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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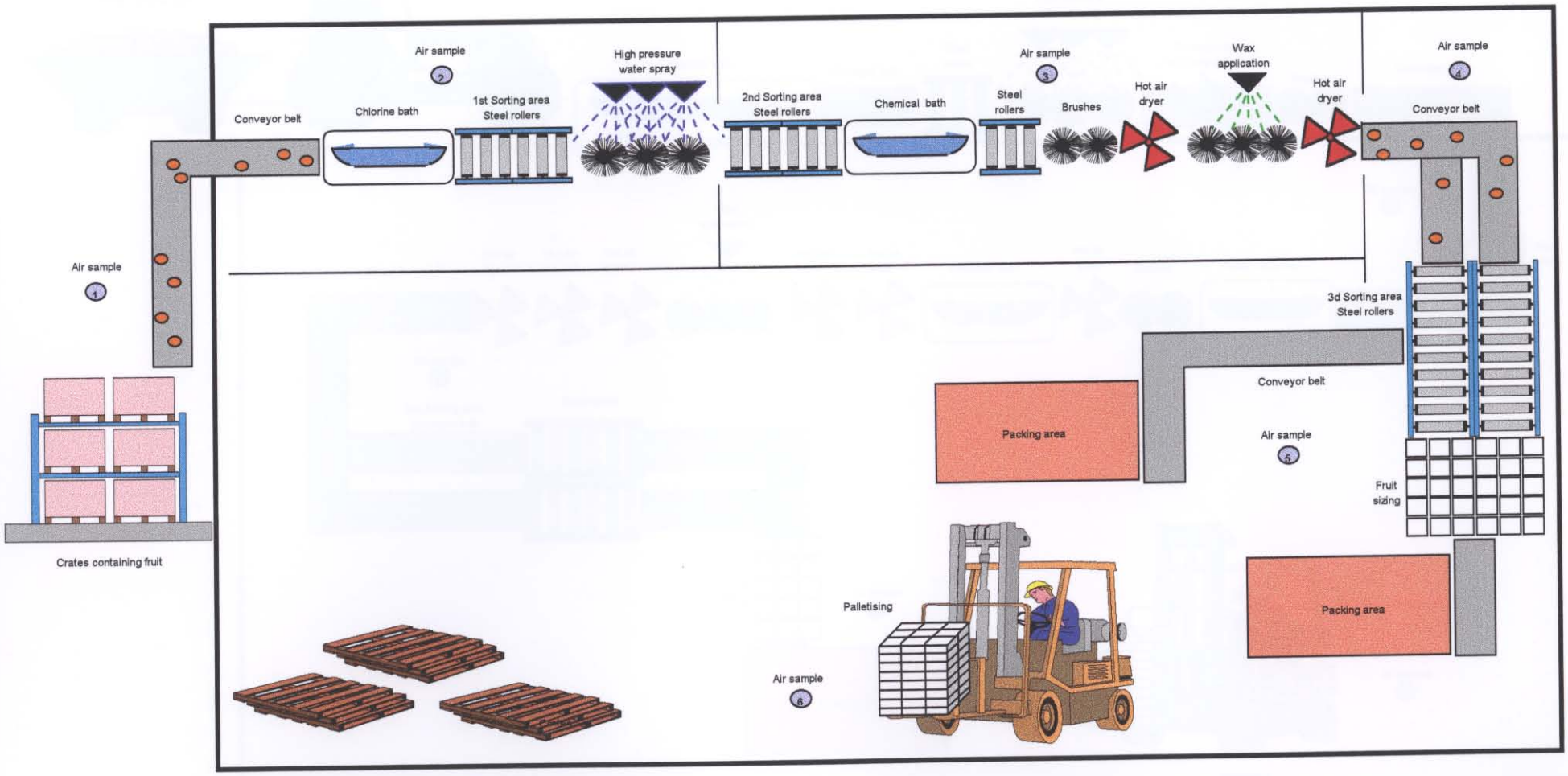

Fig. 1 Schematic representation of Packhouse 1 in Citrusdal.

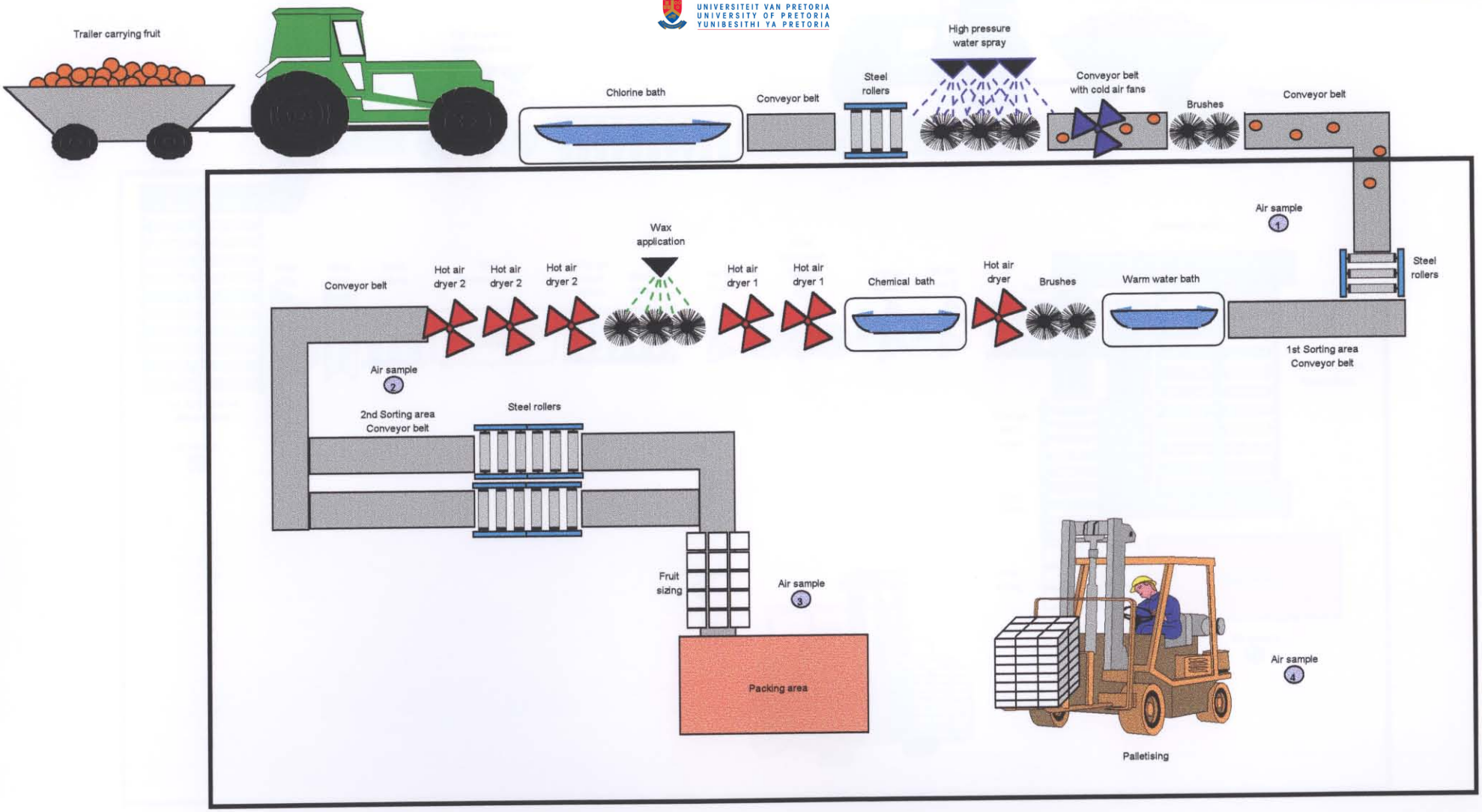

Fig. 2 Schematic representation of Packhouse 2 in Patensie.

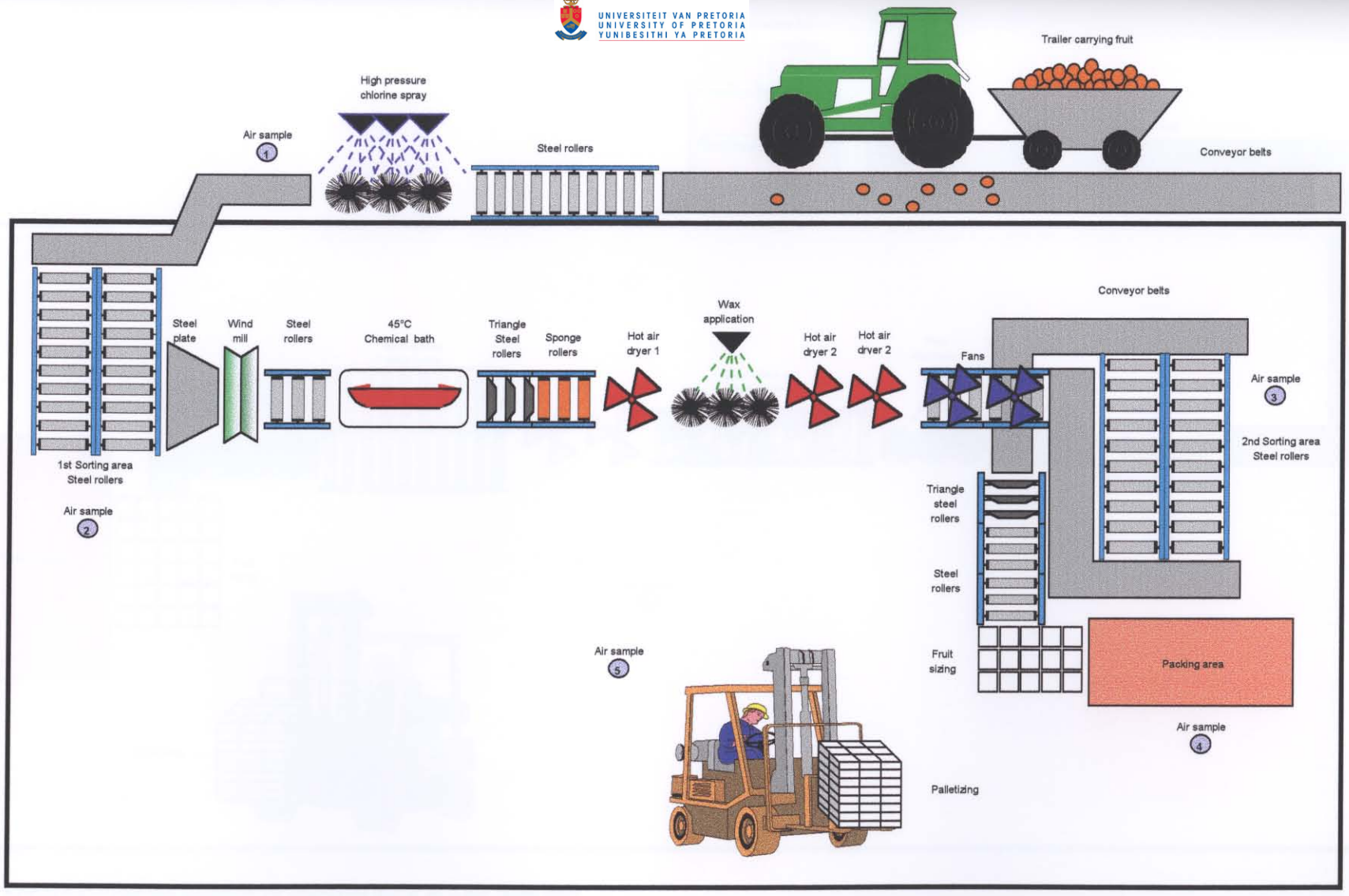

Fig. 3 Schematic representation of Packhouse 3 in Addo.

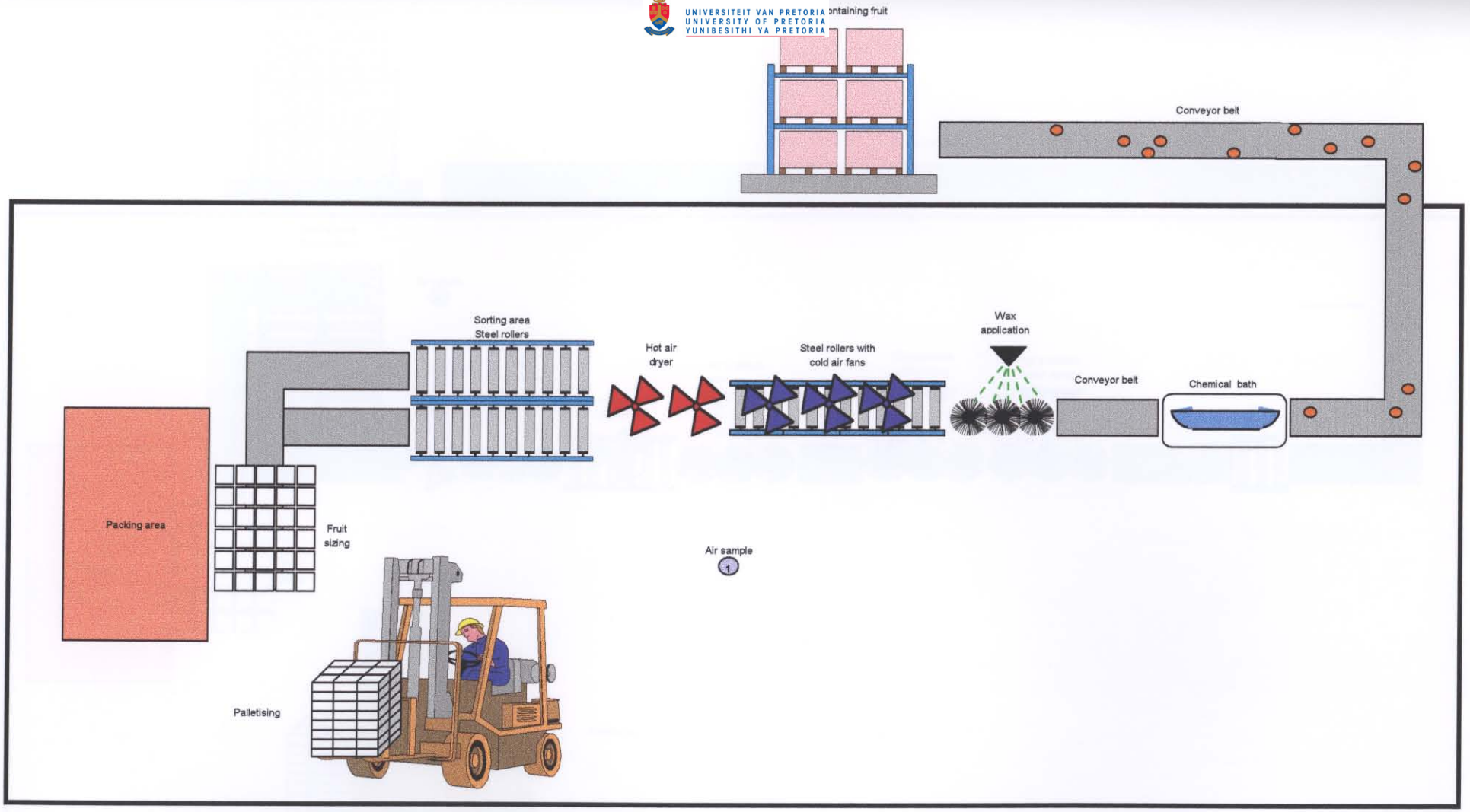

Fig. 4 Schematic representation of Packhouse 4 in Fort Beaufort.

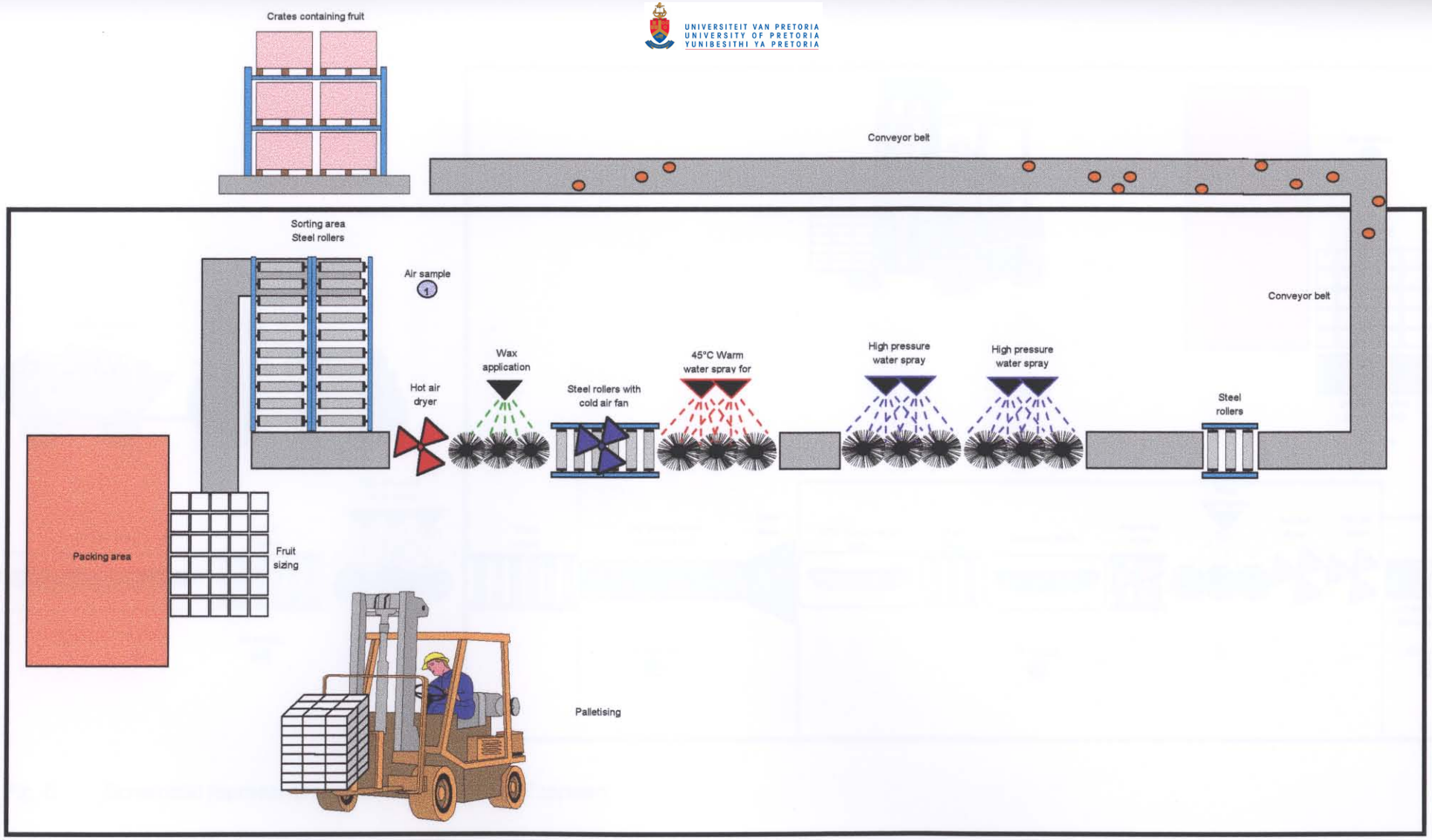

Fig. 5 Schematic representation of Packhouse 5 in Nelspruit.

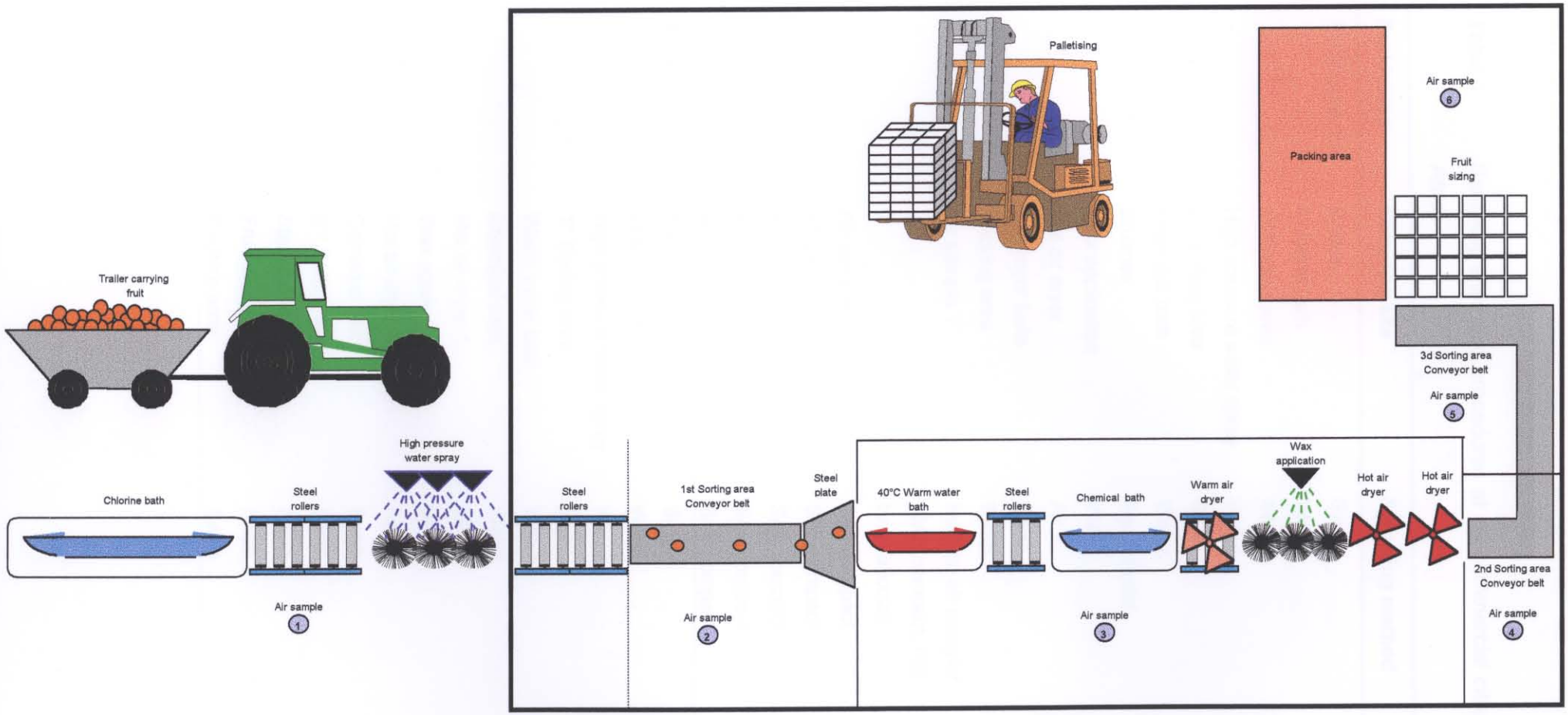

Fig. 6 Schematic representation of Packhouse 6 in Tzaneen.

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).