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Determination of Possible Infection and Alternative Control Application Sites in Avocado Packhouses

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SUMMARY

The extent, to which pathogens associated with post-harvest diseases of avocado can accumulate in the packhouse to cause secondary infections, was investigated. In addition, sanitary conditions in the packhouse were assessed by monitoring numbers of fungi, bacteria and yeasts. Rhizopus stolonifer has been implicated in stem-end rot and was isolated from crates, wax, sorting rollers and trolleys in packhouse I and from the brush rollers, trolleys and air sample I in packhouse 2. No other pathogens were apparent in this study. Sampling methods used in this study were not effective for determining accurate values for species diversity and -richness of fungi, bacteria and yeasts. Although values for species diversity and richness should be seen as indicative rather than quantitative values, unsanitary conditions could nevertheless be identified for crates, chlorine dump tanks and trolleys. Optimisations of sampling methods used for avocado packhouses are needed in order to obtain a true indication of the spread of pathogens and other micro-organisms throughout the packhouse.

OPSOMMING

Die mate waartoe patogene, geassosieer met na-oes siektes van avokado in die pakhuis kan akkumuleer om sekondére infeksies te veroorsaak is ondersoek. Sanitére toestande in die pakhuis is ook ondersoek deur die getalle van fungi, bakterieé en giste te monitor. Rhizopus stolonifer is betrokke by stingel-end verrotting en is geisoleer van kratte, waks, sorteerrollers en trollies in pakhuis I en van borselrollers, trollies en lugmonster I in pakhuis 2. Geen ander patogene is gevind nie. Monsternemings-metodes wat gebruik was tydens die opnames was nie effektief vir die bepaling van akkurate waardes vir spesiediversiteit en -rykheid van fungi, bakterieé en giste nie.Alhoewel die waardes vir spesiediversiteit en -rykheid gesien moet word as ¡ndikatiewe eerder as kwantitatiewe waardes, kon nie-sanitere toestande nogtans geidentifiseer word vir kratte, chloorbaddens en trollies. Optimisering van monsternemingsmetodes vir avokado pakhuise is nodig om 'n ware ¡ndikasie van die verspreiding van patogene en ander mikroörganismes deur die pakhuis te kry.

INTRODUCTION

Of the 7 million cartons of avocados (*Persea americana* Mill) exported in 1994/95, actual waste was determined at approximately 2% (Gavin Turner, Katope, personal

communication). This loss is caused to a large degree by pathogenic micro-organisms which attack the commodity at certain points along the harvesting, handling and processing line (Droby *et al.,* 1991). Since the majority of fruit is exported by sea, which necessitates long storage periods, post-harvest losses are of great concern to the avocado industry (Korsten *et al.,* 1995).

Pathogens associated with post-harvest decay of avocado include: *Colletotrichum gloeosporioides* (Penz..) Penz & Sacc. in Penz., which is associated with anthracnose, *Dothiorella/ Colletotrichum* fruit rot complex (DCC) and stem-end rot (SE); *Dothiorella aromatica* (Sacc.) Petrak & Sydow, which causes DCC and SE; and *Thyronectria pseudotrichia* (Schw.) Seeler, *Phomopsis perseae* Zerova, *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. *Pestalotiopsis versicolor* (Speg.) Steyart, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl, *Fusarium decemcellulare* Brick, *Fusarium sambiicinum* Fuckel, *Drechslern setariae* (Sawada) Subram. & Jain and *Rhizopus stolonifer* (Ehrenb. Fr.) Vuill., all implicated in SE (Darvas *et al,* 1987). Because these pathogens mostly infect in the orchard and remain latent until the onset of fruit ripening (Sommer, 1982), very little has been done thus far to determine whether these pathogens can accumulate at certain points in the packhouse and cause further infections.

The purpose of this study was therefore to determine whether pathogens associated with post-harvest diseases of avocado can be traced in the packhouse and, if so, to monitor their numbers at different sites with the aim of identifying points in the packhouse where further infections may occur and where control of these pathogens may be critical. In addition, the numbers of general micro-organisms (fungi, bacteria, yeasts) at different points in the packhouse, as related to sanitary conditions, was also monitored in order to determine possible application sites for alternative control agents, i.e. disinfectants.

MATERIALSAND METHODS

Packhouse surveys

Populations of fungi, bacteria and yeasts were determined at each stage of fruit handling in two commercial avocado packhouses. Packhouse 1 (Hazyview, RSA) (figure 1) was monitored once during May 1996 and once during June 1996, while packhouse 2 (Tzaneen, RSA) (figure 1) was monitored once during June 1996 and August 1996. Sampling sites, methods and sizes are described in table 1. At each site a sample was taken by using either three replicates of three isolation media, i.e. potato-dextrose agar (Biolab Diagnostics (Pty) Ltd, Midrand, RSA) supplemented with 0,01% chloramphenicol (Chlorcol, Premier Pharmaceutical Co Ltd., Bryanston, RSA) and 2 *mℓ /ℓ* alkylaryl polyglycol ether (Agrowett, 35% a.i., Perskor (Pty) Ltd., Durban, RSA) for isolation of fungi, standard 1 nutrient agar (Biolab) supplemented with 0,01% cycloheximide (Actidione, Merck (Pty) Ltd, Midrand, RSA) for isolation of bacteria and wort agar (Biolab) for isolation of yeasts; or by using three replicates of yeast and mould petrifilm (Microbiology Products, 3M Health Care , St Paul, MN, USA) and three replicates of bacteria petrifilm (Microbiology Products, 3M Health Care). Samples were placed in a coolbox, transported to the laboratory and incubated at 25°C for 5 10 days.

Colonies were counted and the average species diversity and richness calculated for each medium at each site.

Isolation of fungi

Fungal isolation media were carefully examined for colonies resembling those of post-harvest avocado pathogens. Such colonies were subcultured on PDA supplemented with 0,01% chloramphenicol to obtain pure cultures, incubated at 25°C for 5-10 days and identified. Fungi that occurred dominantly through each packhouse was also isolated and identified according to the same procedure.

Table I

Description of sampling sites, methods and sizes for two commercial avocado packhouses

Packhouse	*Sampling site*	*Sampling method*	*Sampling size*
Packhouse 1	Crates	Swab	25cm²
	Chlorine dump tank	Water	100*l*
	Brush rollers	Agar imprint	160cm²
	Tag wax	Wax	100*l*
	Sorting rollers 1	Petrifilm	650cm²
	Sorting rollers 2	Petrifilm	650cm²
	Trolleys	Swab	25cm²
	Air sample	Surface air sampler (SAS compact) (PBI International)	150*l*
Packhouse 2	Crates	Swab	25cm²
	Chlorine dump tank	Water	100u*l*
	Brush rollers	Agar imprint	169cm²
	Conveyor belt	Petrifilm	650cm²
	Conveyor rollers	Petrifilm	650cm²
	Tag Wax	Wac	100u*l*
	Sorting rollers	Petrifilm	650cm²
	Trolleys	Swab	25cm²
	Air sample 1 (reception – sorting area)	SAS compact	150*l*
	Air sample 2 (packing area)	SAS compact	150*l*

RESULTS

With the exception of *R. stolonifer,* populations of pathogens associated with post-harvest diseases of avocado could not be traced in the packhouse. Colonies of *R. stolonifer* were detected from samples taken from crates, wax, sorting rollers 1 and trolleys in packhouse 1 and from brush rollers, trolleys and air sample 1 in packhouse 2.

Sampling methods used in this study were not effective for determining accurate values for species diversity (SD) and especially species richness (SR). Agar plates were often overgrown, making it difficult to distinguish between individual colonies, and to

accurately calculate SD and SR. Where sampling was done by means of petrifilm, fungal colonies could not be clearly distinguished from packing line dirt, while bacteria were not visible on the film at all. Furthermore, low counts obtained from air samples seem to indicate that the sampling size was not sufficient to give a true indication of the numbers of microorganisms that occur in the air. Values for SD and SR should therefore be seen as indicative rather than quantitative values.

Low SD was observed for fungi in both packhouses (table 2). Highest SR was observed for crates (packhouse 1 and 2), chlorine dump tank (packhouse 1 and 2) and trolleys (packhouse 2). Species of *Cladosporium* and *Trichoderma* were found to be dominant in both packhouses.

Compared to that of fungi, bacterial SD was slightly higher for both packhouses (table 2). Highest SR was observed for crates and trolleys (packhouse 1 and 2), and the chlorine dump tank (packhouse 2).

The only other significant trend that could be identified was a dramatic decrease in numbers of fungi and bacteria in Tag wax during the second survey in both packhouses.

Table 2
Species diversity and -richness obtained at different sites in Packhouses 1 and 2

Packhouse	*Sampling*	*Packhouse survey*			
		Replicate 1		*Replicate 2*	
		Species diversity	*Species richness*	*Species diversity*	*Species richness*
Packhoue 1	Crates (per cm²)				
	Fungi	0,2	8,5	0,2	7,7
	Bacteria	0,4	7,9	0,4	5,0
	Chlorine Dump tank (per m*l*)				
	Fungi	23,3	986,7	–	–
	Bacteria	3,3	3,3	–	–
	Brush rollers (per cm²)				
	Fungi	0,03	1,8	0,03	1,8
	Bacteria	0,03	1,8	0,05	1,8
	Wax (per m*l*)				
	Fungi	30	620	13,3	7,0
	Bacteria	16,7	36,7	3,3	3,3
	Sorting rollers 1 (per cm²)				
	Fungi	0,003	0,06	0,005	0,03
	Bacteria	–	–	–	–
	Sorting rollers 2 (per cm²)				
	Fungi	0,003	0,06	0,005	0,03
	Bacteria	–	–	–	–
	Trolleys (per cm²)				
	Fungi	0,02	0,9	0,1	1,5
	Bacteria	0,5	1,5	0,4	12
	Air sample (per *l*)				
	Fungi	0,01	0,2	0,02	0,1
	Bacteria	0,01	0,01	0,01	0,01
Packhouse 2	Crates (per cm²)				
	Fungi	0,2	1,1	0,2	6,8
	Bacteria	0,4	1,9	0,4	12
	Chlroine Dump tank (per m*l*)				
	Fungi	13,3	33,3	26,7	292,3
	Bacteeria	45	3000	50	250
	Brush rollers (per cm²)				
	Fungi	0,02	1,8	0,03	1,8
	Bacteria	0,02	1,8	0,05	1,8
	Conveyor rollers (per cm²)				
	Fungi	0,003	0,1	0,005	0,2
	Bacteria	–	–	–	–
	Wax (per m*l*)				
	Fungi	23,3	86,7	13,3	33,3
	Bacteria	13,3	20	13,3	13,3
	Sorting rollers (per cm²)				
	Fungi	0,004	0,01	0,004	0,3
	Bacteria	–	–	–	–
	Trolleus (per cm²)				
	Fungi	0,1	4,1	0,2	1,3
	Bacteria	0,1	0,3	0,2	8,2
	Air sample 1 (per *l*)				
	Fungi	0,02	0,06	0,01	0,05
	Bacteria	0,02	0,04	0,02	0,08

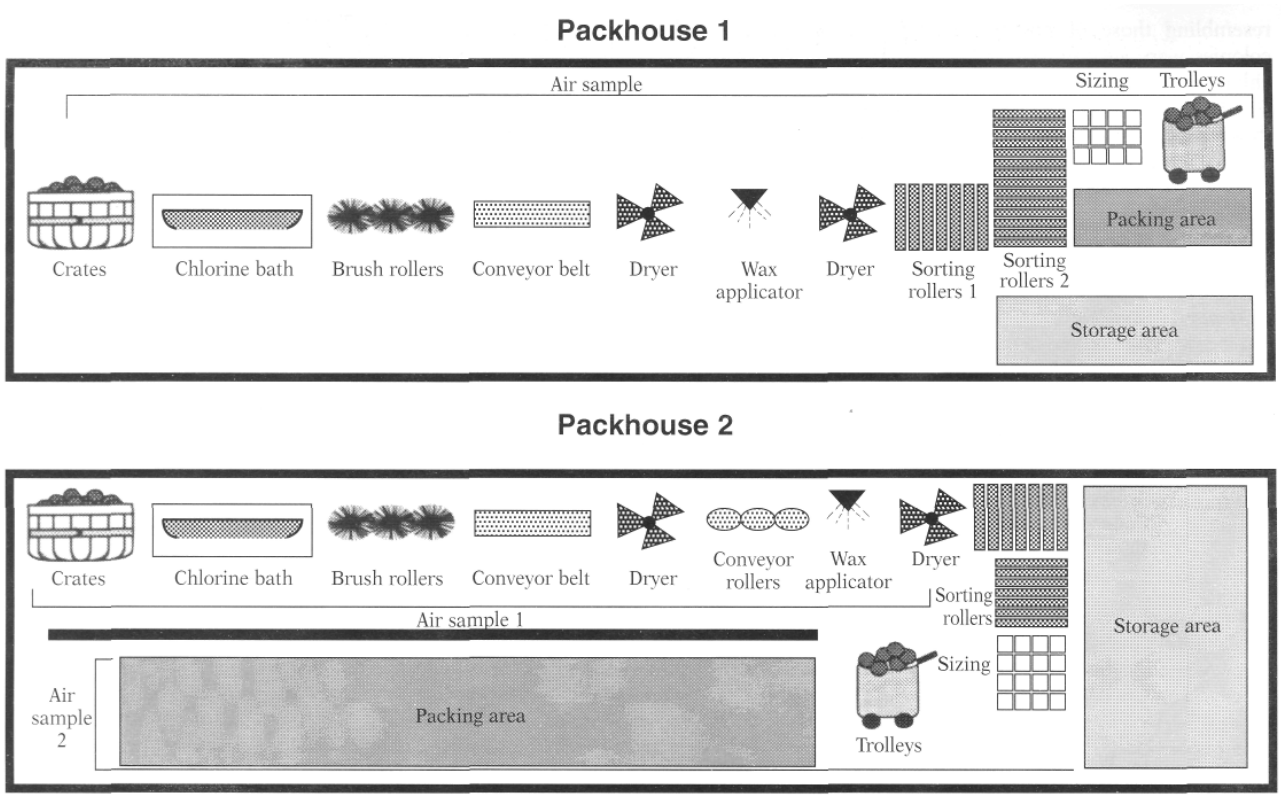

Figure 1
Schematic representation of Packhouse 1 and 2

Although yeasts were monitored in the packhouse, SD and SR could not be determined due to a high incidence of fungal contamination.

DISCUSSION

Although considered to be of minor importance, *R. stolonifer* has been implicated in SE (Darvas *et al,* 1987) and could therefore be involved in secondary infection of fruit. Populations of other pathogens associated with post-harvest diseases of avocado were not apparent in this study. Because post-harvest pathogens of avocado mostly infect in the field and remain latent until fruit ripening (Sommer, 1982), the possibility exists that pathogen populations do not build up to traceable levels in the packhouse. Another explanation could be that pathogens occur in lower numbers than other micro-organisms in the packhouse and are easily overgrown by these organisms so that they would not be detectable by methods used in this study.

Consistent with results from previous studies (Spotts & Cervantes, 1986; 1992), crates and dump tank water were found to carry high numbers of microorganisms, indicating unsanitary conditions. In addition, relatively high counts of fungi and bacteria were found on trolleys. Although all packhouse equipment, floors and walls should be sanitized on a routine basis (Beuchat, 1995), more regular sanitation of the afore-mentioned areas would be advisable.

The decrease in fungal and bacterial counts in Tag wax from the first to the second surveys; possibly indicate regular replacement of the wax as standard packhouse operation (Marius van der Merwe, Westfalia Estates, personal communication).

This study clearly indicates a need for optimisation of sampling methods used for avocado packhouses. Various methods, including dilution plating, should be evaluated on a trial and error basis. Only then will it be possible to obtain an accurate assessment of the spread of pathogens and other microorganisms through the packhouse.

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