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Effect of postharvest practices on the culturable filamentous fungi and yeast microbiota associated with the pear carpoplane

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ABSTRACT

Information regarding the filamentous fungi and yeast microbiota on pear surfaces is limited when compared to other fruits such as grapes and apples. The effect of commercial postharvest practices on pear fruit surface microbiota and species composition is not known, particularly in terms of the presence of postharvest pathogens and potential biocontrol microorganisms. Pear fruit were collected at harvest in the orchards of four commercial farms, after harvest at a communal pack house following chlorine drenching and after modified atmosphere storage. Microbiological analysis showed that during season one the fungal populations on pears from the four farms were significantly lower after CA storage when compared to populations of orchard pears, however during season two, the opposite trend was observed. The yeast populations were either significantly higher or similar after CA storage compared to the orchard pear counts during both seasons. Commercial drenching led to either an increase or reduction in the filamentous fungi and yeast populations, however a definite trend could not be observed. The postharvest practices decreased the number of viable morphologically different yeast and filamentous fungal species. A total of 16 yeast and 24 filamentous fungal species were isolated. A 76% dominance of Ascomycetes was observed. Known postharvest pathogens *Penicilium commune* and *Penicillium crysogenum* were present after CA storage. Potential known biocontrol organisms included *Aureobasidium pullulans, Cryptococcus* sp. and *Sporobolomyces roseus*. Knowledge generated could contribute to development of commodity-specific supply-chain management systems and biocontrol strategies based on scientific data to reduce pear fruit losses and for quality control purposes.

1. Introduction

Pear fruit has a protective tough outer skin (epicarp) which functions as a barrier against the entry of plant pathogenic microbes (Kalia and Gupta, 2006). The fruit surface represents an environment where microbes must not only be able to survive but also compete with other inhabitants. Resident microorganisms have the ability to attach, survive, multiply and grow on these surfaces forming part of the carpoplane microbiota (Hanklin and Lacy, 1992; Lima et al., 2015). The nutrients for microbial growth is mainly provided by exudates and in the case of wounding, wound exudates. Pear fruit surface wounding during the postharvest processing practices could facilitate the entry of residing microbes and colonisation of the less protected internal soft tissue (Kalia and Gupta, 2006) . This could contribute to a change in the microbe diversity and composition and ultimately to fruit decay if there are postharvest pathogenic species present on the carpoplane (Van Deventer, 2011). Should a large plant pathogen antagonistic population reside on the fruit surface, the reverse may be true due to their protective ability against plant pathogens (Janisiewicz et al., 2014).

The carpoplane microbiota orginates from inoculum introduced from soil, insects, the air, animals, rain (Beuchat, 2002), or the environment within the tree canopy or the orchard, or later in the packhouse, or during interventions such as harvesting, transportation, washing or chemical treatment or through equipment or contact surfaces (Thompson, 2008). Generally all these sources or processes have an effect on the microbial diversity as some microbes are better adapted to survive the adverse environmental conditions they are exposed to throughout the postharvest system (Kubo et al., 2012).

Resident fungal microbiota on pear fruit surfaces included species such as *Rhizopus*, *Aspergillus*, *Penicillium*, *Eurotium*, and *Wallemia*, while the dominant yeasts included species such as *Saccharomyces*, *Zygosaccharomyces*, *Hanseniaspora*, *Candida*,

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Debaryomyces and *Pichia* (Kalia and Gupta, 2006). Within the postharvest environment *Penicillium* spp. remains the dominant pathogenic group on pome fruit (Andersen et al., 2004; Louw and Korsten, 2014). On pear fruit *Penicillium expansum*, *P. crustosum*, and *P. solitum* have been described as the most important postharvest pathogens causing decay in South Africa (Louw and Korsten, 2014).

Knowledge of the biodiversity and ecological function of yeasts is limited compared to those of other microorganisms (Herrera and Pozo, 2010). The known ecological functions of yeasts include amongst others: the production of antifungal metabolites that kill or inhibit pathogenic fungi, the production of extracellular polysaccharides, which aids and enhances their survivability and restrict the growth of pathogens and their ability to utilize nutrients rapidly and proliferate at a faster rate than pathogens (Little and Currie, 2008).

Within specific habitats yeast species face numerous challenges such as temperature, pH, and UV light as well as osmotic and oxidative stresses (Sui et al., 2015). These factors will eventually determine the inclusion, persistence and relative abundance of a particular yeast species in that habitat. To persist and become a resident on the fruit carpoplane the organism must be able to be deposited, attach, be capable of growing, colonising and successfully compete and survive to ensure further dispersal (Sui et al., 2015). The use of yeasts for the biological control of field and postharvest diseases has been reported frequently in the literature (Liu et al., 2013; Janisiewicz et al., 2014). Moreover, known biocontrol yeast strains are based on yeasts originally isolated from the fruit microbiota (Janisiewicz et al., 2014).

The aim of this study was to determine the effect of key commercial postharvest practices on the culturable filamentous fungi and yeasts on pear fruit surfaces to determine the biocontrol and decay potential within this microbiota. Knowledge of the changes in viable microbial populations due to post harvest practices will aid development of commodity-specific supply-chain management systems and biocontrol strategies based on scientific data to reduce pear fruit losses and for quality control purposes.

2. Materials and methods

2.1. Sites and process flow

Pears were collected from four Global G.A.P. accredited commercial pear production farms near Grabouw in the Western Cape, South Africa. After harvesting in the orchards the pears are transported in crates on trucks to a central packhouse (within a 30 km radius). At the packhouse the pears are drenched once with water containing 75 ppm chlorine. The chlorine drench water is managed according to standard commercial practices according to pome fruit postharvest guidelines and the pH adjusted to 6.5-7.7 as required in order to provide high concentrations of microbicidal hypochlorous acid. After drenching the pears are stored in controlled atmosphere (CA) for 12 weeks, at oxygen levels set at 2–5 kPa, carbon dioxide levels at 2–5 kPa and temperature set at −0.5 °C (with variation at front and back of the room 0.2 °C to −0.7 °C), and subsequently exported.

2.2. Sample collection

Pyrus communis cv Packhams Triumph pears were collected at harvest in the orchards of the four farms, after harvest following chlorine drenching at the communal packhouse as well as after CA storage for two consecutive seasons during 2013 and 2014. During sampling environmental conditions (i.e. temperature and humidity) were recorded in the orchards. Twenty fruit in total were collected in a random selection strategy from five trees in four rows (regarded as replicates) per orchard for each of the four farms. After chlorine drenching at the packhouse five pears each were sampled randomly from four crates (five pears, four replicates, n = 20) for each farm. Similarly, following CA storage five pears each were sampled randomly from four bulk bins (five pears each, 4 replicates, n = 20) for each farm. Sampled pear fruit were placed in labelled brown paper bags and kept in cold storage (±5 °C) until shipment for laboratory analysis within 24 h.

2.3. Sample processing

Individual pear samples (five pears each) were placed in 500 mL of 0.25× Ringer's solution amended with 0.02% Tween 80 (Sigma, Johannesburg) and sonicated in an ultrasonic bath (EUmax®, Labotec., Johannesburg) for 5 min to facilitate detachment from the pear fruit surfaces. Volume displacement (vd) was also recorded for each of the fruit and converted to area (cm^2) using the following equation ($A = 4.84\,[(vd)^{1/3}]^2$ (De Jager, 1999; Collignon and Korsten, 2010). Wash liquid from the sonicated samples were then filtered through a sterile nitrocellulose membrane with a pore size of 0.45 μm (Sartorius Stedim, Biotech, Germany). The filters were aseptically cut into smaller pieces, added to 9 mL sterilized peptone buffered water (Biolab Diagnostics, Johannesburg) and vortexed to remove the organisms from the filter membrane. Ten-fold serial dilutions of each sample was prepared, a 100 μL of each dilution plated in duplicate onto malt extract agar (MEA) (Merck, Johannesburg) and incubated at 25 °C for five days. Filametous fungal and yeast population counts were recorded and data was converted to $\log_{10}(x + 1)$ CFU/cm^2.

2.4. Isolation of filamentous fungi and yeasts from pear fruit surfaces

Filamentous fungi and yeasts colonies were isolated randomly from MEA culture plates based on different phenotypic characteristics and prevalence as described by Janisiewicz et al. (2014). Isolated colonies were purified by triple streaking, preserved in 15% (yeasts) and 10% (filamentous fungi) glycerol (Merck, Johannesburg) and stored at −70 °C.

2.5. Molecular identification of filamentous fungal and yeast isolates

The purified filamentous fungi and yeast isolates were cultured on MEA plates and the DNA extracted using the ZR fungi/bacterial DNA miniprep™ kit (Zymo Research, USA) according to the manufacturer's protocol. The DNA concentration of individual isolates was determined using the Qubit® 2.0 fluorometer (Invitrogen, Life Technologies, USA). Each PCR reaction contained 15–20 ng/μL template DNA, 10 μM of each primer, 5U/μL MyTaq™ DNA Polymerase (Bioline, USA), 5× MyTaq™ reaction buffer (Bioline, USA, containing dNTPs, $MgCl_2$, stabilizers and enhancers), and nuclease free water (Thermo scientific) in a total volume of 25 μL. For fungal and yeast isolate identification, universal primers Internal Transcribed Spacer (ITS) 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used for PCR amplification (Fell et al., 2000). The PCR analysis was performed using a BioRAD T100™ thermal cycler (BioRAD). The PCR conditions were as follows: an initial denaturation of 95 °C for 2 min, followed by 35 cycles of 30 s at 94 °C, 45 s at 57 °C, and 90 s at 72 °C, with a final extension period of 7 min at 72 °C. The amplified PCR products were purified from a 2% (w/v) agarose gel using a Geneclean kit (Zymo Research, California, USA—manufacturer's protocol). The PCR products were directly sequenced with both the forward (ITS1) and reverse (ITS 4) primers using BigDye Terminator v3.1 cycle sequencing on an ABI 3500XL sequencer (Inqaba Biotec, Pretoria, South Africa). The