the various procedures could provide a management strategy, which is economically viable, readily implemented and

environmentally sound.

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CONTROL STRATEGIES FOR CITRUS POSTHARVEST DISEASES

by

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RESUMÉ

A study was undertaken to evaluate biological, warm water and integrated treatments as alternatives to fungicides for control of citrus postharvest diseases. Possible infection sites and unsanitary conditions in packhouses, and the use of surfactants/disinfectants for sanitation of fruit surfaces, packhouses and packhouse equipment were also investigated. The following transpired from the study:

1. *Bacillus subtilis* (B246) (isolated from avocado) and *B. licheniformis* (B250) (isolated from mango), effectively inhibited *in vitro* growth of citrus postharvest pathogens, *Alternaria citri*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Geotrichum citri-aurantii*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*. However, *B. licheniformis* (B251) (isolated from mango) and *B. subtilis* (B248) (isolated from avocado) were the most effective antagonists against *P. digitatum* in *in vivo* experiments. *Bacillus licheniformis* (B254) (isolated from litchi) provided only moderate *in vitro* inhibition, but was more effective than B246 and B250 in postharvest experiments.
2. Antagonist treatments were neither as effective nor as consistent as full-strength chemical

treatments (guazatine-1000 ppm, thiabendazole -1000 ppm, 2,4-D - 500ppm, imazalil -500 ppm) in reducing decay. However, when integrated with quarter-strength chemical application, B246 and B254 provided the same control as full-strength chemical treatment.

3. Warm water treatment of artificially inoculated fruit was as effective as fungicide treatments in controlling citrus postharvest diseases. Optimal temperatures determined *in vivo* for warm water were 36 and 40 °C for 1, 2.5 and 5 min. This was confirmed in packhouse experiments. No decay, off-tastes or smells resulted from exposure of fruit to the temperatures.

4. *Aspergillus niger*, *P. digitatum*, *P. italicum* and *Rhizopus stolonifer* were the only citrus postharvest pathogens detected in packhouses. Crates/trailers and dip tank water were identified as the main sources of fungal accumulation.

5. Several surfactants inhibited conidial germination and growth of *P. digitatum in vitro*, the most effective ones being Multichlor and Tronic at an inoculum level 10^3 conidia ml^{-1} and Armoblem, Ecosanitizer (Handwash + Low foam), Frigate, G49, QA5DP, Terminator and Tronic at 10^5 conidia ml^{-1}.

BEHEERSTRATEGIË VIR SITRUS NA-OESSIEKTES

deur

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SAMEVATTING

'n Studie is onderneem om biologiese, warmwater en geïntegreerde behandelings te evalueer as alternatief vir chemiese beheer van sitrus na-oessiektes. Moontlike infeksie-areas en onhigiëniese toestande in pakhuise en die gebruik van oppervlakspanningsverlagings- en ontsmettingsmiddels vir ontsmetting van vrugoppervlaktes, verpakkingstoerusting en pakhuise is ook ondersoek. Die volgende is gevind:

1. *Bacillus subtilis* (B246) (geïsoleer vanaf avokado) en *B. licheniformis* (B250) (geïsoleer vanaf mango) het *in vitro* groei van die sitrus na-oes patogene, *Alternaria citri*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Geotrichum citri-aurantii*, *Penicillium digitatum*, *P. italicum* and *Trichoderma viride*, effektief geïnhibeer. *Bacillus licheniformis* (B251) (geïsoleer vanaf mango) en *B. subtilis* (B248) (geïsoleer vanaf avokado) was egter die effektiefste anatagoniste teen *P. digitatum in vivo*. *Bacillus licheniformis* (B254) (geïsoleer vanaf lietsjie), wat slegs 'n mate van beheer *in vitro* gegee het, was meer doeltreffend as B246 en B250 in pakhuis eksperimente.
2. Antagonisbehandelings was nie so doeltreffend of konsekwent soos volsterkte chemies

behandelings (guasatien-1000 dpm, thiabendasool -1000 dpm, 2,4-D - 500 dpm, imasalil - 500 dpm) nie. Beide B246 en B250 het egter dieselfde mate van beheer gegee as volsterkte chemiese behandeling wanneer geïntegreer met die toediening van die chemiese middels teen kwart sterkte.

3. Warmwater-behandeling van kunsmatig-besmette vrugte het vrugbederf net so doeltreffend as chemiese behandeling beheer. Optimale temperatuur vir warmwater-behandeling is *in vivo* bepaal as 36 and 40 °C vir 1, 2.5 and 5 min, en is bevestig in pakhuiseksperimente. Geen bederf, vreemde smake of reuke is waargeneem by vrugte wat aan hierdie temperature blootgestel is nie.

4. *Aspergillus niger*, *P. digitatum*, *P. italicum* and *Rhizopus stolonifer* was die enigste sitrus na-oespatogene wat in pakhuise aangetref is. Kratte/sleepwaens en dooptenkwater is geïdentifiseer as die hoofbronne van swamakkumulasie.

5. Verskeie oppervlakspanningsverlaging- en ontsmettingsmiddels het konidiumontkieming en groei van *P. digitatum in vitro* beperk. Die effektiefste middels was Multichlor and Tronic by 'n inokulumvlak van 10^3 konidiums ml^{-1} en Armoblem, Ecosanitizer (Handwash + Low foam), Frigate, G49, QA5DP, Terminator and Tronic by 10^5 konidiums ml^{-1}.

I have fought the good fight,

I have finished the race,

I have kept the faith.

2 Timothy 4:7