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Penicillium air mycoflora in postharvest fruit handling environments associated with the pear export chain

Ilonka Scholtz, Nazareth Siyoum, Lise Korsten*

University of Pretoria, Department of Plant and Soil Sciences, Plant Pathology Laboratories, Pretoria, Gauteng, South Africa

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ABSTRACT

Penicillium is a well-known airborne fungal contaminant that is prevalent in indoor air. In this study, the total air mycoflora was determined in postharvest fruit handling environments. The study included eleven indoor environments from the packhouse to the final retail outlet. Standard active and passive air sampling methods were used over a period of three years to obtain a profile of air quality. A total of 6047 and 5849 *Penicillium* colonies were counted of which 1123 and 508 isolates were obtained using active and passive sampling methods respectively. Ultimately, 25 dominant *Penicillium* spp. were identified from active air samples. The five most prevalent species isolated were: *P. glabrum* (31.88%); *P. expansum* (14.18%); *P. crustosum* (13.42%); *P. chrysogenum* (10.35%) and *P. brevicompactum* (10.25%). Furthermore, a total of 22 *Penicililum* spp. were isolated from passive air samples with *P. glabrum* (23.72%); *P. italicum* (16.45%); *P. brevicompactum* (14.22%); *P. crustosum* (13.80%) and *P. chrysogenum* (11.76%) being most prevalent. The presence of pathogenic *Penicillium* spp. in the air of fruit handling environments was profiled. Counts of total air mycoflora were significantly higher in the re-pack facilities than in all other environments sampled and are significantly higher than the proposed baseline threshold value. This study clearly shows the importance of air quality in facilities that regularly handle different fruit types. Re-pack and retail facilities should therefore be cleaned more effectively to reduce the potential air inoculum that can induce decay of fruit at the market-end. Finally we propose an air quality standard for fresh produce environments.

1. Introduction

Aerosols are microscopic solid or liquid particles that are suspended in air (Pasquarella et al., 2000). Airborne particles containing living organisms known as bio-aerosols often represent a contaminant of indoor air (Kim et al., 2007). Viruses, fungal propagules, bacterial endospores, plant pollen and segments of plant tissues are constitutes of a bio-aerosol (Kalwasińska et al., 2012). Bio-aerosols are mainly sampled to evaluate the potential of environments to contaminate products due to the fallout potential of an area (Pasquarella et al., 2000). Physical (particle size, -density and -shape) and environmental factors (air currents, relative humidity and temperature) are the main parameters that affect bio-aerosol settling (Stetzenbach et al., 2004).

Air currents have been recognised as the principal physical factor responsible for the detachment and dispersion of fungal propagules, particularly *Penicillium* and *Aspergillus*, in indoor environments (Sivasubramani et al., 2004). Airborne fungal contaminants have progressively gained importance as health hazards and are also known to contribute to postharvest losses of fresh produce (Kakde and Kakde, 2012). The likelihood of *Penicillium* infection to occur through inoculum presence spores settling onto fruit surfaces and subsequent infection increases during optimal environmental conditions (Amiri and Bompeix, 2005). Other factors that influence the probability of *Penicillium* infection are the presence of high inoculum levels and environmental conditions favouring infection (condensate, vapour, temperature fluctuation), as well as respiration rate of fruit stored together, age of fruit and wounding frequency. The increased probability of *Penicillium* infection subsequently contributes to substantial postharvest losses due to decay (Amiri and Bompeix, 2005).

In general, the need to decrease pathogenic airborne microorganisms and their fallout has increased in various industries such as agriculture, health care, production and food processing plants (Shintani et al., 2004). Data accumulation and knowledge of microorganisms present within the air is of great importance before attempting to reduce microbial fallout (Yau et al., 2012). The

* Corresponding author.
E-mail address: lise.korsten@up.ac.za (L. Korsten).

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approaches used for setting or reviewing microbial air quality standards vary between countries and industries. Differences between methodology, monitoring strategies, data interpretation and levels deemed acceptable prevent comparative analysis (Pasquarella et al., 2012; Spickett et al., 2013). The need for the establishment of a national threshold value of fungal loads in the air (pathogenic and non-pathogenic fungi) in both indoor and outdoor air environments have previously been reported (Fischer and Dott, 2003). Fungi have been considered as useful biological indicators of indoor air quality to monitor the health of an environment or ecosystem (Cabral, 2010). A method to distinguish air quality in fruit handling environments has not been developed.

Active and passive air sampling methods are commonly used to monitor microbial aerosols in different environments (Altunatmaz et al., 2012; Pasquarella et al., 2000). Active air sampling is used to determine total fungal loads in a specified volume of air, while passive air sampling provides a better representation of mycoflora that has the potential to settle onto fruit surfaces. The aim of this study was therefore to first determine the total air mycoflora in various postharvest fruit handling environments along the pear export chain using active and passive air sampling methods. This study further aimed to identify the ratio of *Penicillium* spp. within the air myco-environment, group the species. according to their postharvest disease causing potential and to develop a potential decay indicator (pdi) to monitor pathogenic *Penicillium* spp. in fresh produce environments. Finally to propose a more realistic air quality standard for fresh produce handling facilities.

2. Materials and methods

2.1. Sampling approach and selected methods

Air myco-environments were monitored by using both active- and passive air sampling methods. Active air sampling was done by using an automated SAS SUPER 100™ Compact Surface Air System® (PBI International, Italy) to collect airborne fungi by means of impaction on 65 mm malt extract agar (MEA) (Merck, Johannesburg) contact plates. Passive air sampling was performed by exposing 90 mm MEA settle plates to the environment for 20 mins. Air sampling was conducted locally (SA) and internationally (UK) over three years to achieve a reasonable profile of air quality in various facilities following the pear export chain. Local sampling areas included two commercial large scale pear packhouse facilities, at least three regular- and controlled atmosphere storage rooms at each packhouse and an export fruit container before packing pallets with pear fruit cleared for export. International sampling areas sampled included the same fruit export container after opening on the overseas market (average of 21 days later), two fresh fruit re-pack facilities handling the exported fruit, at least two cold storage rooms containing amongst others pears at each re-pack facility, a distribution centre (various fruit and vegetables ready for retail sales) and a retailer outlet where the pears are temporarily stored and displayed for sales. Between nine and twenty air samples were collected (depending on the size of the room) at randomly selected points in all the facilities sampled. Points and number of samples collected per room were the same for active and passive sampling. Active air samples were collected from the corners of a specific room and the sampler was directed inward towards the centre of the facility. The automated air-sampler was always held at the same height (one metre) above the ground. A total of 1380 air sampling plates were obtained (834 from active air sampling and 546 from passive air sampling). Eleven facilities were selected for sampling in the supply chain. Table 1 indicates the number of samples taken for active and passive air in each major facility sampled following the pear export chain as well as the average temperature and humidity typically encountered in these environments.

2.2. Sample processing and determining total air mycoflora loads

All air sampling plates were incubated at 25 °C for approximately five days directly after sampling and transportation. Colony forming units per cubic metre of air (CFU/m^3) and per 20 min (CFU/20 min) were calculated based on total viable counts (TVC) of mycoflora for active and passive air sampling respectively. Even though no international consensus exists on limits for microbial contamination, the standard index of microbial air contamination in environments as described by Pasquarella et al. (2000) was used in this study as benchmark value to determine the microbial air quality. *Penicillium* colonies that were visually distinctive from each other (colour, texture, reverse colony colour etc.) were counted and recorded for each sample. Conidia was isolated from a single visually distinctive *Penicillium* isolate per sample and purified on 65 mm MEA plates for further identification.

2.3. Identification of Penicillium species and data analysis

Methods used for quantification, isolation, grouping and identification of isolated *Penicillium* spp. were described in Scholtz and Korsten (2016). *Penicillium* colonies were counted and isolates were grouped according to similar cultural characteristics such as: colony size, colour, texture and formation; mycelia coloration and formation; reverse plate coloration and the production of exudates. DNA was extracted from representative isolates using DNeasy® Plant Mini Kit from Qiagen (Southern Cross Biotechnology, Johannesburg). Amplification of a partial beta-tubulin

Table 1
Areas sampled in the pear export chain and number of samples obtained from each area over three years as well as the average temperature and humidity typically encountered in these environments.

Facility sampled	Number of active air sampling plates	Number of passive air sampling plates	Average temperature (°C)	Average relative humidity (%)
Packhouse facilities (SA)	180	80	19.6	65.00
CA coldstorage (SA)	144	68	3.85	79.60
RA coldstorage (SA)	200	88	2.9	73.75
Container before export (SA)	13	13	13.75	65.55
Container after export (UK)	15	15	8.5	79.9
Receival area (UK)	18	18	7.8	72.9
Re-pack facilities (UK)	57	57	20.9	55.3
RA coldstorage (UK)	102	102	4.6	71.1
Distribution centre (UK)	69	69	11.2	66.5
Retail storage (UK)	18	18	19.1	45.5
Retail display (UK)	18	18	19.1	68.5
Total	834	546		

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