

**IVth INTERNATIONAL SYMPOSIUM ON  
POSTHARVEST PATHOLOGY**

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**BOOK OF ABSTRACTS**

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# ORAL PRESENTATIONS

## 2 Optimizing protocols to assess the efficacy of nanoparticles as antifungal agents

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**Keywords** zinc oxide, postharvest, turbidimetry, predictive mycology

### ABSTRACT:

Several types of fungal growth inhibition assays have been developed in the past for synthetic antifungal compounds. However, most of the traditional antifungals have now been reviewed or banned in postharvest disease management. A class of materials which have recently received considerable attention as antifungals are metallic nanoparticles. Antifungal tests, therefore, need to be optimised in order to be suitable for this new category of compounds. The properties of Zinc Oxide nanoparticles (ZnO NPs) at concentrations ranging from 0 mM up to 18 mM were assessed against selected fungal isolates, i.e. *Aspergillus niger* and *Aspergillus terreus*, by: (i) agar disk-diffusion test, (ii) mycelium growth inhibition and (iii) turbidimetry techniques. All the techniques were investigated by making the proper adjustments to the protocols and to the experimental design, in order to obtain the most reproducible and consistent results upon repeated experiments. Potato Dextrose Agar (PDA) obtained from raw potatoes was used for all the techniques, fungal spores suspensions were prepared starting from  $10^5$  spores/mL. The binary response (growth/no growth) and the radial growth against time were recorded for the disk-diffusion and the mycelium inhibition test respectively; while, for the turbidimetric assay, plots of optical density (O.D.) against time were produced. The diameter growth rate and the O.D. rates were analysed by mathematical prediction models. This was performed by non-linear regression analysis. The obtained results demonstrate that methods such as disk-diffusion assay may not be applied to nanoparticles as *in situ* degree of inhibition and NPs concentrations cannot be precisely assessed. Techniques such as mycelium growth inhibition and turbidimetric assay can instead be considered and complemented by predictive mycology tools.



## 8 Effects of Putrescine, acetic acid and hot water treatments on quality and shelf life of apricot fruit

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**Keywords** Apricot fruit; putrescine; acetic acid; hot water

### ABSTRACT

Apricot is a highly perishable fruit due to its high water content and high respiration rate after harvest. Reducing the ripening rate and thus delaying the senescence of apricot fruit in order to increase its shelf life is a desirable trait for apricot fruit. In this study, the effects of coating apricot fruits with Putrescine (Put) (0.5, 1.5 and 2 mM), Acetic Acid (AA) (1 and 2 %) and hot water (HW) (20, 45 and 55 °C) on postharvest quality improvement and also on the increase in apricot fruit shelf life were investigated. Following treatments, fruits were stored in a commercial warehouse at 4 °C and 80 ± 5% relative humidity for 40 days. Fruits traits such as decay percentage, weight loss, firmness, total soluble solids (TSS), titratable acidity (TA), TSS/TA, pH, ascorbic acid content and skin color ( $L^*$ ,  $a^*$ ,  $b^*$ ) were monitored at 10 days intervals up to 40 days. Results showed that in all treatments, fruits weight loss occurred over storage time. The weight loss was the highest in untreated fruits (control). After 40 days of storage period, the fruits weight loss, decay and softness were the lowest in fruits treated with 55 °C HW or 2 mM Put, while the highest were occurred in untreated fruits. Fruits total soluble solids (TSS) increased during the storage time. The highest fruits TSS content were observed in untreated (control) fruits. Titratable acidity (TA %) decreased during storage. The highest fruits TA % was observed in fruits treated with HW and Put, and the lowest was in control fruits. The  $L^*$ ,  $a^*$  and  $b^*$  values of skin color in all treated and untreated fruits increased during storage. In general, the results of this study support the beneficial effects of putrescine and hot water application on postharvest improvement of apricot fruit quality

## 12 Effect of cinnamon essential oil- loaded nanostructured lipid carriers (NLC) against *Penicillium citrinum* and *Penicillium expansum* involved in tangerine decay

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**Keywords** Cinnamon essential oil, Nanostructured lipid carriers, *Penicillium citrinum*, *Penicillium expansum*, Decay, Tangerine.

### ABSTRACT

The effect of cinnamon essential oil (CEO) at 0.3 and 0.6 g/L concentrations loaded in the nanostructured lipid carriers (NLC) against *Penicillium citrinum* and *Penicillium expansum* involved in tangerine decay was investigated. The minimum inhibitory concentrations (MIC) of the CEO and CEO-loaded NLC against both *P. citrinum* and *P. expansum* were about 0.425 and 1 g/L, respectively. Moreover, the minimum fungicidal concentrations (MFC) of the CEO and CEO-loaded NLC were 0.675 and 1.5 g/L, respectively and the values were approximately the same for *P. citrinum* and *P. expansum*. The tangerines were inoculated with *P. citrinum* and *P. expansum* spores and percentage of the infected wounds and chemical characteristics changes of the fruits were evaluated during 25 d storage at 25 °C. The CEO-loaded NLC reduced the fungal spoilage of tangerine fruits during storage significantly (from 100% on the day 10 to 31% and 33% on the day 25 for *P. citrinum* and *P. expansum*, respectively). No detrimental effects on the chemical properties such as titratable acidity, pH and total solids due to the treatment with CEO-loaded NLC were observed. Therefore, it is possible to introduce this method as a new one for increasing tangerine shelf life.

## 15 Plasma activated water; an indirect method of cold plasma application for the control of *Colletotrichum* species associated with anthracnose of avocado

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**Keywords** Postharvest pathogen control

### ABSTRACT

Cold plasma has been accepted as an effective method for decontamination of food from microorganisms, such as *Escherichia coli* and *Salmonella*. In addition to its direct sterilisation ability, cold plasma can be used to produce Plasma Activated Water (PAW) which also demonstrates excellent antibacterial properties. In this study, we tested PAW on an important postharvest pathogen, *Colletotrichum alienum*, isolated from avocado. Tap water, deionised water and distilled water were used to generate PAW by treating each with gliding arc plasma for up to 60 minutes. A spore suspension of *C. alienum* ( $1 \times 10^6$  conidia/ml) was then added to one of the three PAW solutions in one of three ratios: 1:1, 1:2 or 1:3 (spore suspension: PAW) and the percentage spore germination was measured every three hours. The most effective treatment, as measured by reduction in spore germination, was PAW produced following treatment of deionised water and distilled water with plasma for 60 minutes, at all three ratios, with a significant reduction in germination occurring 3 hours after exposure to PAW. PAW produced following treatment of deionised water for 30 minutes had significantly higher spore germination, except at the highest ratio (1:3) where spore germination was similar to that of the 60 minute treatments. Interestingly, when incubated for up to 24 hours, all of the combinations tested reduced spore germination except the 1:1 ratio of PAW produced from tap water for 30 minutes. These results indicate that the efficacy of PAW depends on both the ratio of spore suspension to PAW and the type of water used to generate the PAW, as well as the length of exposure to PAW. This study demonstrates the potential of PAW for the control of anthracnose in avocado, and provides a novel method for fruit treatment to reduce postharvest diseases.

## 16 BioSpectra 100SC: A new biorational fungicide to control postharvest diseases of fruits

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**Keywords** biofungicides, decay control, exempt-from-tolerance, natamycin, pimaricin

### ABSTRACT

BioSpectra 100SC is a newly registered biorational fungicide for postharvest use in the US with exempt-from-tolerance. The active ingredient, natamycin, is a naturally occurring antifungal agent produced by fermentation of *Streptomyces natalensis*. It binds to ergosterol in fungal cell membranes and causes leakage of the cell wall, leading to cell death. The effectiveness of the fungicide was evaluated when applied alone and/or in combination with other postharvest fungicides on various fruit crops. In laboratory and packingline studies, BioSpectra 100SC effectively controlled the most important postharvest diseases of citrus, green mold and sour rot incited by *Penicillium digitatum* and *Galactomyces citri-aurantii*, respectively. It effectively controlled green mold caused by resistant strains of *P. digitatum* to thiabendazole, fludioxonil, and pyrimethanil. Mucor rot, a newly emerged postharvest disease on mandarin, was also significantly reduced. However, reduced efficacy was observed on late season lemons and oranges. Further studies are being conducted on various fruit crops, including stone fruits, sweet cherries, pome fruits, and tropical fruits. Therefore, it is a valuable tool for broad spectrum decay control and fungicide resistance management in postharvest fungal pathogens.

## 18 Prevalence and distribution of *Penicillium* sp. and *Botrytis cinerea* in apple packinghouses across Washington State and their sensitivity to the postharvest fungicide-pyrimethanil

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**Keywords** Postharvest, Blue mold, Gray mold, fungicide resistance, *Malus domestica*

### ABSTRACT

Blue and gray molds, caused by *Penicillium* sp. and *Botrytis cinerea*, respectively, are the two major postharvest diseases of apple worldwide. To evaluate their prevalence in Washington State, decayed apples were collected from 140 grower lots (50 fruit per lot) distributed throughout 10 counties in central Washington from February to June of 2016 on fruit harvest in 2015. Blue- and gray-mold symptomatic fruit were used to collect *Penicillium* sp. and *B. cinerea* isolates on Petri plates containing potato dextrose agar (PDA) acidified to pH 3.5. Isolates were purified and identified using key morphological traits. *Penicillium* sp. was found in 148 (98.5%) of the 140 lots surveyed versus 114 lots (81.4%) for *B. cinerea*. Interestingly, only one of the two pathogens was predominant in a single grower lot. Differences in geographic distribution of the two pathogens were observed. In total, 860 *P. expansum* isolates and 780 *B. cinerea* isolates were collected, stored as purified cultures and tested for their sensitivity to the anilinopyrimidine-pyrimethanil using a germ tube elongation inhibition assay. The sensitivity of *P. expansum* was assessed on L-asparagine agar amended with 0.5 µg/ml pyrimethanil and on 0.5% sucrose agar amended with 1 and 25 µg/ml pyrimethanil for *B. cinerea* isolates. These doses were reported to discriminate resistant from sensitive isolates. The overall statewide resistance frequencies were 15.5 and 10.4% in *P. expansum* and *B. cinerea*, respectively. Resistant populations of *P. expansum* and *B. cinerea* were found in 51.8 and 54.2% of grower lots surveyed, respectively, at frequencies ranging from 2.6 to 100%. These two pathogens are continuous threats to apple storability and the increasing selection for populations resistant to pyrimethanil can result in major control failure unless adequate rotation programs are implemented quickly.

## 19 Increased anthocyanin in mango fruit peel are associated with cold and pathogen resistance

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**Keywords** *Colletotrichum gloeosporioides*, Abiotic stress, Antifungal activity

### ABSTRACT

Mango fruit of eighty-three collection cultivars from all over the world were inoculated with *Colletotrichum gloeosporioides* or stored for two weeks at sub-optimal temperature. Interestingly, red cultivars that accumulate high amount of anthocyanin in their cuticle were overall more resistant to both biotic (anthracnose) and abiotic (chilling) stress. In order to validate that anthocyanin and red color peel of mango fruit are correlated to biotic and abiotic tolerance, red and the green 'Shelly' mango fruit from the same trees were evaluated. Mango developing at the exterior of tree canopy is exposed to sunlight and acquires a red peel color on the sun-exposed side compared to the green peel fruit that develop within the canopy. Measurements of the red mango peel showed a significant increase in total anthocyanin, flavonoids and antioxidant accumulation, while the ripening parameters of both red and green mango fruit were similar. However, after three weeks of cold-storage in suboptimal temperature the 'green fruit' developed significantly more chilling injury symptoms than the 'red fruit'. Furthermore, 'red fruit' were found to be more resistant to a challenge of *C. gloeosporioides* inoculation and showed reduction in general decay incidence both at the red and green side of the fruit. Organic extraction of red fruit peel showed more antifungal activity and inhibition of spore germination when compare to green fruit. Thus, red mango fruit that accumulate high amount of anthocyanin showed increase resistance to chilling and pathogens. The results point to new agro-technological approaches to extend shelf life and quality of mango fruit

## 20 Postharvest microbiota dynamics of the mango fruit stem-end in response to light, temperature and during storage

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**Keywords** endophytic microbiota, anthocyanins, Chitinophagaceae bacteria

### ABSTRACT

Stem-end rots (SER) develop in mango fruit after harvest as fruit ripens and results in significant losses. SERs are caused by pathogenic fungi (e.g. *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Lasiodiplodia theobromae*, *Neofusicoccum Dothiorella*, *Phomopsis mangiferae*, and others) that endophytically colonize the fruit stem during fruit development in the orchard and remain quiescent until the onset of fruit ripening. The present work was conducted to characterize the endophytic microbiota in mango fruit-stems and study the effect of different postharvest treatments on the composition of bacterial and fungal populations in the fruit stem-end tissue. Microscopic observations showed that during the quiescent stage, fungi colonize the phloem of the fruit stem-end, and after switching to the pathogenic stage colonize the fruit parenchyma surrounding the stem, causing SER. Fruits that were subjected to high light in the orchard developed less SER after storage. These fruits accumulated anthocyanins leading to a red colored peel, which was correlated with resistance to both anthracnose and SER. The composition of microbiota in the stem-end of red and green mango fruit stored at different temperatures was determined using universal bacterial and fungal primers (16S and ITS respectively). Data analysis indicated that mango stem-end fungal and bacterial microbiota population is significantly modified during storage in response to different storage temperatures and in response to high light in the orchard. For example, in green, susceptible fruit that was not exposed to sunlight or during storage and fruit ripening Pleosporaceae (*Alternaria*) was the most abundant fungi. The change in fungal composition was accompanied with increased occurrence of SER. Prior to the development of SER, the increased amount of fungi was correlated with an increase in the abundance of chitin degrading Chitinophagaceae bacteria. Collectively, our results indicate that pre- and post-harvest treatments modify microbial populations in the stem-end of mango and may be associated with reducing postharvest SERs.

## 21 The MADS-Box transcription factor *Bcmads1* is involved in sclerotia production and pathogenicity of *Botrytis cinerea*

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**Keywords** *Botrytis cinerea*; MADS-Box Transcription factor; Sclerotia; Pathogenicity

### ABSTRACT

The MADS-box family of transcription factors is widely present and highly conserved in eukaryotes and has been demonstrated to be crucial for numerous life processes in model organisms. The function of the MADS-box largely remains to be understood in *Botrytis cinerea*, a notorious fungal pathogen with a wide host range. In the present study, the regulatory network of the MADS-box transcription factor *Bcmads1* in *B. cinerea* was characterized and it was demonstrated that *Bcmads1* plays an important role in sclerotia production and pathogenesis. Sclerotia serve as survival structures under stress conditions or act as the female parent in sexual reproduction and form in a light dependent manner. Results of RT-qPCR suggested that *Bcmads1* influenced sclerotia formation by regulating the expression of light-responsive genes. In addition, *Bcmads1* was involved in the pathogenicity of *B. cinerea*. Comparative proteomic analysis identified 55 differential proteins that are potential targets of *Bcmads1* including *Bcsec14* and *Bcsec31*, which are associated with vesicle transport. Knockout of *Bcsec14* and *Bcsec31* repressed virulence and protein secretion of *B. cinerea*. These results implied that *Bcmads1* may influence sclerotia formation by modulating light responsive gene expression and regulate pathogenicity by its effect on the protein secretion process.



## 22 Carbon regulation of environmental pH by secreted small molecule effectors modulates pathogenicity of fungi in ripening fruits

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**Keywords** Fruit Pathogenicity, Fruit Ripening

### ABSTRACT

Fruit pathogens can contribute to acidification or alkalization of the host environment. This capability has been used to divide fungal pathogens into acidifying and/or alkalizing classes. Here we show that diverse classes of fungal pathogens—*Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans*, and *Fusarium oxysporum*—secrete small pH-affecting molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonizing strategies and induce the expression of PACC-dependent genes. We show that in many organisms, acidification is induced under carbon excess, i.e. 175mM sucrose (the most abundant sugar in fruits). In contrast, alkalization occurs under conditions of carbon deprivation, i.e., less than 15mM sucrose. The carbon source is metabolized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification, whereas catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2*) results in the secretion of ammonia. Functional analyses of  $\Delta$ *gdh2* mutants showed reduced alkalization and pathogenicity during growth under carbon deprivation, but not in high-carbon media or on fruit rich in sugar, whereas analysis of  $\Delta$ *gox2* mutants showed reduced acidification and pathogenicity under conditions of excess carbon. The induction pattern of *gdh2* was negatively correlated with the expression of the zinc finger global carbon catabolite repressor *creA*. The present results indicate that differential pH modulation by fruit fungal pathogens is a host-dependent mechanism, affected by host sugar content that modulates environmental pH to enhance fruit colonization.

**31 Evaluation of the efficacy of the bio-fungicide Timorex Gold in the control of anthracnose (*Colletotrichum gloeosporioides*) in avocados cv 'Hass', Cabildo, Valparaiso Region, Chile 2015**

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**Keywords** Post Harvest, *Antrachnose*, *Colletotrichum*, BioFungicides

**ABSTRACT**

One of the most important problems facing avocado industry is postharvest diseases. Losses of 36% due to anthracnose and 13% due to stem-end rot (SE) have been recorded on the overseas market (Bezuidenhout, 1983). The most common fungi associated with these diseases include *Colletotrichum gloeosporioides* (Penz.) Sacc. *Thyronectria pseudotrichia* (Schw.) Seeler, *Phomopsis perseae* Zerova, *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. and *Dothiorella aromatica* (Sacc.) Petr. & Syd. (Darvas, 1985). Reasonable control of the diseases has been achieved by preharvest sprays with copper oxychloride or benomyl (Darvas, 1982), or postharvest treatment with prochloraz (Darvas, 1985). However, the use of these fungicides is facing challenges due to visible spray residues on harvested fruit, build-up of pathogen resistance of continued use of these compounds and residues limitations. Exploring new alternatives for post-harvest disease control is required. Biological control as an alternative strategy for the control of postharvest diseases can be a complementary solution to prevent avocado fruit diseases. Two trials with post-harvest treatments were conducted in Chile between December 2014 and January 2015 considering natural inoculum from the field. For these trials were considered a CRD with 4 treatments and 6 replications (1 box of 4 Kg per replication per treatment). Evaluations were done before post-harvest treatments and after 42 days of cold storage at 0°C. Post-harvest pathogen isolations from rotten avocados and isolate identifications were conducted in the laboratory for post-harvest disease at the University of Chile. Timorex Gold was investigated in two different concentrations 1 and 2% compare to difenoconazole at 0.5%. All fungicides treatments were statistically different to the untreated control that showed disease incidence of 12.5 % after 42 days of cold storage at 0°C Timorex Gold 2% was the most effective treatment with 100% of control followed by Timorex Gold 1% and difenoconazole 0.5% with a level of 88,8 and 77,7 % of control respectively.

## 32 Postharvest grey mould development was suppressed by *Origanum dictamnus* oil vapours in tomato, pepper and eggplant fruit

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**Keywords** vegetables; essential oil; *Botrytis cinerea*; fungal growth

### ABSTRACT

Worldwide, significant postharvest losses of fruit and vegetables due to attack by microorganisms is evidence, while chemical applications is of great consumer concerns regarding food safety. Alternative sanitizers are explored, with natural compounds such as essential oils (EOs) to achieve scientific and consumer's interest for the preservation of fresh produce. In the present study, the efficacy of dittany (*Origanum dictamnus* L.) essential oil for the control of *Botrytis cinerea*, a common postharvest pathogen of three economically important vegetables, tomato, pepper and eggplant was examined. Pathogen development (vegetative or reproductive phase) in culture medium or in fruits was evaluated after treatment with dittany EO (0, 50, 100, 250 ppm) *in vitro* and *in situ* when stored at 12 °C and 95% RH during or following exposure to EO volatiles. *In vitro*, fungal development was completely inhibited by the application of 100 or 250 ppm of EO volatiles. In inoculated fruits, the application of 50 ppm EO resulted in suppressed disease development by reduced lesion growth and fungal sporulation, where increasing EO concentration led to greater effects. Pre-exposure of the three fruits to volatiles, before fungal inoculation, revealed reduced lesion growth, indicating that dittany EO probably caused induced resistance of fruits against the pathogen. Moreover, EO application did not affect quality-related characteristics of fruits in general, while skin lightness and pulp lightness of eggplant fruits were improved under the presence of dittany EO volatiles. Overall, the results suggest that dittany EO volatiles may be considered as an alternative food preservative treatment, significantly reducing or eliminating *B. cinerea* infection during fruit storage.

### 36 *Bacillus lipopeptides* for a novel postharvest disease control technology

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**Keywords** *Bacillus lipopeptides*; production; purification; biofungicide efficacy

#### ABSTRACT

Postharvest diseases caused by fungal phytopathogens account for about 40% of global food losses. Biocontrol strategies for postharvest disease are particularly attractive as they are environmentally benign and non-toxic. Current biocontrol strategies employ microorganisms as the biocontrol agents, with application under controlled environmental conditions mandatory for cell viability. Here, a novel biocontrol technology has been developed which instead uses microbially produced antifungal lipopeptides, namely fengycin and iturin. These do not require environmental control and further, have the potential to be manipulated into bespoke products with efficacy against specific phytopathogen targets. Antifungal lipopeptides were produced by *Bacillus amyloliquefaciens* DSM 23117 and quantified by reverse phase high pressure liquid chromatography (RP-HPLC). Using discrete ratios of ammonium to nitrate, the nitrogen source was shown to exhibit a significant influence on the production kinetics. The antifungal lipopeptide concentration in the culture supernatant exhibited a general downward trend with decreasing amounts of nitrate with a 2.6-fold difference in the maximum and minimum lipopeptide concentrations in the cultures containing only nitrate or ammonium respectively. A similar trend was observed in cell concentration which exhibited a 3.3-fold reduction, and a corresponding 1.3-fold increase in specific antifungal concentration, over the same range. Purification of the culture supernatant was effected by acid precipitation to pH 1, 2, 3 and 4. Precipitates were recovered by centrifugation and drying and the lipopeptides quantified by RP-HPLC. High recoveries (70-80 %) were attained at pH 1-3, with optimal purity at pH 3. Thin layer chromatography confirmed the low purity at pH 4 was due to contaminating proteins. Antifungal efficacy of a 10-fold concentrate was confirmed for *Alternaria brassicicola*, *Botrytis cinerea*, *Rhizopus stolonifera* and *Penicillium expansum* in zone clearing assays, demonstrating the successful production of a concentrated and partially purified biofungicide effective against postharvest phytopathogens.

## 37 Effect of Chitosan-based natural product (chitoplant) on the major postharvest diseases and the defence related enzymes in avocado

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**Keywords** Decay, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, Chitinase and  $\beta$ -1,3-glucanase

### ABSTRACT

Avocados are attacked by pathogenic fungi that penetrate the mesocarp tissue through the stem-end of the unripe fruit. This study is aimed at determining the eliciting effect of chitoplant or its combination on the defence related enzymes in artificially inoculated 'Fuerte'. In this study, concentrations of stand-alone chitoplant solutions (0.2% - 0.05% w/v) alone and their combinations with citral, thyme oil, and prochloraz were screened against *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*. The optimal concentration of 0.2% w/v chitoplant was applied *in vivo* as a stand-alone treatment or in combination with 50% prochloraz as a dip application. The fruit was incubated for 6 and 8 days at 20 °C (curative method), and 14 days at 10 °C, and afterwards held for 3 days at 20 °C (preventative method). The preventatively treated fruits were subjected to biochemical analysis to assess the induced defence related enzymes. Chitoplant solutions or their combinations exhibited lower inhibitory effects, except for 50% prochloraz + 0.2% w/v chitoplant that completely inhibited the radial mycelial growth of *C. gloeosporioides* and *L. theobromae*. The fruits treated (curative and preventative) with prochloraz alone and 50% prochloraz + 0.2% w/v chitoplant significantly reduced the stem-end rot and anthracnose incidence compared to other treatments. However, the activity of defence related enzymes such as phenylalanine ammonia-lyase (PAL) was lower in untreated control fruit and higher in prochloraz treated fruit (anthracnose and stem-end rot) respectively. The pathogenesis-related enzymes: Chitinase and  $\beta$ -1,3-glucanase, activities were lower in the untreated control fruit and higher in stem-end rot infected fruit treated with 50% prochloraz + 0.2% w/v chitoplant while in anthracnose infected fruit. Chitinase and  $\beta$ -1,3-glucanase activities were higher in prochloraz treated fruit. Conclusively, this study indicates that the presence of prochloraz in chitoplant solutions played a key role in the inhibition of mycelial growth of *C. gloeosporioides* and *L. theobromae* and the control of anthracnose and stem-end rot in 'Fuerte'.

### 38 The effect of thyme oil vapours exposure on defence mechanisms for the control of anthracnose in avocados (*Persea americana* Mill.)

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**Keywords** Postharvest decay, lipoxygenase, phenylalanine ammonia-lyase, chitinase

#### ABSTRACT

Avocado production in South Africa is export-driven, with the European Union being the most important market requiring high-quality fruit. However, postharvest losses, mostly due to anthracnose disease caused by *Colletotrichum gloeosporioides*, impact on the profitability of export consignments and the eating quality of the fruit. The effective use of thyme oil (TO) vapours (24 h), was investigated for efficacy during simulated cold storage conditions. The effect of TO vapours on gene transcripts of chitinase (CHI), phenylalanine ammonia-lyase (PAL) and lipoxygenase (LOX) during low-temperature storage was further investigated using real-time PCR. The gas chromatography-mass spectrometry analysis of specific phenolic acids, flavonoids, in the mesocarp of the fruit was also carried out and the untreated and prochloraz dipped fruit were included for comparison. Preventative TO vapours reduced the incidence of anthracnose by ~20% compared to prochloraz and ~64% when compared to the untreated control in both cultivars evaluated (Hass and Ryan). The TO vapours applied at harvest-induced the highest and earliest increases in the levels of CHI and PAL, and the lowest and delayed levels of the lipoxygenase genes. The PAL is the key gene in the phenylpropanoid pathway and its up-regulation consequently results in higher levels of phenolic acids (*p*-coumaric, caffeic and ferulic acids) and flavonoids (+)-catechin and (-)-epicatechin contents. The above data provide an important insight towards residual elicitation effect of TO when applied in the vapour phase for effective control of anthracnose. The application of TO in the vapour phase provides a potential alternative strategy for anthracnose disease control in avocados.

## 39 Citrus sour rot management by propiconazole drench application

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**Keywords** Citrus sour rot, propiconazole, drench

### ABSTRACT

Guazatine was previously used as drench application to control citrus sour rot before degreening. Its use has been banned because of technical reasons and difficulty in measuring its residue load on fruit. Propiconazole (PPZ) is a newly registered fungicide for postharvest treatment of sour rot on citrus. The aim of the study was to test the efficacy of the fungicide for the control of sour rot and green mould as a drench in citrus. Untreated lemon and clementine fruits were inoculated with a sensitive and less sensitive *Galactomyces* spp. (N=2) and a sensitive and less sensitive *Penicillium digitatum* (N=2) isolates. Isolates used were tested for PPZ sensitivity *in vitro*. Curative drench application of PPZ was studied to determine the timeframe (6 h, 14 h, 18 h, 24 h) in which the fungicide has to be applied when fruit arrives from the orchard in the packhouse. Additionally, the exposure time (1 min, 2 min, and 3 min) required to obtain adequate coverage of the fungicide applied to the fruit was tested. Average residue loaded on lemons and clementines was measured using liquid chromatography-mass spectrometry (LC-MS). Lemons infected with a sensitive *Galactomyces* spp. isolate had infection levels that indicated that PPZ can effectively control sour rot when treatment is applied at 600 mg/l within 14 h inoculation at 1 min exposure time (flow rate approximately 600 L/min), achieving 98.7% control. To reach maximum effect on clementines, treatment had to be applied within 6 h of infection. Green mould infection levels were similar to sour rot resulting in a PPZ treatment window in lemons and clementines of no more than 14 hours. Propiconazole residue was similar to the maximum residue limit (MRL) of 5 ppm immediately after treatment with 1 min exposure time, which is expected to be lower after degreening and also partially removed on the packline (i.e. through brushing).

**50 Curative activity against citrus postharvest green mold of composite hydroxypropyl methylcellulose-beeswax edible coatings with zeolites containing Ag-nanoparticles**

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**Keyw ords:** *Penicillium digitatum*, alternative postharvest disease control; nanotechnology

**ABSTRACT**

Two types of commercial Na-zeolites, LTA and faujasite (FAU) were prepared with different percentage of silver that was incorporated by ion exchange using a solution of AgNO<sub>3</sub>. The physico-chemical properties of the samples were established using different techniques as atomic emission spectroscopy with inductive coupled plasma (ICP-AES), X-ray diffraction (XRD), electronic microscopy (SEM, TEM and EDX) and X-ray absorption spectroscopy (XAS). It was observed that in the pore of the zeolites were formed nanoparticles and nanoclusters of silver. The curative activity of these materials was first evaluated as water dispersions in *in vivo* primary screenings by placing a drop (30 mL) in a rind wound of 'Valencia' oranges that had been inoculated 24 h before with a conidial suspension of *Penicillium digitatum*. Selected Ag-zeolites were incorporated as antifungal ingredients at different concentrations into composite hydroxypropyl methylcellulose (HPMC)-beeswax (BW) edible emulsions prepared with up to 10% total solid content with stearic acid as emulsifier and glycerol as plasticizer. Depending on the experiment, these coatings were applied 24 h after fungal inoculation to 'Valencia' or 'Barnfield' oranges as drops in rind wounds or covering the entire fruit. Controls included inoculated but untreated fruit and inoculated fruit treated with HPMC-BW without Ag-zeolites. After incubation of treated fruit at 20°C for up to 9 days, coatings amended with Ag-zeolites reduced the incidence and severity of green mold by more than 80%, showing high potential for effective disease control. Curative activity depended on Ag concentration and FAU zeolites were more effective than LTA zeolites, probably because of their larger pore apertures and higher Si/Al ratio that favored Ag diffusion. However, coatings with the highest Ag content were phytotoxic causing dark blemishes on the fruit rind. Further work is needed to optimize non-phytotoxic coatings with the highest curative activity.



## 52 Induced resistance as a sustainable tool to control postharvest diseases of fruit and vegetables

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**Keywords** biostimulants, elicitors, induced systemic resistance, resistance inducers, systemic acquired resistance

### ABSTRACT

A large part of harvested fruit and vegetables are lost after harvest, during handling, storage, transports, shelf life or at the consumer's home, mainly due to postharvest decay. During the last decades, there has been growing interest in the use of alternatives to synthetic fungicides for postharvest disease management. Induced resistance was among most recent alternatives investigated. This approach is regarded as an eco-friendly and safe mean to reduce postharvest losses while keeping the fruit free of synthetic fungicides residues. The natural resistance of fruit and vegetables can be increased by various means, such as biocontrol agents or their secreted elicitors. Alternatively, physical means, such as UV-C, ozone, and heat treatment, can prime plant resistance through abiotic stress. Moreover, various defense-related phytohormones, biological elicitors, non-organic elicitors, and volatile organic compounds have been shown to induce plant resistance. Recently, new technologies have enabled the evaluation of gene expression, such as quantitative real time PCR and the most recent next-generation sequencing, thus the quantification of physiological changes, have revealed new knowledge about pre-harvest and post-harvest induced resistance in response to various treatments. These tools allow optimization of postharvest application of the control means. The use of induced resistance as alternative to the synthetic fungicides meets the requirements of integrated disease management that is implemented in the EU through Directive 128/2009 on sustainable use of pesticides. Lastly, induced resistance usually leads to increased levels of nutraceutical compounds in the plant tissues, which often have antioxidant properties that are highly beneficial to humans.

## 54 Biochemical and mitochondria proteomic evidences on sodium silicate regulates energy metabolism and ROS production during induced resistance of muskmelon

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**Keywords** Muskmelons, mitochondria, induced resistance, energy metabolism, silicate, proteomic

### ABSTRACT

Muskmelon (*Cucumis melo* L.) is one of the major economically important crops in Northwest of China. However, the fruit is quite perishable and susceptible to postharvest rot caused by various fungi. Pinkrot caused by *Trichothecium roseum* is among the well-known postharvest pathogens of muskmelon in China. In this study, the effects of postharvest sodium silicate (Si) treatment on pinkrot and its possible mechanisms of action were investigated in muskmelon fruit (cv. Yujinxiang). Si treatments at 100 mM resulted in lower decay incidence and severity in treated fruit challenged with *T. roseum*. The treated fruit had higher activity of H<sup>+</sup>-adenosine triphosphatase, Ca<sup>2+</sup>-adenosine triphosphatase, succinic dehydrogenase and cytochrome oxidase. The treatment also kept a higher intracellular level of ATP. Additionally, the treatment induced the accumulation of hydrogen peroxide and promoted the generation of superoxide anion. The co-localization of fluorescent signals confirmed that mitochondria were involved in Si and pathogen induced ROS burst at 24 hours after treatment. Based on iTRAQ proteomics analysis, a total of 51 proteins were significantly altered in mitochondria observed in different treatment groups. These proteins mainly involved in respiratory chain, tricarboxylic acid cycle, glycolytic process, oxidation-reduction process, defense and stress responses, mitochondria carriers, protein and amino acids metabolism. Collectively, the results suggest that the priming of defense responses is involved in Si-induced disease resistance, in particular energy metabolism and ROS production of mitochondria.

## 59 New and emerging postharvest diseases in pome fruit in the Netherlands

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**Keywords** Storage; Pathogens; Fruit crops

### ABSTRACT

Postharvest diseases of apple and pear are caused by a range of fungal pathogens, and often result in significant economic losses during storage. In general, this group of pathogens infects developing fruits and remains quiescent without causing symptoms during the growing season and after harvest during the first weeks in storage. Typically, symptoms of disease occur after several months in cold storage with controlled atmosphere. Common pathogens causing such late post-harvest losses are *Neofabraea* spp. (lenticel rot or bull eye's rot); *Neonectria galligena* (Nectria rot; blossom-end rot); *Colletotrichum acutatum* species complex (bitter rot); *Phytophthora* spp., *Alternaria* spp., and *Stemphylium vesicarium*. A survey of apple and pear fruit lots in the Netherlands in 2012-2016 revealed a number of new and emerging postharvest diseases. The most important pathogens were *Cadophora luteo-olivacea* causing side rot on pears, and *Fibulorhizoctonia psychrophila* as the causal agent of lenticel spot on apples and pears. Also new problems with sooty blotch were noticed as well as several pathogens not earlier described in the Netherlands on apple or pear, such as *F. avenaceum* on pear and apple, *Neonectria candida* and *Neofabraea kienholzii* on pear, and *Colletotrichum godetiae* and *Truncatella angustata* on apple. The survey revealed strong season effects with different incidences and severities of the various pathogens in different years.

## **60 Abscisic acid in the susceptibility of citrus fruit to *Penicillium digitatum* infection. Implication in the LED Blue light-induced reduction of postharvest decay**

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**Keywords** abscisic acid, hormones, induced resistance, infection, light emitting diodes, *Penicillium digitatum*

### **ABSTRACT**

*Penicillium digitatum* (Pers.:Fr.) Sacc, is the major pathogen causing postharvest disease in citrus fruit grown under Mediterranean conditions. Abscisic acid (ABA) may be an important player in resistance of plants against pathogenic fungi. However, the role of this plant hormone in the response of citrus fruits to *P. digitatum* infection remains unknown. The aim of this study was to understand whether ABA may be involved in the defense response of this crop to the fungus, and also to decipher whether the hormone may participate in the LED blue light (LBL)-elicited resistance against *P. digitatum*. To that end, we have examined the effect of exogenous ABA and of inhibitors of ABA biosynthesis in the 'in vitro' fungal growth and on the susceptibility of sweet oranges, exposed or not to the LBL-elicitation treatment, to be infected by *P. digitatum*. Moreover, we have compared the ability of *P. digitatum* to infect fruits of the wild-type 'Navelate' orange (*Citrus sinensis* L. Osbeck) and its spontaneous ABA-deficient mutant 'Pinalate'. Results showed that the 'Pinalate' orange was more susceptible than its parental to infection and that exogenous ABA reduced both the percentage of infected fruits and also the maceration zone without affecting the 'in vitro' fungal growth. These results, together with those obtained from examination of changes in endogenous ABA in response to LBL and to infection, indicate that ABA may play a defensive role in citrus fruits against infection caused by *P. digitatum* but it does not play a critical role in the LBL-elicited response.

## 61 Postharvest losses of apples by fungal decay and physiological disorders in southern Brazil

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**Keywords** Cryptosporiopsis perennans; Penicillium sp.; Botrytis cinerea; Malus domestica;

### ABSTRACT

Postharvest losses of apples by fungal decay and physiological disorders were quantified for fruit treated and untreated with 1-MCP, over four years in three experiments. The frequency of causal pathogens on rotted apples was determined. Apples from 15 orchards of 'Gala' (Experiment 1) and 17 orchards of 'Fuji' (Experiment 3) cultivars were picked in a 15 days-harvest window for long-term storage and sampled at three commercial packing houses, in each year, within 48 h after harvest, for analyses of production losses during and after storage. Losses in twenty samples of ~380 kg of apples (20 bins) per orchard and year were analyzed during storage; ten samples (10 bins) were treated with 1-MCP and ten remain as untreated. Fruit samples of each orchard and year were stored in an individual Controlled Atmosphere storage room, at ~0.7 °C, for 150 to 240 days and 170 to 270 days for 'Gala' and 'Fuji' cultivars, respectively. One sub-sample of 100 healthy apples taken from each sample on the opening day of storage room was kept at 22 °C for seven days and then analyzed to quantify losses after storage. For experiment 3, decayed apples from ten storage rooms of each cultivar ('Gala' and 'Fuji') and year (4) were sampled at pre-sizing sorting line, after storage. Ten samples of 100 decayed apples were taken throughout the sorting period for each storage room and the causal pathogenic fungi identified for each fruit by main symptoms. Losses of apple during storage varied from 4% to 14% for Gala and 6.5 to 8.5% for Fuji. Deterioration by decay was approximately 60% and 80% of total losses of Gala and Fuji cultivars during storage, respectively. Additional losses of apple after storage by decay varied from 8% to 19% for Gala and 10.7% to 27.3% for Fuji depending on the year. Senescent breakdown and superficial scald were the second most important causes of Gala and Fuji cultivars losses during and after storage, respectively. 1-MCP treatment did not affect losses of 'Gala' and 'Fuji' cultivars by decay during and after storage. *Cryptosporiopsis perennans*, *Penicillium sp.*, *Botrytis cinerea* and *Alternaria alternata* were the main pathogens for 53%, 23%, 10% and 14% of decayed Gala, respectively and for 48%, 13%, 12% and 8% of decayed Fuji cultivars, respectively.

## 62 Development and application of a combined methodology based on propidium monoazide with real-time PCR to quantify viable conidia of *Monilinia fructicola* in stone fruit

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**Keywords** conidia viability; fungal populations; detection; brown rot; molecular tools

### ABSTRACT

Brown rot caused by *Monilinia* spp. is an economically important fungal disease of stone fruit worldwide and is caused by *Monilinia laxa* and *M. fructicola*. Currently the main approach used to prevent fruit decay is focused on an integrated strategy that combines spraying programs on orchards, good agricultural practices and suitable storage conditions also in packinghouses and during distribution to remove or avoid *Monilinia* conidia, which are one of the most important inoculum sources of brown rot. Therefore, new methodology to detect and identify viable *Monilinia* conidia are needed. Conventional methods used to identify *M. fructicola* are based on phenotypic characteristics and the quantification is not always accurate enough. In contrast, methodologies based on molecular tools improve its characterization and identification but are not able to differentiate between live and dead conidia. In this work, propidium monoazide (PMA) combined with qPCR methodology was developed, optimized and validated in artificially inoculated samples and used to quantify *M. fructicola* viable conidia in naturally infected samples. The conditions of PMA pre-treatment were 60 µM of PMA for 20 min of incubation and 30 min of LED exposure that combined with qPCR, measured accurately live conidia without overestimation of dead conidia. Using this methodology in naturally infected samples, we quantified *M. fructicola* live conidia from other associated microbiota and it was observed that these conidia were only distinguished by PMA-qPCR method. The developed methodology based in the combination of PMA-qPCR will suppose a great advance and help to assess the environmental fate of *M. fructicola* in stone fruit.

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## 63 Infection timing for *Colletotrichum acutatum* and *Phomopsis* sp. causing postharvest rots of avocado in New Zealand

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**Keywords** *Persea americana*, fruit rots, fungicides, benomyl, conidia, germination, infection criteria, stem-end rots, body rots

### ABSTRACT

In New Zealand avocados are infected by a number of different fungi, including *Colletotrichum acutatum* and *Phomopsis* sp., to cause postharvest rots. Temperatures required for > 50% spore germination are between c. 10 - 25°C for *C. acutatum*, and greater than c. 12°C for *Phomopsis* sp. Monthly spray inoculations using Nit isolates of both fungi to wounded and unwounded sites on the side of 'Hass' fruit in the field for eight months showed that *Phomopsis* does not infect through the sides of the fruit, and that *C. acutatum* infects once temperatures are above a threshold of c. 10°C. *Phomopsis* sp. populations on flowers were reduced after application of a systemic fungicide, and fruit harvested from these trees showed fewer stem-end rots from which *Phomopsis* sp. was isolated. Isolations from postharvest rots on avocado fruit yielded *Colletotrichum acutatum* from body rots and stem-end rots, and *Phomopsis* sp. only from stem-end rots. We therefore propose that avocado fruit are infected by *C. acutatum* after a temperature threshold is exceeded to cause body rots, and that *Phomopsis* sp. infects flowers in spring to cause stem-end rots. The mechanism by which *C. acutatum* causes stem-end rots may be by contamination of the picking wound at harvest.

## 64 Effect of a postharvest treatment with natural fungicides on the epiphytic populations of *Geotrichum candidum* on nectarines

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**Keywords** Stone fruits, Sour rot, biofungicides

### ABSTRACT

Sour rot of stone fruit caused by *Geotrichum candidum* is a frequent problem for Chilean growers especially in fruit destined for international markets. The epiphytic population on the fruit is reduced by fungicides sprayed in the orchard, but most of the packed fruit harbors the pathogen due to recontamination in the packinghouse, and postharvest fungicides actually used on stone fruits have no effect on *G. candidum*. The objective of the study was to spray natural fungicides Serenade® or Timorex Gold®, along with fludioxonil during fruit waxing with the aim to reduce the epiphytic population of *G. candidum* and to evaluate their possible effect on the shelf life of nectarines. Experiments were conducted in commercial packinghouses in nectarines cvs. Summer Fire and Venus. The natural fungicides were used at 2% and 3% mixed with fludioxonil at a rate of 2,500 cc/100 L of wax. Eight boxes of 48 fruits each were taken at the end of the packing line; half of them were cold stored (0 +/- 1 °C) for 40 days plus 10 days at room temperature simulating commercial conditions while the other half were frozen at -17 °C for 4 days and let to defrost for 4 days. Thereafter, the number of fruits with saprophytic development of *G. candidum* were counted. The effect of the treatments was measured as the percent of fruits with development of the pathogen. Data was analyzed with Anova and mean values separated with Fisher LSD ( $p \leq 0.05$ ). Ninety-one percent of the untreated control fruit (treated only with fludioxonil) had development of *G. candidum*. Serenade at 2% reduced the epiphytic population to 62.1% while Timorex Gold had no effect. The control fruit stored for 40 days plus 10 days at room temperature had 100% of total rot, while the rot was reduced to 48 and 25% when treated with fludioxonil mixed with Serenade and Timorex Gold, respectively.



## 71 Novel film forming formulations for *Candida sake* CPA-1 to improve their biocontrol efficacy on grapes

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- Keywords** Fluid-bed spray-drying, biocontrol, *Candida sake*, coatings, *Botrytis* spp.

### ABSTRACT

*Candida sake* CPA-1 is a well-studied biocontrol agent and its efficacy has been demonstrated against various diseases caused by *Penicillium expansum*, *Botrytis cinerea* or *Rhizopus stolonifer* on fruit. Nevertheless, *C. sake* adherence and distribution on fruit surface improved when it was applied with a food coating and therefore its efficacy was also enhanced. However, food coatings have to be applied separately and are usually expensive products that increase treatment cost. For this reason, to find an economical alternative included in the final product would be interesting. The aim of this research was to achieve a novel film forming formulation of CPA-1 to be applied independently of any product. A novel drying system, fluid-bed spray-drying was optimised to dry CPA-1 together with biodegradable coatings. During the optimisation, several substances were tested as carriers and binders, and also drying temperature was optimised. The survival of *C. sake* during the dehydration process could be improved by adding protective compounds for this reason some products were tested with this purpose. The optimal temperature of drying was 55°C and two film forming formulations were obtained. Potato starch and maltodextrin were the best products to use as carriers and/or binders. Skimmed milk and sucrose were added as protective compounds at one of the optimised formulations. When the formulations were optimised, biocontrol efficacy was demonstrated against *Botrytis* bunch rot on table grapes and products shelf life was studied.

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## 74 Role of proteases and iron metabolism on the virulence of *Penicillium digitatum*

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**Keyw ords** *Penicillium digitatum*, citrusfruit, proteases, pathogenicity, iron

### ABSTRACT

*Penicillium digitatum* is the major postharvest pathogen of citrus fruit. It is a necrotrophic fungus with a very narrow host range that penetrates the fruit through injuries inflicted during harvesting and handling. In the last years there has been an increasing interest in trying to find out what are the mechanisms used by this ascomycete to be such a successful pathogen. We have previously identified a set of fungal genes induced during pathogenesis. Gene ontology analysis of differentially expressed genes pointed to acidic proteases and metal ion homeostasis as two important factors during pathogenesis. In order to elucidate the possible role of proteases and metal ion metabolism we have undertaken a functional genomics approach. Proteases comprise different families of proteins each one containing many redundant enzymes. So, we have knocked out the gene *prtT*, which is the ortholog of the transcription factor that in *Aspergillus niger* regulates the expression of acidic proteases. We will present the characterization of the  $\Delta prtT$  null mutant as well as the expression of the two major acidic proteases expressed during infection of citrusfruit. On the other hand, iron is the most abundant metallic ion in nature and plays fundamental roles in living organisms. In aerobic environments, iron is mostly present as insoluble salts of  $Fe^{3+}$ . Fungi rely on two mechanisms for iron acquisition: a reductive iron assimilation and a siderophore mediated assimilation. We have obtained and characterized single and double knockout mutants in both pathways, as well as on the major transcription factor that regulates the expression of iron dependent genes under iron-limited conditions. We will present the characterization of these mutants and discuss their role on the virulence of *P. digitatum*.

## 76 Obtention and characterization of a *Penicillium digitatum* non-ethylene producer knockout mutant

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**Keywords** *Penicillium digitatum*, citrusfruit, ethylene, pathogenicity, EFE

### ABSTRACT

Infection of citrus fruit by *Penicillium digitatum* is accompanied by a large increase in ethylene production. Classical studies conducted more than 30 years ago have shown that this phytohormone is produced both by the fruit and the pathogen. There are three known routes for ethylene production. In plants, ethylene is produced in a two-step reaction from methionine via S-adenosyl-methionine (SAM), which is converted into aminocyclopropane-1-carboxylate (ACC) by AAC synthase. Then, ACC oxidase converts ACC into ethylene and cyanide. In addition to plants, many microorganisms also produce ethylene, but using two different pathways. In one of them, methionine is also the precursor compound, but in this pathway it is transaminated into 2-keto-4-methylthiobutyric acid (KMBA), which is spontaneously oxidized to produce ethylene. The last known pathway for ethylene production utilizes  $\alpha$ -ketoglutarate (AKG), which derives from glutamic acid, and arginine as substrates in a reaction catalyzed by an ethylene-forming enzyme (EFE), and enzyme that requires ferrous iron and oxygen, yielding ethylene, carbon dioxide, succinate, 1-pyrroline-5-carboxylic acid and guanidine. These last two pathways have been shown to be present in *P. digitatum*. Under *in vitro* growth conditions, the KMBA pathway is the predominant in shake cultures grown in liquid medium, whereas the AKG pathway is predominant in static cultures. In order to elucidate the contribution of EFE to ethylene production during infection of citrus fruit by *P. digitatum*, we have obtained a *P. digitatum* $\Delta$ *efeA* knockout mutant. In this communication, we will present the characterization of this mutant as a valuable tool to understand the role of ethylene in *P. digitatum* infection.

## 78 A metagenomic approach to assess *Neofabraea* infection and dynamics on stored apples

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**Keyw ords** Microbiome, Metagenomics, Bull's eye rot, *Neofabraea*

### ABSTRACT

Microbes are an integral part of the biome and interact in many ways with the plant products that humans produce for food. While the detrimental effect of postharvest pathogens on fresh produce can be characterized by classical microbiology, little is known about the infection dynamics of plant pathogens before symptoms appear. This holds especially true for *Neofabraea* spp., the causative agent of Bull's eye rot on apple, which is responsible for major losses in stored apples. Important aspects about the life cycle and infection dynamics of this pathogen are still unknown. Here, we characterize the infection levels of apples with *Neofabraea* spp. at the time of harvest and the microbial dynamics that occur during storage in a controlled atmosphere facility by characterizing the apple skin microbiome using a metagenomic approach. While the classical microbiology approach consistently failed to detect *Neofabraea* spp. on asymptomatic apple skin tissue, the metagenomic approach was able to detect the occurrence of *Neofabraea* spp. before bull's eye rot symptoms became visible. Furthermore, the quantification of *Neofabraea* spp. using the metagenomic approach correlated with the extent of post-storage symptoms across two different apple varieties and across three different orchard management practices. Due to the inherent variability of fungal infection loads between locations and years, the experiment will be repeated to gain additional confirmation of the results. Nonetheless, the data provided here suggests that metagenomic data can serve as a monitoring tool to pre-symptomatically address the infection load of *Neofabraea* spp. and assess the risk of developing bull's eye rot symptoms during storage. Metagenomic sequencing may provide a valuable tool that can be used in the near future to inform practitioners on disease risks and prevent post-harvest losses.

## 80 Diagnostic survey on the occurrence of pineapple fruitlet core rot in Réunion Island

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**Keywords** pineapple, fruitlet core rot, phenolic acids, *Fusarium ananatum*, *Talaromyces stollii*

### ABSTRACT

Fruitlet core rot is the major postharvest disease affecting 'Queen' pineapple cultivar in Réunion Island and South Africa. The sector is currently facing increasing susceptibility to the disease which leads to important economic losses. The two fungi responsible *Talaromyces stollii* and *Fusarium ananatum* cause black spot in the flesh of the fruit. These internal damages make the FCR difficult to diagnose for the producers. To get a better idea of the extent of the disease, we conducted a survey of agricultural practices throughout the island. Pineapple sampling allowed us to determine the occurrence of the disease. During the winter season, almost all pineapples had the FCR symptoms. Some pineapple fruits were inoculated with a solution of *Fusarium ananatum* spores ( $10^3$  sp/ml). Phenolic acids were monitored in the healthy and infected fruitlets. All the infected fruitlets react to the fungal infection with a high accumulation in free and bound phenolic compounds. The links between certain mechanisms of resistance and cultural conditions are discussed.

## 81 Pre- and postharvest alternative approaches to control *Alternaria* brown spot of citrus

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**Keywords** Alternative control means, *Alternaria*, Mandarin cv Fortune, tangerine, phenolic compounds, chitosan

### ABSTRACT

*Alternaria* brown spot (ABS) is one of the most important diseases of tangerines and their hybrids worldwide. Although its control mainly relies on fungicides, their use is facing troubles related to the development of resistant pathogen populations and adverse effects on human and environmental health. The present study was undertaken to find possible alternative control means against ABS. In particular, the effectiveness of organic and inorganic compounds of plant and animal origin were tested by pre- and postharvest applications. Phenolic compounds, that is hesperidin, naringin, quercetin, and umbelliferon were tested *in vitro* against *A. alternata* and *in vivo* on mandarin cv. Fortune. In *in vitro* tests, naringin and umbelliferon reduced colony diameter by 60 and 36%, respectively, while, *in vivo*, hesperidin and quercetin reduced disease incidence by 32 and 53%, respectively, at 14 days post inoculation. Moreover, chitosan (Iko-Hydro, Italy), Fortisol (Citrosol, Spain), and potassium phosphite (Decco, Italia) were applied before and after harvest for two consecutive years on mandarin cv. Fortune. In preharvest, plants were treated monthly and disease incidence was recorded on leaves and fruit. On fruit, the best results were obtained by chitosan, which reduced disease incidence by 59%. On leaves, chitosan and fortisol reduced the disease incidence by 63 and 75%, respectively and disease control was comparable to that obtained by fungicides. In *in vitro* trials, the three substances showed a direct effect on mycelial growth of *A. alternata*, with chitosan being the most effective (93% reduction). Although the obtained results need further confirmation, the tested compounds might represent interesting alternatives to synthetic fungicides against citrus ABS, to be applied in the field and/or during the postharvest phase.

## 82 Development of active packaging solutions with natural antimicrobial compounds for organic leafy greens

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**Keywords** eugenol, carvacrol, trans-anethole, wild rocket, iceberg lettuce, shelf life, organic produce

### ABSTRACT

Increased consumer awareness has led to the introduction of active packaging solutions with natural compounds that do not only preserve and protect the produce, but also has the function to maintain quality by its antimicrobial and antioxidant properties. The use of active packaging relies on a steady release of volatile active compounds into the atmosphere of the packages. A procedure for the development of active packaging solutions was developed for two model systems: i) unwashed, organic wild rocket (*Diplotaxis tenuifolia* L.) stored for 6 days at 5 °C and ii) ready-to-eat, shredded, organic iceberg lettuce (*Lactuca sativa* L.) stored for 5 days at 4 °C. The starting point for the work was *in-vitro* testing of eugenol, carvacrol, and *trans*-anethole against storage rot and human pathogens. Results showed that all compounds had high antimicrobial activity *in-vitro*. The next step was to develop emitting sachets with active compounds. To test their efficacy *in-vivo* under laboratory condition, pellets were loaded with eugenol, carvacrol, and *trans*-anethole and placed in packages inside sachets. The results showed that carvacrol and *trans*-anethole masked the odour of rotten wild rocket and had minor effects on the microbial load. The next step was to test the efficacy of the compounds *in-vivo* at industrial scale. Trays with organic wild rocket containing sachets at the bottom of the package showed that eugenol and *trans*-anethole could mask the off-odours produced by spoiling rocket, but had little effect on the microbial load, which was initially very high. This result showed that further optimization of sachet placement was necessary to allow better release of active compounds during storage. Sachets attached to the upper layer of pillow packaged ready-to-eat organic iceberg lettuce demonstrated that quality was maintained by providing microbiological safety, reducing oxidation and improving sensorial quality. The results demonstrate that further scaling up of active packaging with natural compounds is very important in developing new packaging, however further careful optimization may lead to development of promising solution for maintaining quality of organic fresh produce.

## 83 Postharvest application of disinfecting agents for controlling fruit and vegetable diseases: a brief review

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**Keywords** chlorine, chlorine dioxide, ozone, ethanol, hydrogen peroxide, organic acids, electrolyzed water

### ABSTRACT

Disinfection of fresh fruit and vegetables is an essential step of postharvest handling. The minimal requirement is to maintain commodities and facilities free from fungal postharvest pathogens and bacterial human pathogens thus improving food safety. Since postharvest pathogens accumulate on fruit surface before and during harvest, disinfection can occasionally prevent decay by itself. The current review includes historical, chemical, and regulatory background on some of the major disinfectants available today. These include chlorine, chlorine dioxide, ozone, ethanol, hydrogen peroxide, organic acids, and electrolyzed water. Some of the disinfectants described herein are in wide usage since many years and some are at an initial stage and thus considered 'alternative'. Information is given on their experimental reports, practical application, phytotoxicity, residues, advantages or disadvantages and mode of action. The conclusion is that disinfection could be an important tool to manage postharvest decay of fresh produce. Indeed, in some cases disinfection is a precondition to successful implementation of other major postharvest technologies and occasionally it can become the major technology. An important aspect arising from this review is also that most of the bad reputation of chemical disinfectants is unjustified, since they leave non-toxic residues and their environmental impact is minimum in view of their potential benefits.



## 89 Essential oils to control postharvest diseases of apples and peaches: elucidation of the mechanism of action

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**Keywords** Biofumigation, *Botrytis cinerea*, induction of resistance, *Monilinia fructicola*, savoury, thyme

### ABSTRACT

There is great interest on essential oils due to their potential to control postharvest pathogens of pome and stone fruit. Most essential oils have been studied for their efficacy *in vitro* but only few of them have been investigated *in vivo*. They can be applied by dipping the fruit in or spraying on the fruit surface or through fumigation. Biofumigation with thyme and savoury essential oils was effective in controlling brown rot caused by *Monilinia fructicola* in nectarines and peaches. The effect of biofumigation was also useful in reducing weight loss and preserving carotenoid and vitamin C content. The efficacy of essential oils in the control of fungal pathogens is often due to their antimicrobial activity and synergy of different chemical components. Apples treated with thyme oil at 1% concentration showed lower grey mould (*Botrytis cinerea*) incidence. The expression of the pathogenesis-related gene PR-8 was slightly higher in response to thyme oil treatment in apple fruit tissue. Essential oils efficacy depends both on their antimicrobial activity and on their ability to induce resistance in the host (fruit).

## 92 From seeds to postharvest: The impact of the plant microbiome on health

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<b>Keywords</b>	The Microbiome in Postharvest Pathology			

### ABSTRACT

Today plants have to be considered as co-evolved species assemblages. Consequently, plant species harbor a specific microbial diversity consisting of bacterial, archaeal, and diverse eukaryotic species. Breeding changed the entire plant holobiont and resulted thousands of cultivars and even in cultivar-specific microbiota. We have shown diversification of the microbiota for lettuce and pumpkin cultivars (Cardinale *et al.* 2015; Adam *et al.* 2016). The lettuce microbiota was dominated by *Proteobacteria*, *Bacteroidetes*, *Chloroflexi* and *Actinobacteria*. Cultivars share 50% of this diversity, while specificity comprised 12.5% of the species. For Styrian oil pumpkin genotypes, strong genotype-specific microbiomes were detected for seeds but not for the rhizospheres. Although we figured out a strong impact of breeding on the composition the plant microbiome in both studies, all analyzed microhabitats (seeds, rhizosphere, phyllosphere, endosphere) were characterized by specific bacterial communities. Seeds of oilseed pumpkin were characterized by the lowest diversity and dominant members of *Enterobacteriaceae* including potential pathogens (*Erwinia*, *Pectobacterium*). Beneficial bacteria like *Lysobacter*, *Paenibacillus* and *Lactococcus* could be also detected in significant abundances. The composition of the microbiome was linked to the susceptibility/resistance against pathogens. The "postharvest microbiome" showed a reduced but still impressive microbial diversity. In this stage, diversity of *Enterobacteriaceae* plays a crucial role (Erlacher *et al.* 2015). Plant microbial diversity is an important factor for plant health. This diversity has to be considered in all control strategies.

Adam *et al.* 2016. *The Cucurbita pepo seed microbiome: genotype-specific composition and implications for breeding. Plant Soil.* doi: 10.1007/s11104-016-3113-9.

Cardinale *et al.* 2015. *Bacterial networks and co-occurrence relationships in the lettuce root microbiota. Environ Microbiol.* 17:239-52.

Erlacher *et al.* 2015. *Biotic stress shifted structure and abundance of Enterobacteriaceae in the lettuce microbiome. PLoS One* 10:e0118068.

### 93 The prevalence of *Botrytis cinerea* in plum and weed tissue: An investigation to elucidate pathogen ecology, for new decay control strategies

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**Keywords** *Botrytis cinerea*, detection, grey mould, decay, plums

#### ABSTRACT

*Botrytis cinerea*, the cause of gray mould decay in plums, is of commercial importance in the South African export fruit industry. Despite various efforts, decay control failures leading to financial forfeitures to producers, continue to occur. Such failure to control decay may imply that knowledge of the ecology of the fungus is incomplete. The aim of this study was to investigate the prevalence of *B. cinerea* in both pre- and post-harvest plum tissue, as well as in weeds, over the plum production season. The occurrence of *B. cinerea* in different tissue types may give insight into the ecology of the fungus. This, in turn, could reveal critical points for decay control. An orchard with a high incidence of Botrytis gray mold, and high weed infestation, was identified. Samples of blossoms, fruit at harvest and fruit during and after storage were evaluated for the presence and quantity of *B. cinerea* DNA, using molecular techniques. Weeds were also screened for the presence of *B. cinerea*. Results indicate that *B. cinerea* was almost omnipresent in blossoms. In fruit, despite fungicide applications, both *B. cinerea* DNA and fruit decay increased in a linear relationship as storage time progressed. Notably, the amount of *B. cinerea* DNA in blossoms and fruit also revealed positive correlations to percentage fruit decay. Additionally, most weeds tested were positive for the presence of *B. cinerea*. However, Potato Dextrose Agar isolations from fruit or weed tissue failed to successfully isolate *B. cinerea*. From this study, it was clear that *B. cinerea* ecology in plums could be more complicated than generally held. Alternative gray mould decay control strategies should be considered, potentially with a stronger focus on fungicide protection of blossoms from primary infection and eradication of weeds, which act as bridging hosts for the fungus to survive and ultimately lead to subsequent fruit infections.

## 94 Optimisation of postharvest fungicide application in citrus packhouses: Low-tech but high impact

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**Keywords** Drench application, Imazalil, Curative control

### ABSTRACT

Postharvest decay leads to significant economic losses, considering that these losses typically occur at the end of the value chain. In most citrus fresh fruit producing industries, postharvest decay is managed through postharvest application of fungicides and cold storage. In South Africa, fungicides are applied by means of various systems, including pre-packhouse drench, inline dip, spray or wax coatings. However, specifications of these application systems and treatment protocols vary significantly. Since 2008, our research group studied the optimisation of postharvest fungicide application, specifically focussed on citrus green mould control. The project was initiated by a comprehensive survey of application systems, which indicated that pre-packhouse drench, in-line fungicide dip and wax coating application were most commonly applied. Drench application was generally inferior to dip application, especially when using fungicides alone; mixtures of fungicides demonstrated improved and synergistic action and were recommended as a potential control to fungicide resistance development. Dip application showed excellent curative, but relatively poor protective control. Curative control was not affected following the residue stripping effect of post-dip brushing. Imazalil application in wax coatings demonstrated very good preventative, but poor curative control. This supported the recommendation for double application of imazalil in dip and wax coatings. More recently, we investigated imazalil application using a flooder applicator, which demonstrated excellent curative as well as preventative control and sporulation inhibition. In South Africa, the imazalil sulphate formulation is applied most commonly, and the effects of pH and temperature of application solutions were modelled to allow prediction of residue loading and green mould control. Fungicide resistance led to control failure and integrated programmes for fungicide applications were recommended to South African packhouses.

## 97 Complex and emerging challenges facing citrus postharvest pathology

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**Keywords** Citrus exports, Phytosanitary, Maximum residue limit

### ABSTRACT

Production and export of citrus around the globe is a challenging and demanding enterprise, which face an inimitable constellation of problems that, in various combinations, can potentially ruin exports. In South Africa (SA), about 120 million 15 kg equivalent cartons were exported in 2016, totalling 67% of the production and earning more than ZAR 14 billion. Citrus exports contribute significantly to the country's GDP and job creation; any failure or success of South African exports will affect approximately 125 000 job opportunities and as many households. One of the unique aspects of SA and most southern hemisphere citrus producers is our distance from the markets. Ranging between 3 and 5 weeks by sea, travel time places excessive demands on fruit physiology. Tight protocols need to be followed by both producers and exporters to ensure the optimum arrival of the fruit subjected to these protracted export periods. Over time, these protocols were optimised resulting in successful export of high quality product. However, importing markets frequently impose severe constraints over and above scientifically determined phytosanitary and fruit physiological requirements, which have to be met to ensure continued trade on international markets. Additionally, in the last seven years, the South African citrus industry has dealt with the loss of morpholine in wax coatings, quaternary ammonium compounds as sanitising agents, and guazatine for green mould and sour rot control. Producers and packhouses have to appease an ever-increasing cohort of auditing bodies, each with their own set of standards. Additionally, certain supermarket chains limit the number of active ingredients that can be used on exported fruit and restrict residue levels at as much one third of the maximum residue limit. These requirements are based on consumer perceptions rather than science and good agricultural practice which have a direct impact on sustainable crop protection. The timeous manner in which the South African citrus industry has dealt with these challenges will be discussed.

## 98 Discovery and characterization of NLP effector family genes in *Penicillium expansum* and *Penicillium digitatum*

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**Keywords** Necrotrophic pathogens, molecular mechanisms, Effector proteins

### ABSTRACT

*Penicillium expansum* and *P. digitatum* are important postharvest, necrotrophic pathogens of apple and citrus fruit, respectively. *P. expansum* has a wide host range, while *P. digitatum* is specific to citrus fruit. Elucidating the molecular mechanisms associated with the pathogenicity of both pathogens, as well as their interactions with fruit during the early stages of infection and subsequent decay development, will greatly facilitate the development of new effective and safe management technologies. In this regard, numerous studies have demonstrated the essential role that effector proteins play in a variety of plant-pathogen systems. Effector proteins are typically unique to each species and have little or no similarity to known proteins. Some effector families, however, have been found to play an important role in the pathogenicity of a wide range of fungal species. Nep1-like proteins (NLP) are taxonomically widespread and mostly found in plant-related prokaryotic and eukaryotic microorganisms. Nep1-like proteins (NLP) are best known for their cytotoxic activity in dicot plants, although, they have also been shown to play a role in fungal vegetative growth and asexual reproduction. In the present study, a single gene (*PdNLP*) coding for NLP was identified in the genome of *P. digitatum* and two genes (*PeNLP1* and *PeNLP2*) in the *P. expansum* genome. *PdNLP* and *PeNLP1* are type 1 NLPs

and are induced during fruit infection. In contrast, *PeNLP2* codes a type 3 NLP and is expressed mainly in spores. *PeNLP2* was found to undergo alternative splicing. Targeted deletion of *PdNLP*, *PeNLP1*, and *PeNLP2* in either pathogen had no effect on the *in vitro* growth rate, spore production, or germination. The deletion of *PdNLP*, however, significantly compromised virulence of *P. digitatum* on lemon and grapefruit fruits and significantly delayed conidia formation on the decayed fruit, relative to the wild-type pathogen. *P. expansum* virulence on apple fruit was also impaired following *PeNLP1* deletion, but to a lesser extent. The interaction of *PeNLP* with host proteins was investigated using a yeast-two-hybrid system. Potential *PeNLP1* protein-protein interactions were revealed and may shed some light on its functional role during *P. expansum* infection.

## 99 Identification of quantitative trait loci controlling resistance to *Penicillium expansum* in *Malus sieversii*

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**Keywords** Blue mold, single nucleotide polymorphism, Apple

### ABSTRACT

Blue mold caused by *Penicillium expansum* is the most important postharvest disease of apple worldwide and results in significant financial losses. There are no defined sources of resistance to blue mold in domesticated apple, however, resistance has been described in wild *Malus sieversii* accessions, including plant introduction (PI) 613981. The objective of the present study was to identify the genetic loci controlling resistance to blue mold in PI613981. We describe the first quantitative trait loci (QTL) reported in the Rosaceae tribe Maleae conditioning resistance to *Penicillium expansum* on genetic linkage group 3 (qM-Pe3.1) and linkage group 10 (qM-Pe10.1). These loci were identified in a *Malus domestica* 'Royal Gala' X *M. sieversii* PI613981 family (GMAL4593) based on blue mold lesion diameter (7 days post-inoculation) in mature, wounded apple fruit inoculated with *P. expansum*. Phenotypic analyses were conducted in 169 progeny over a 4 year period. PI613981 was the source of qM-Pe3.1, a QTL with a major effect on blue mold resistance, accounting for 27.5% of the experimental variability. It mapped from 67.3 to 74 cM on linkage group 3 of the GMAL4593 genetic linkage map while qM-Pe10.1 mapped from 73.6 to 81.8 cM on linkage group 10. It had little effect on resistance, accounting for 14% of the experimental variation, and 'Royal Gala' was the primary contributor to the resistance effect of this QTL. Both parents, however, appeared to contribute to the least square mean blue mold lesion diameter in an additive manner at qM-Pe10.1. A GMAL4593 genetic linkage map composed of simple sequence repeats (SSRs) and 'Golden Delicious' single nucleotide polymorphism (SNP) markers was able to detect qM-Pe10.1 but failed to detect qM-Pe3.1. The subsequent addition of genotyping-by-sequencing markers to the linkage map provided better coverage of the PI613981 genome on linkage group 3 and facilitated the discovery of qM-Pe3.1



## 100 Perspectives and challenges of microbial application for postharvest diseases management

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**Co-author(s)** None

**Keywords** Alternatives, Biocontrol, '-omic' technologies

### ABSTRACT

Developing novel approaches or strategies to control postharvest diseases that are based on effective and safe alternatives to chemical fungicides is still a challenging task that has not been achieved at the commercial level. In most cases, there are inherent problems in the biocontrol systems related to poor performance and inconsistency under commercial conditions. The need for alternatives, however, is still valid and the outlook for microbial biocontrol products overall is still very promising. The current paradigm that is based on the premise that a single microbial antagonist may no longer be appropriate given our current knowledge on the complex multitrophic interactions taking place between all the components of the biocontrol system (plant host, the antagonist, the pathogen, and the microbiome). Although these interactions have been the subject of research for over thirty years, our understanding is still incomplete and a significant gap still exists between basic research underlying the selection of biocontrol agents and their use as commercially-successful products. This is because of the difficulties associated with the study of complex interactions and the lack of appropriate research tools and technologies. In this context, the multitrophic interactions that occur in postharvest biocontrol systems, and the potential development and use of synthetic microbial communities for postharvest biocontrol will be discussed and preliminary data about this new paradigm for postharvest biocontrol will be presented. The potential of utilizing microbial consortia in a beneficial manner has greatly increased due to advances in '-omic' technologies (metagenomics, metabolomics, transcriptomics, etc.) and bioinformatics. Additionally, using new technologies and cutting-edge microbial network modeling algorithms, may provide a compositional and functional understanding of the microbiome of fresh fruits and vegetables. This knowledge will enable a rational, science-based design of host-specific microbial consortia that can be used to manage postharvest decay in harvested fruit crops more effectively than the prevailing use of single antagonists.

## 101 The role of microbial volatiles in plant protection

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**Keywords** Indigenous microbiota, Inter-species interactions Volatile Organic Compounds

### ABSTRACT

The indigenous microbiota of plant hosts fulfils a central role in terms of protection against biotic stresses. A variety of microorganisms produce highly efficient metabolites to block pathogen attacks and thus maintain plant health under unfavourable conditions. The utilization of volatile organic compounds (VOCs) is a powerful strategy to overcome distance boundaries. Bioactive VOCs are employed by beneficial microorganisms to establish and maintain a 'protective shield' around the host. It was demonstrated that distinct plant-associated bacteria, e.g. the genera of *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, and *Paenibacillus*, are major contributors to the recruitment of volatile and highly active antimicrobial substances. Identified diazines from the *Bacillus* and *Paenibacillus* clusters drastically reduced the viability of the pathogenic fungi *Botrytis cinerea*, *Verticillium dahliae*, and *Candida albicans*. Moreover, analogous diazine derivatives were demonstrated to inhibit the growth of various human pathogenic bacteria including *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* when applied in low concentrations. We also demonstrated that various volatiles can be employed as long-distance messengers and play a central role in inter-species interactions. In the environmental context we found that VOCs-driven effects highly depend on i) the availability of nutrients, ii) the development stage of the microorganism, and iii) strain-specific interactions, e.g. synergistic effects of compatible microorganisms. The overall findings provide strong evidence for the importance of microbial VOCs in the maintenance of host wellbeing and additionally increase the repertoire for upcoming biotechnological applications.

## 102 Fruits and shoots! Exploring the microbiome of apple

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**Keywords** Nanopore platforms, rRNA gene, Fungi

### ABSTRACT

Technological advances in DNA sequencing, such as the Illumina and Nanopore platforms, have revolutionized the ability to sequence large numbers of samples in a high-throughput and cost-effective manner. In this regard, next-generation sequencing technologies combined with the use of PCR-primers designed against conserved ITS region of rRNA genes in fungi and the 16S bacterial rRNA gene has also allowed for unprecedented taxonomic characterization of microbial communities. Microbiome-based studies are revolutionizing our understanding of how organisms interact with the microbes that inhabit them (both epiphytes and endophytes). Our recent research documenting the microbiome of apples, revealed spatial differences in the composition of the microbiota. Our data indicates that specific microbes dominate stem-end and calyx-end portions of apple fruit, relative to either the peel or wounded tissues. Surprisingly, a number of yeast species related to dermal infections were also observed, though absolute identification of these species still needs to be confirmed. We have also asked the question as to whether or not different cultivars of apple harbour unique microbial assemblages. Preliminary data indicate that genetically-related genotypes harbour shoot microbial communities that are more similar than genotypes that are more distantly related. Different rootstocks also appear to harbour distinctly-different microbiota which has an influence on the composition of the shoot microbiome. A major international project is underway to document the impact of management practices, genotypes, and environment on the composition of the microbiome of apple fruit. Additional research will provide a model of the community-level interactome that exists within the apple fruit microbiome and examine how variations in the composition of the microbiome impact the microbial metabolome.

## 104 Imazalil resistance management for sustainable citrus green mould control: limited options and alternatives

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**Keywords** Pyrimethanil, *Penicillium digitatum*, effective residue

### ABSTRACT

Imazalil (IMZ), a demethylation inhibitor (DMI) fungicide, with its excellent curative, preventative and anti-sporulant abilities is used by the postharvest citrus industry as the main control strategy for green mould (caused by *Penicillium digitatum*). Green mould is the major cause of decay losses in citrus. Resistance development against IMZ is incremental and eventually leads to control failure. Monitoring for resistance development is therefore an important management tool. However, *in vitro* sensitivity levels (EC<sub>50</sub> values) do not always correlate with practical resistance levels (ER<sub>50</sub> values). In some cases, resistant isolates could be partly controlled with higher residue levels, while control failure was observed for moderately resistant isolates in other cases. Resistance management strategies should therefore employ alternative fungicides or their mixtures with the existing one. The recent registration and implementation of another DMI, propiconazole (PPZ), for the control of sour rot further compromises the sustainable use of IMZ as cross-resistance is possible and since PPZ is less effective against green mould. Thiabendazole (TBZ) and fludioxonil (FLU) are co-application options, but field resistance against TBZ and relatively poor green mould control by FLU renders them less suitable partners. Pyrimethanil (PYR) stands out as a more suitable alternative given very good curative control following dip treatments. Compared to other alternatives PYR was shown to be superior in controlling IMZ resistant isolates. PYR activity in drench and wax application was poor, but very good synergistic activity was observed when PYR was used with IMZ, TBZ and/or guazatine. In cases where IMZ resistance is prevalent, inoculum levels could be effectively managed through the use of effective partner fungicides, such as PYR, in treatment programs that implement sanitation, optimum fruit quality management and fruit treatment within 24 h after harvest.

## 108 Potential uses of lactoperoxidase against post-harvest diseases on fruits

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**Keywords** natural biological protection system, *Penicillium expansum*, *Botrytis cinerea*, *P. italicum*, *P. digitatum*

### ABSTRACT

Intensive cultivation methods worsen the impact and the frequency of plant diseases leading to a frequent utilization of chemical pesticides. Moreover the decrease in the number of authorized chemical products coupled with the demand for environmentally and human safe products calls for the search for safer alternatives. The development of a low impact biopesticide could be one of the tools to reach that goal particularly in postharvest handling of commodities. Lactoperoxidase is an enzyme present in a variety of mammal secretions including bovine milk. The bovine milk lactoperoxidase system (LPS) is a natural biological protection system based on the oxidation of thiocyanate and iodide by the enzyme to produce the antimicrobial ions hypothiocyanate (OSCN<sup>-</sup>) and hypoiodide (OI<sup>-</sup>). A broad spectrum of antimicrobial activity against gram-positive and gram-negative bacteria, as well as viruses and fungi [1, 2] has been described for the LPS. We developed an aqueous solution without the enzyme and containing only active ions OSCN<sup>-</sup> and OI<sup>-</sup>. The solution was then tested *in vitro* against postharvest pathogens of pome, citrus and banana fruits as well as tubers. Inhibition was noticed to range between 70 and 100%. Furthermore *in vivo* trials demonstrated high efficacy of OSCN<sup>-</sup> and OI<sup>-</sup> solutions against *Penicillium expansum* and *Botrytis cinerea* in apples and against *P. italicum* and *P. digitatum* in citrus fruits at the postharvest stage. The results will be discussed as well as the potential practical application of this novel technique.

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**109 Preliminary study of the interactions between the apple microbiota and *Pichia anomala* strain K, a biocontrol yeast against wound diseases of postharvest apples**

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**Keywords** Microbiota; Strain K; Apple

**ABSTRACT**

Apple postharvest diseases caused by *Gloeosporium* spp., *Penicillium expansum* and *Botrytis cinerea* are still of economic importance with 25% of losses due to fruit decay. Biological Control Agents (BCA) are sustainable candidates to control these pathogens. Progress has been made during the past two decades to understand the modes of action of the isolated BCA *Pichia anomala* strain K through various studies (microbiology, enzymatic, genomic, transcriptomic and proteomic). Nevertheless, BCA commercial application has been hampered by low or non-reliable efficacies in comparison to fungicide treatments (Droby et al., 2016). This is the case with *P. anomala* strain K. Once applied on the fruit surface, BCA will face a complex microbiota where ecological interactions such as parasitism, mutualism and commensalism occur thus affecting the BCA efficacy. However, next generation sequencing has enabled microbiota profiling making it possible to study these interactions in detail. Massart et al. (2015) have put a new alternative forward to improve BCA efficacy: the selection of helper strains and prebiotics of biocontrol present in the microbiota. Helper strains are microorganisms that effectively enhance BCA activities whereas prebiotics of biocontrol are molecules that modulate the microbiota composition to favour the biocontrol agent. In this regard, apple epiphytic microbiota appears to be a good alternative in improving the efficacy of strain K against *Botrytis cinerea* in apples. Apple fruit samples were collected and their microbiota harvested to generate a diverse microbiota bank made up from seventeen varieties, four localizations and four disease management practices. Biological trials on wounded apple involving each microbiota, the strain K and *Botrytis* are ongoing. The results will shed more light on the interaction between strain K and the apple microbiota.

*Droby, S., Wisniewski, M., Teixidó, N., Spadaro, D. & Jijakli, M.H. 2016 The science, development, and commercialization of postharvest biocontrol products. Postharvest Biology and Technology, in press, pp.22–29.*

*Massart, S., Martinez-Medina, M. & Jijakli, M.H., 2015. Biological control in the microbiome era: challenges and opportunities. Biological Control, 89, pp.98–108.*

## 110 Exploring new pathways in the host response of apples and citrus fruit against *Penicillium* spp

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**Keywords** blue mold; green mold; fruit-pathogen interaction; disease control

### ABSTRACT

Apples and oranges are some of the most valuable fruit crops worldwide. However, postharvest diseases caused mainly by fungi belonging to *Penicillium* spp. affect the postharvest quality of the fruit during the storage period. Specifically, *Penicillium digitatum* and *P. expansum* are the most devastating pathogens of citrus and pome fruits respectively, causing important economic losses during postharvest handling. Despite the economic impact of both fungal pathogens in apple and citrus fruit, there is poor knowledge on fruit-pathogen interactions. To develop new rational and environmental friendly control alternatives, it is fundamental to elucidate the factors that modulate the interaction between each fruit and each specific pathogen. The main goal of this research was to obtain a better understanding of fungal diseases in apples and oranges taking advantages that *P. digitatum* is a specialist species with relative host specificity and primarily attacking citrus fruits, whereas *P. expansum* can explore a broad host range including apples amongst many others. Studies were conducted using pathological, biochemical and molecular approaches. In particular, the current availability of high-throughput molecular methods provided an unprecedented opportunity to conduct global screenings of the major determinants of the fruit defence mechanisms by transcriptomic and proteomic studies in apples infected by the compatible (*P. expansum*) and the non-host (*P. digitatum*) pathogens. The knowledge obtained in this study will help us to obtain a better understanding of fruit-pathogen interactions and then establish the basis towards the improvement of disease control strategies in postharvest fruit.

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**111 Effect of nano silver particle, aluminum sulfate and hydroxyquinoline citrate on vase solution microbial contamination and postharvest properties of *Alstroemeria* cv. 'Vanilla'**

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**Keywords** Biocide, chlorophyll content, microbial proliferation, *Trichoderma harzianum*, visual quality, water relation

**ABSTRACT**

Vase solution microbial contamination affects postharvest behaviour of cut flowers mainly through water relation interruption, dehydration and consequently vase life termination. Proliferation of vase solution microbe could affect vase life in different ways such as vascular blockage, secretion of bacterial by-product, toxic compounds, and ethylene production (in ethylene sensitive flowers). Therefore controlling vase solution microbial proliferation is a prerequisite in postharvest studies of cut flowers. In order to find a suitable biocide, 'Vanilla' cut *Alstroemeria* flowers were treated with Nano Silver Particles (NSP), Aluminum Sulfate (AS) and Hydroxyquinoline Citrate (HQC) as vase solution preservatives. Subsequently, some microbiological features of vase solution such as microbial proliferation, growth and identification were studied. Besides, some postharvest physiological parameters such as vase life, side effect, weight change, solution uptake, and chlorophyll content were studied. NSP and HQC were effective in controlling microbial proliferation until day-6, while AS controlled microbial proliferation until day-4. Two kinds of fungal colonies, and six kinds of bacterial colonies were isolated from AS vase solutions. Later the fungi were identified as *Trichoderma harzianum* and *Fusarium solani* and the bacteria identified as *Bacillus* sp., *Coccus* spp. and *Streptomyces* sp. Results of the physiological study indicate beneficial effects of NSP and AS on vase life and most physiological parameters of cut 'Vanilla' *Alstroemeria* specially chlorophyll content and visual green appearance of leaves. The AS and NSP treatments improved solution uptake and fresh weight retention. Although HQC completely controlled microbial population, its side effect was unsuitable and it also reduced vase life. Results suggest nano silver application as a biocidal preservative solution for cut *Alstroemeria* flowers is as effective as other conventional biocides. The compound is effective in controlling vase solution microorganisms has ethylene antagonistic effect and therefore retards senescence.



## 112 Use of elicitors as a postharvest tool to reduce decay during marketing

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**Keywords** Postharvest diseases, signaling compounds, induced defence

### ABSTRACT

Globally, the increase of postharvest losses is attributed to fruit susceptibility to several fungal attacks, which impacts the shelf life and commercial value of the fruit during the supply chain. In developing countries, food loss can reach up to 50%, with fungal attack accounting for much of the losses occurring during storage and transportation. In order to reduce such losses, postharvest fungicides are applied immediately after harvest at the pack houses. Although control of major postharvest pathogens can be achieved with synthetic chemical fungicides, the growing concern for human and environmental health risks associated with pesticides, coupled with the development of fungicide-resistant strains and the ban on many fungicides, has prompted the industry to search for alternative approaches. The deposits of the fungicide residues on the fruits and their negative effects on the environment and consumer health have raised concerns regarding food safety. Furthermore, in Europe, application of fungicides at postharvest level is limited and the consumer demand is higher for pesticide-free fruits. Apart from the above-mentioned reasons, the use of synthetic fungicides is not approved by the organic fruit industry. Moreover, the food suppliers and food catering agents are also expected to adopt sustainable agriculture and food safety practices. All the aforementioned concerns have directed research towards the development of alternative approaches to combat major postharvest diseases in the fruit industry. The use of alternative control strategies aims at controlling postharvest diseases by adopting treatments that are less harmful to human health, environmentally friendly and cost effective in order to replace or reduce the use of synthetic fungicides. The application of postharvest elicitors or essential oils can trigger induced defences in the host tissue, resulting in distinct changes in the plant's secondary metabolism thus enriching the phytochemicals, providing protection against the pathogen and improving the fruit health. Hence, this review provides an overview of the research carried out over the last five years on eliciting the signalling effect of thyme oil, *methyl salicylate*, and *methyl jasmonate* vapours in avocado and peach fruits.

### 113 Efficacy and mechanisms of action of a pomegranate peel extract in controlling postharvest citrus rots

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**Keyw ords** Postharvest-diseases; Biological control; Plant extracts; Pomegranate peel; Mechanisms of action; Citrus diseases

#### ABSTRACT

A pomegranate peel extract (PGE) was extensively evaluated as a natural antifungal preparation for the control postharvest rots of citrus. It showed a broad spectrum of activity, strong and direct antimicrobial effects and both curative and preventive activities with long persistence even when applied in commercial conditions. Several evidences indicated a very quick activation of defence responses in treated fruit. This included a significant reduction of rots without a direct contact between PGE and pathogens. An increased ROS activity and expression of genes is involved in activation of defence responses in plants (CHI, CHS, MAPK, MAPKK and PAL). Furthermore, the analysis of the whole transcriptome in treated and untreated Valencia oranges confirmed a strong overexpression of most genes. In large-scale experiments conducted on lemons, Valencia oranges and clementines, PGE reduced the incidence of rots after storage and increased shelf life by 70-90%, according to the concentration used. In all experiments, PGE proved to be significantly more effective than the chemical control Imazalil. Pre-harvest treatments applied the day before harvesting were particularly effective. PGE is obtained from a waste product of the processing factories (the pomegranate peel) and is extracted and stabilized using safe chemicals (food grade ethanol and citric acid). This extract did not cause any apparent phytotoxic effect on treated fruit. It therefore, has a high potential to be implemented in postharvest control strategies as a natural safe and eco-friendly alternative.

## 114 Effective use of disinfectants in a postharvest environment

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**Keywords** Sanitation, CT values, Aerobic microbes, Fungal populations

### ABSTRACT

Sanitation is a critical aspect of the handling of fresh products, to minimize decay and risks from microbes of food safety concern, and disinfectants are the primary tools employed to accomplish this. An initial step in development of a disinfectant application is quantification of disinfectant potency to the organism of interest in vitro, where a level of mortality is defined by a concentration x time product (CT), which combines the concentration and the length of exposure where mortality occurs into a single value (eg. mg-L<sup>-1</sup>-hours, ppm-minutes, etc.). For example, if a spore is inactivated by a CT of 100 mg-L<sup>-1</sup>-minutes, mortality would occur after one minute with 100 ppm, two minutes with 50 ppm, and four minutes at 25 mg-L<sup>-1</sup>, etc. CT values greatly facilitate the development of commercial practices and equipment. In the citrus industry, in vitro assays concluded that a sodium hypochlorite CT value of 400 mg-L<sup>-1</sup>-minute was effective to cause >99% mortality of conidia of *Penicillium digitatum* (cause of green mold) and arthrospores of *Geotrichum citri-aurantii* (cause of sour rot) at a solution pH of 10, although mortality was faster at lower pH. This knowledge enabled the use of hypochlorite to sanitize the relatively alkaline sodium bicarbonate solutions used to control postharvest decay. A second application of CT values is the expression of sulfur dioxide toxicity to *Botrytis cinerea*, cause of gray mold. Packages of table grapes are fumigated with sulfur dioxide, either applied externally or released from generator devices inside the packages. An issue for externally applied gas is to know if sufficient sulfur dioxide has diffused into the package. CT values to control aerial mycelial growth and conidia germination were determined to be approximately 50 and 100 µL-L<sup>-1</sup>-hours, respectively, and a colour change dosimeter was developed and enabled managers to know if sufficient gas had diffused into their packages. More recently, they were used to quantify gas released from generator devices inside the packages over long periods. Peracetic acid is another sanitation tool of renewed interest in California owing to approvals that allow higher rates of its use before harvest, acceptance by organic growers, and its compatibility with postharvest fungicides. Applied the day before harvest, peracetic acid reduced aerobic microbe and fungal populations by 1-2 log<sub>10</sub> units. Its use in near-harvest applications for table grapes and citrus fruit of peracetic acid for the control of postharvest decay.

## 121 Fresh produce safety in a postharvest perspective

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**Keywords** Foodborne pathogens, microbiological quality, microbial biome, antibiotic resistance

### ABSTRACT

The microbiological safety of fresh produce can be compromised by poor quality faecal contaminated irrigation water. Fresh produce are mostly consumed raw and limited postharvest interventions are used to ensure product integrity. Raw produce are therefore no longer automatically considered a low risk product and are increasingly seen as potential vehicles of foodborne pathogens. The prevalence of foodborne pathogens and its presence as part of the natural microbial biome makes it difficult to mitigate risks associated with its consumption. In South Africa, data on foodborne pathogen outbreaks is limited but several studies confirmed the regular presence of foodborne pathogens in the formal and informal food system. Pathogens could be detected on fruit and plant surfaces using traditional and next generation methods including whole genome sequencing. Pathogens such as *Escherichia coli*, *Salmonella* spp. and to a lesser extent *Listeria* spp. could sporadically be detected in low concentrations in agricultural and food environments. The microbiological quality of fresh produce and the associated link with antibiotic resistance genes provides a sobering perspective on future food safety and public health challenges. The current status of food safety assurance in South Africa will be presented in the context of postharvest science and technology.

## 122 Microbial contamination source tracking in fresh fruit and vegetable supply chains

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**Keywords** Food safety, irrigation water, fresh produce

### ABSTRACT

An overview of importance of contamination source tracking and antimicrobial resistance surveillance in agroecosystems will be presented. Discussion points are as follows 1) Potential contamination points from farm to fork and areas where water, soil and fresh produce are typically sampled 2) Results obtained and main conclusions reached regarding the potential link between the irrigation water quality and the microbiological quality and safety of fresh produce 3) Details of current USAID/DST and Water Research Commission funded projects 4) Current research focus on informal and formal fresh produce supply chains 5) Enumeration of hygiene indicator bacteria not only coliforms and *Escherichia coli*, but Enterobacteriaceae as well 6) Characterisation of multidrug resistant Enterobacteriaceae and preliminary results will also be presented.

# POSTER PRESENTATIONS

### 3 Effect of thyme oil vapour exposure on the brown rot infection, phenylalanine ammonia-lyase (PAL) activity, phenolic content and antioxidant activity in red and yellow skin peach cultivars

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**Keywords** Prunus persica, Brown rot, Essential oils, Phytochemicals, Induced disease resistance

#### ABSTRACT

Influence of thyme oil vapour on the incidence of brown rot (*Monilinia laxa*) was

investigated in the yellow-flesh peach cultivars consisting of red skin 'Jim Dandy', 'Novadonna', 'Scarlet Rich' and 'Transvalia', and yellow skin 'Earli Gold', 'Kakamas', 'Kesie' and 'Summer Sun'. Fruit were exposed to thyme oil ( $96 \mu\text{L L}^{-1}$ ) vapour for 24 h, subsequently inoculated with the pathogen ( $10^5$  spores  $\text{mL}^{-1}$ ), and stored for 14 d at  $0^\circ\text{C}$ , followed by 6 d shelf-life conditions at  $15^\circ\text{C}$ , 75 % RH. Four varieties, i.e. 'Scarlet Rich', 'Transvalia', 'Earli Gold' and 'Kesie' were then selected to study the effect of thyme oil vapour on the phenylalanine ammonia-lyase (PAL) activity and hydroxycinnamic acids content. The effect of thyme oil vapour on brown rot incidence, phenolic content and antioxidant activity was also investigated in naturally infected fruit. Thyme oil vapour reduced the incidence of brown rot by increasing the activity of PAL, the content of catechin, chlorogenic and caffeic acids, and via enhancing antioxidant scavenging capacity. However, the key finding from this study was that thyme oil vapour exposure is far more suitable for red skin peaches than their yellow skin counterparts.

## 5 Biocontrol capabilities of selected *Candida* spp against *Penicillium digitatum* on Citrus: Study with respect to their mode of action

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**Keywords** Biological agent – Green mold disease – Citrus – Yeasts – Control method

### ABSTRACT:

Seventeen yeast fungi isolates were recovered from surface of healthy fruits of Orange, Lemon, Cucumber and Tomato, identified and evaluated for their biocontrol capabilities against *Penicillium digitatum* on Citrus. Four species of yeast fungi were found to be strongly antagonistic to *P. digitatum* on Citrus. *Candida wickerhamii* and *Candida glabrata* reduced green mold disease incidence by approximately 85%, *Candida mucilagina* reduced disease incidence by 70% while *Candida fructus* caused 54% disease reduction. Co – culturing of living, autoclaved cells and culture filtrates, each with *Penicillium digitatum* spore suspension (in vitro), showed higher efficacy of living cells on spore germination than culture filtrate, while autoclaved cells had no efficacy. Antagonism study on solid agar media showed marked developed inhibition zones by the four yeast strains against *Penicillium* growth (in vitro). The examined antagonist yeasts exhibited high level of extra cellular B-1,3 gluconase enzyme ( in vitro). *Candida wickerhamii* produced the highest amount (150 nktal). Scanning electron microscope (SEM) observations revealed general attachment and colonization of *Penicillium digitatum* by yeast cells two hours before application each of *Candida fructus*, *Candida mucilagina*, *Candida glabrata* and *Candida wickerhamii*.



## 6 Assessment of eleven South African peach cultivars for susceptibility to brown rot and blue mould

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**Keywords** *Prunus persica*, Total phenols, anthocyanin, *Penicillium expansum* *Monilinia laxa*, postharvest decay

### ABSTRACT

Peach is a popular fruit, mainly because of its sweet taste and nutritional content.

Consumption of peaches can suppress reactive oxygen species in human plasma and provide protection from chronic diseases. Peaches, however, are susceptible to various postharvest fungal pathogens including *Monilinia laxa* (brown rot) and *Penicillium expansum* (blue mould). The use of synthetic chemical fungicides in controlling postharvest diseases is becoming a challenge for various reasons, including consumer preference for chemical-free fresh produce and concerns about environmental pollution. Therefore, this study was conducted to identify the peach cultivars that are resistant to postharvest pathogens causing brown rot and blue mould in order to reduce fungicide application on peaches for fresh consumption. Eleven peach cultivars (five red and six yellow) were screened for resistance or susceptibility to brown rot and blue mould. The disease incidence and severity in artificially inoculated peaches was determined after 14 and 21 days of cold storage (0°C). Total phenols, anthocyanins, total antioxidant activity and phenylalanine ammonia-lyase (PAL) were also analysed. The results showed that of the red skin cultivars, 'Temptation' had a significantly lower brown rot disease incidence and severity, while the yellow skin cultivar 'Suncrest' had a higher disease incidence after both 14 and 21 days of cold storage. In addition, the reduction of brown rot incidence was greater than that of blue mould rot in 'Temptation'. Cultivar 'Temptation' also showed higher PAL activity, total phenolics, anthocyanin content and total antioxidant activity. Therefore, the findings of this study suggest that cultivar 'Temptation' is adequately resistant to the brown rot pathogen.

## 7 Development of coatings based on whey protein concentrate and fennel essential oil for anthracnose control and improvement of papaya postharvest quality

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**Keywords** *Carica papaya*, biodegradable coating, postharvest ripening, fruit quality, antifungal activity, essential oil

### ABSTRACT

Papaya (*Carica papaya* L.) is a climacteric fruit that has high transpiration rate and intense metabolism during ripening. Therefore, it senesces quickly and it is difficult to store for long periods and consequently postharvest losses are high. This situation is aggravated by postharvest decay caused by *Colletotrichum gloeosporioides*. This research aimed to develop a coating based on whey protein concentrate (WPC), fennel essential oil (FEO), calcium chloride (CC) and glycerol (G) to extend shelf life and maintain the quality of papaya 'Golden'. Besides chemical analysis by GC-MS of the essential oil extracted from *Foeniculum vulgare* Mill, its fungicidal and/or fungistatic effects against *C. gloeosporioides* were investigated. Essential oil was extracted by means of hydro-distillation and afterwards GC/MS analysis was performed to identify their components. Trans-anetol, methyl chavicol, fenchone and limonene were detected as main constituents of the essential oil from *F. vulgare*. *In vitro*, minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of FEO to reduce mycelial growth of *C. gloeosporioides* were 0.2% (w/v). Coatings formulated with FEO concentrations of 0.2 and 0.4% combined with different WPC (10, 12 and 14%), CC (1%) and G (5%) were evaluated using the analysis of contact angle, average particle diameter, zeta potential and microscopy. It was found that the most stable coatings were those containing a lower percentage of WPC (10%) and higher FEO concentrations (0.2 and 0.4%). Overall, a whey protein concentrate-based coating formulation (10% of WPC/1% CC/5% G) formulated with 0.2 or 0.4% FEO showed potential to extend the shelf-life and maintain quality of papaya 'Golden'.

## 9 Effect of cinnamon essential oil on reducing the postharvest decay of strawberry fruit caused by *Rhizopus oryzae*

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**Keywords** *Rhizopus oryzae*, decay, infection, strawberry

### ABSTRACT

The aim of this study was to evaluate the non-contact effect of cinnamon essential oil (CEO) on postharvest decay of strawberry fruit. The fruits were inoculated with *Rhizopus oryzae* spores. Micro-volumes of CEO at four quantities (0, 66, 132 and 198  $\mu$ L) were dropped on filter papers and were placed in the fruit containers with the volume of about 0.5 L (no contact of CEO with fruits). All of the treated fruits were stored in two types of containers. One set of the containers were punched for ventilation and the other set had no ventilation holes and was sealed to prevent gas exchange. Treated fruits were kept for 15 days at 4 °C and 70-75 % relative humidity. The percentage of infected fruits and also sensory characteristics were evaluated at days 5, 10 and 15 of storage. Results showed that non-contact application of cinnamon essential oil at three applied quantities with or without ventilation, totally inhibited the infection of strawberry fruits by *Rhizopus oryzae* after 15 days of storage. Sensory characteristics such as taste, colour, odour and overall acceptance were improved in treated fruits with cinnamon essential oil compared to untreated fruits.

## 10 Application of selected plant volatiles to reduce postharvest diseases in Fuerte avocado

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**Keywords** *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, alternative control

### ABSTRACT

'Fuerte' is one of the important green-skinned cultivars of avocado exported from South Africa to the European markets. The shelf life and quality of avocado are majorly affected by postharvest diseases caused by *Colletotrichum gloeosporioides* (anthracnose) and *Lasiodiplodia theobromae* (stem-end rot). Application of synthetic fungicides has been employed to manage postharvest diseases but recently poses a high risk to the consumers and environment. Development of an eco-friendly novel alternative has piloted this research which is focused on investigating the antifungal activities of selected plant volatiles in aqueous phase on the control of anthracnose and stem-end rot in 'Fuerte'. In experiment 1, the artificially inoculated fruit was subjected to preventive treatment (14 days at 10 °C and thereafter kept for 3 days at 20 °C). In experiment 2, the fruits were curatively treated for 6 and 8 days at 20 °C. The following treatments were adopted in this study: (i) 0.1% v/v Thyme oil (ii) 0.1% v/v Citral (iii) 80% Prochloraz (iv) Y-extract (v) 50% Prochloraz + Y-extract (vi) 50% Prochloraz + 0.1% v/v Thyme oil (vii) 50% Prochloraz + 0.1% v/v Citral and air-dried. Stem-end rot and anthracnose incidence was significantly lower in fruit treated (preventive method) with [80% prochloraz, 50% prochloraz + 0.1% v/v citral, 0.1% v/v citral] and [0.1% v/v thyme oil] respectively. However, the incidence of stem-end rot was reduced to 56.25% in fruits treated (curative method) with [50% prochloraz + 0.1% v/v citral] and 18.75% in [0.1% v/v citral] for 6 and 8 days respectively. The incidence of anthracnose was reduced to 18.75% in [0.1% v/v citral, 80% prochloraz, 50% prochloraz + Y-extract] and 12.5% in [0.1% thyme oil] for 6 and 8 days respectively. Fruits treated with 0.1% v/v citral and 0.1% v/v thyme oil was firmer compared to other treatments. It was evident that citral and thyme oil in an aqueous state could play an important role in reducing postharvest diseases of 'Fuerte' during storage.

## 11 Exposure of Fuerte and Hass avocados to volatile citral as an alternative to control stem-end rot

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**Keywords** Postharvest decay, *Lasiodiplodia theobroma*, *Persea americana*, plant volatiles

### ABSTRACT

Stem-end rot is a postharvest disease caused by several endophytic fungi such as *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Thyronectria pseudotrichia*, *Phomopsis perseae* and *Dothiorella aromatic* in tropical and subtropical fruits. Avocado is consumed for its nutritional benefits; however, it is difficult to control stem-end rot during the supply chain. *Lasiodiplodia theobromae* undergoes a latency stage of infection which renders it difficult to detect in the pack line. Due to the characterisation of plant volatiles as GRAS (Generally Recognised as safe) additives, this study was aimed at investigating the antifungal activities of citral in the vapour phase against stem-end rot in 'Fuerte' and 'Hass' *in vitro* and *in vivo*. Citral, octanal, hexanal and thymol were screened against *Lasiodiplodia theobromae*, and the conidia were exposed to the same volatiles for 24 h, 48 h and 72 h. For the *in vivo* trials, 'Fuerte' and 'Hass' were exposed to citral and dipped in prochloraz, and thereafter stored for 6 days and 8 days at 20 °C (curative method) and 14 days at 10 °C and afterwards kept for 3 days at 20 °C (preventative method). It was observed that volatile citral completely inhibited the radial mycelial growth of *Lasiodiplodia theobromae* at a minimum inhibitory concentration of 4 µL L<sup>-1</sup>, while thymol exhibited a lower inhibitory effect. Conidia exposed to hexanal and thymol showed 80% inhibition of conidial germination after 24 h while octanal exhibited 90% inhibition after 48 h compared to citral that completely inhibited the conidia germination after 72 h. Exposure of artificially infected 'Fuerte' and 'Hass' avocados to volatile citral delayed stem-end rot development during storage with the lowest incidences of 25% (preventative method) and 70% (curative method). Furthermore, volatile citral delayed the development of stem-end rot in both cultivars, but mainly in 'Fuerte' and could be considered to be a biological fumigant in managing stem-end rot of avocado.

## 14 Direct application of cold plasma to *Colletotrichum* species in-vitro reduces their growth and germination

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**Keywords** Postharvest pathogens, anthracnose, cold plasma

### ABSTRACT

Cold plasma (CP) is widely used in the biomedical field, especially in dentistry and skin care but also for surface sterilisation of medical equipment. It has also been successfully used for the decontamination of fresh produce from microorganisms, particularly bacteria that cause food-borne illness. There are limited reports of CP to control plant pathogenic fungi. In this study we investigated the direct effect of CP on three postharvest fungal plant pathogens, belonging to the *Colletotrichum* genus that we isolated from avocados (*C. gloeosporioides*, *C. alienum* and *C. acutatum*). CP using air as the feed gas was tested for its anti-fungal efficacy against pure cultures and spore suspensions of *C. gloeosporioides*, *C. alienum* and *C. acutatum* following treatment for 3 or 6 minutes duration at a distance of 5 cm. The 6 minute CP treatment significantly reduced the radial mycelial growth of freshly inoculated cultures compared to the control. However, when applied to actively growing cultures the fungicidal efficacy was not significant. In addition, the 6 minute CP treatment of spore suspensions resulted in almost 100% reduction of spore germination, immediately after treatment. In contrast, the 3 minute CP treatment did not significantly reduce the radial mycelial growth of fresh or actively growing cultures, but did suppress the germination of spores within 3 hours of treatment. These results demonstrate the potential of CP for the control of *Colletotrichum* species associated with avocado.

## 17 Control postharvest diseases by thermo-fogging fungicides

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**Keywords** apples, decay control, pears, pome fruit, recirculating drenches

### ABSTRACT

Postharvest diseases are the most challenging barriers to overcome in tree fruit industry worldwide. Recirculating drenches have been widely used for decades to apply fungicides to control the diseases in storage, particularly in pome fruit. Although it is highly effective when applied immediately after harvest, disadvantages are accumulation of decay fungal pathogens, reduction of efficacy due to organic loads in the solution, and environmental concern of solution disposal. More recently, the concern is that human pathogens might be transferred from bin runners into the drench solution and could potentially contaminate tons of sound fruit. Alternative methods, such as pre-harvest fungicide sprays in the orchard substantially reduce the diseases compared to untreated fruit; however, these methods are not as effective as the recirculating drenches. Thermo-fogging is an innovative technology that applies postharvest fungicides without compromising the efficacy and does not have the disadvantages of recirculating drenches previously addressed. In experimental and commercial trials, thiabendazole, fludioxonil, and pyrimethanil applied by thermo-fogging significantly reduced major postharvest diseases on pome fruit. Further investigations are in progress to show the efficacies on other fruit crops.

## 23 The effect of crop rotation under till and no-till practices on maize pathogens in sandy soil in South Africa

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**Keywords** Root and crown rot; Maize; qPCR

### ABSTRACT

Maize serves as staple food crop in South Africa. Diseases such as root and crown rot on maize can cause yield decline of  $1.81 \text{ t ha}^{-1}$  for every 25% disease severity observed. For high and sustainable yields, conservation agriculture (CA) such as crop rotation and limited tillage practices are being implemented. However, implementation of CA raises concern that increased plant residue in the soil could lead to an increased risk of root and crown rot diseases resulting in increased yield losses. The aim of this study was to evaluate the occurrence of 12 commonly occurring soilborne plant pathogens in maize roots and crowns, planted under CA practices using qPCR technology. Maize was rotated with soybean or sorghum under till and no-till practices (NT) in the Free State province (Kroonstad) during 2014/15 and 2015/16. The trials were replicated twice. Root and crown rot severity ratings were performed on 30 randomly selected maize plants per plot (totally 8 plots) at 100 days after planting using a root disease index (RDI). Schoeman et al. (2016) designed primers specific to these 12 selected fungal spp. These primers were used in a quantitative real time PCR to determine the target DNA concentrations of these 12 fungal species within diseased maize roots and crowns. Statistical analysis was performed using ANOVA, Levene's and the Shapiro-Wilk test. The RDI for both seasons were significantly higher in the roots (highest index score: 220) compared to the crowns (highest index score: 53.02). The maize/soybean rotation had the highest RDI average (152.5) and the maize/sorghum rotation had significantly lower RDI averages in the roots and crowns under NT. qPCR analyses showed that *Phoma* spp., *F. chlamydosporum*, *Pythium* spp. and *F. oxysporum* were the most prominent of the fungi tested in the current study. Significant 2-way interactions between season x plant tissue were observed for *F. oxysporum*, *F. equiseti*, *F. chlamydosporum*, *M. phaseolina*, *Pythium* spp., *Phoma* spp., *R. solani* and *E. pedicellatum*. *C. eragostidis* had a significant 2-way interaction between plant tissue x cultivation practice. *F. graminearum* and *Trichoderma* spp. had significant 2-way interactions for season x cultivation practice and *F. verticillioides* had a significant 3-way interaction between season x plant tissue x cultivation practice. None of the significance obtained pointed to a viable management strategy for the fungi analysed that was consistent over both seasons. The current study demonstrated that multiple seasonal data are required in order to obtain better insight into the effect of cultivation practices and rotational crop on soilborne pathogens and their resultant impact on root and crown rot development.



## 24 Evaluation of PCR-RFLP to distinguish between FGSC members occurring on South African maize

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**Keywords** FGSC, maize, species-specific PCR, PCR-RFLP

### ABSTRACT

In South Africa maize is a staple food crop and an important source of vitamins and carbohydrates for millions of people. This crop is commonly infected by the *Fusarium graminearum* species complex (FGSC), which consists of 16 phylogenetic species and is the causal agent of Gibberella root-, crown-, stalk- and ear rot. Members of the FGSC are also known to produce deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) mycotoxins, which can be harmful to humans and animals. Thus, food security and safety are at risk and therefore understanding species and their mycotoxins occurring on maize is very important. In this study, species-specific PCR and PCR-RFLP techniques were used to assign each isolate to one of the six FGSC species associated with South African grains. Six enzymes, *Bsa*HI, *Bfal*, *Eat*I, *Mse*I, *Spe*I and *Dra*I, were used to digest the translocation elongation factor 1- $\alpha$  (*TEF1*) and histone amplicons together with species-specific primers to identify *Fusarium graminearum* s.s., *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. meridionale* and *F. acaciae-mearnsii*. The restriction fragment length patterns were obtained by running a 3% agarose gel. The different enzymes were able to differentiate between the six FGSC isolates. For example, digestion with enzyme *Bsa*HI on the translocation elongation factor  $\alpha$ -1 (*TEF1*) amplicon was able to produce patterns that distinguished between three of the six FGSC species (*F. graminearum* s.s., *F. brasilicum* and *F. meridionale*). The use of PCR-RFLP may offer an alternative method to distinguish between closely related FGSC occurring in South Africa. However, these PCR-RFLP must be tested on all 16 FGSC members in order to ensure accuracy.

## 28 Thyme oil treatments to control internal rot caused by *Fusarium verticillioides* in pineapple fruit (*Ananas comosus* var. MD-2)

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**Keywords** *Ananas comosus* var MD-2, *Fusarium verticillioides*, postharvest, essential oil

### ABSTRACT

Pineapple (*Ananas comosus* var. MD-2) is one of the most perishable fruit during export, therefore its restricted postharvest life needs to be prolonged. Essential oils like thyme oil have the potential to improve the postharvest life on various fruits in order to control the germination of several postharvest pathogens. However, little information is known about the effect of thyme oil to control internal fruit rot caused by *Fusarium verticillioides* on pineapples during cold storage. Consequently, the effect of different concentrations of thyme oil (100 and 1000 ppm) was assessed in order to reduce internal fruit rot occurrence during the postharvest period. Results showed that fruits treated with the chemical fungicide prochloraz ( $3 \text{ cm}^3 \text{ L}^{-1}$ ) presented 45.7% of disease reduction after 21 days at 8°C, 100 ppm of thyme oil showed 21.7% of reduction, whereas the reduction of internal fruit rot at 1000 ppm of thyme oil was 48.7%, all treatments compared with non-treated fruits. Moreover, during storage (21 days at 8°C) treatment with 1000 ppm of thyme oil did not affect weight loss and firmness of pineapple and slowed the changes in solid soluble content (SSC), titratable acidity (TA) and pH. These results suggest that thyme oil (1000 ppm) may be potentially used for controlling internal fruit rot produced by *Fusarium verticillioides* in pineapple during postharvest conservation without negative effect on its physicochemical quality.

### 33 Potassium levels affected spearmint's (*Mentha spicata* L.) essential oils antioxidant and antibacterial activities

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**Keywords** antioxidants; antibacterial; essential oil; *Mentha viridis*; foodborne pathogens

#### ABSTRACT

Plant extracts and essential oils (EO) are gaining great interest as chemical/commercial sanitizers. However, their biocidal activity depends on the mineral content. Little information is available about the effect of potassium supplementation on the activity of plant extracts/EO. The present study aims to determine the effects of potassium level (K: 275-300-325-350-375 mg/L) on the antioxidant and antibacterial activities of spearmint (*Mentha spicata* L.) and its essential oil. High K application increased spearmint polyphenols content and antioxidant activity (DPPH, FRAP), while K>325 mg/L induced oxidative stress (increased H<sub>2</sub>O<sub>2</sub>), followed by the activation of antioxidant enzymes (SOD, APX, CAT), which provide a protective action to the plant. EO, total phenolics and antioxidants were affected by different K levels. All the EOs were more effective against Gram-positive (*L. monocytogenes* and *S. aureus*) than Gram-negative (*E. coli* and *S. enteritidis*) bacteria under different K applications, possibly due to the differences on their cell wall structure. Pure chemical carvone showed greater antimicrobial activity than limonene. Considering greater carvone content, the 325 mg/L K treatment could be appropriate for spearmint cultivation and production of essential oil, improving their antioxidant and antibacterial activity against foodborne pathogens.

### **34 Assessment of mint and pomegranate extracts/oils as antimicrobial agents to inhibit growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on shredded carrots**

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**Keywords** antioxidants; antibacterial; essential oil; plant extracts; foodborne pathogens

#### **ABSTRACT**

Among minimally processed vegetables, shredded carrots are particularly popular; however, they are perishable products and can be contaminated throughout the food chain. Chlorine, the most commonly used disinfectant agent in the washing water, fails to reduce the microbial load of fresh produce and furthermore it can adversely affect human health. The aim of this study was to evaluate the antimicrobial activity of different washing treatments with aqueous solutions of mint essential oil (EO) (1:1000) and mint hydrosol (1:10) and pomegranate juice (1:10) against two major food borne pathogens on shredded carrots as well as their effect on carrot's quality. The results of this study indicate that the tested washing treatments resulted in a small but significant decrease of the microbial load of *Escherichia coli* and *Listeria monocytogenes* on the sixth day of storage. Pomegranate juice and mint hydrosol were more effective against *L. monocytogenes*, whereas mint EO was more effective against *E. coli*. Furthermore, an increase in carrot's total phenolics and antioxidants was observed with the application of mint hydrosol and pomegranate juice, whereas mint EO resulted in a decrease on the sixth day of storage. Additionally, a decreased chroma and an increased whiteness index of the shredded carrots were observed during the application of mint hydrosol on the sixth day. Additional efforts are necessary to evaluate and optimize this process.

### 35 Efficacy of lime and vinegar powder as a disinfectant for tomato during storage

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**Keywords** tomato, sanitizer, lime, vinegar, microbial

#### ABSTRACT

A study is being conducted to determine the efficacy of using food and plant antimicrobial disinfectants during postharvest storage of tomato. Tomato is the highly grown vegetable in Malawi due to its economic importance as a source of food and cash for small scale farmers. Current studies show that losses during postharvest range from 20-30%, and postharvest diseases account for 30% of the total losses. Observations show that farmers do not wash and sanitize their produce after harvest, and this is one of the causative agents for postharvest diseases. Globally, there have been concerns for the potential hazards associated with chlorine reaction by-products and issues of wastewater disposal, which has heightened for research in the use of food and plant based antimicrobial sanitizers. Use of Lime (*Citrus aurantifolia*) and Vinegar as some of the food products in sanitization has been reported. However, there is limited knowledge on the optimum concentrations for tomato during washing, hence the need for carrying out this study. Mature Green tomato varieties of 'Mountain Fresh' from Iowa Horticulture Research Farm and 'Tengeru' from Bunda Campus Horticulture Research Farm in Lilongwe are being used for the experiment. The experiment has been laid out as a factorial with sanitizers and three dipping concentrations as factors. Data is being collected on surface microbes such as yeast and mold, *E. coli* and coliforms, Enterobacteriaceae and aerobic bacteria; postharvest weight loss, color change, lycopene and ascorbic acid content on tomato during storage period. It is being envisaged that the results of this experiment will help farmers by providing them with the right sanitizers for tomato to reduce postharvest diseases.

#### 40 Baseline sensitivity and molecular identification of *Galactomyces citri-aurantii*

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**Keywords** *Galactomyces citri-aurantii*, propiconazole

#### ABSTRACT

South Africa's citrus industry is the third largest horticultural industry and contributed R9.69 billion to total gross value of South African agricultural production during the 2013/14 season. Propiconazole, which is a demethylation-inhibiting triazole fungicide, is currently registered for postharvest use on many crops including citrus and has previously been found to be highly effective for managing sour rot of citrus. The aim of the project was to test the propiconazole sensitivity of South African *Galactomyces citri-aurantii* isolates causing sour rot on citrus. Further-more the species identity of the fungal pathogen isolates (N=52) was characterized using published species specific primers as well as phylogenetic analysis. A multi-gene phylogeny of the internal transcribed spacer of ribosomal RNA region (ITS1 and 2), translation elongation factor one alpha gene (TEF1), ribosomal polymerase subunit B1 (Rpb1) gene and the polygalacturonase (PG) gene indicated the PG gene was less suitable for species identification possibly due to inter species variation in the primer site, whereas the other gene regions could be combined into one phylogenetic tree. Propiconazole was shown to be highly efficient and effective in decreasing mycelial growth of *G. citri-aurantii* *in vitro*. The mean EC<sub>50</sub> value for the 52 isolates was 0.384 µg/ml (0.004-1.089 µg/ml) and the mean EC<sub>95</sub> value was 0.516 µg/ml (0.007-1.093 µg/ml). The discriminatory dose (DD) was calculated to be 5.16 µg/ml (calculated DD= 10 x EC<sub>95</sub>). The information delivered in this study will be used for monitoring of propiconazole fungicide resistance of *G. citri-aurantii* in the packhouse.

## 41 Prevalence of foodborne pathogens on fresh produce from informal retailers in Tembisa, South Africa

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**Keywords** Postharvest food safety; Informal market

### ABSTRACT

Fresh produce is becoming a larger proportion of people's daily dietary intake mainly due to the perceived health benefits. The consumption of fresh produce has increased globally and so has the number of foodborne pathogen outbreaks. Global outbreaks, largely involve *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. which are linked to various food products including fresh produce. Typically, in South Africa food is purchased from a number of different types of traders in the formal sector, which includes retailers, fresh produce markets and convenience stores, and the informal sector, which includes spaza shops, street-vending green grocers and mobile trolleys. Whilst the formal sector is largely regulated, the informal sector is not. The unregulated informal sector has uncontrolled hygiene and sanitation with limited and sometime improper storage which could lead to cross-contamination of fresh produce. The aim of this scoping study was to determine the level of food safety associated with the informal sector (street-vending green grocers). Fresh produce samples, including cabbage, spinach, tomato, apple and carrots, were therefore purchased from the informal market in Tembisa and Ivory Park, Ekurhuleni. Coliforms and *E. coli* on fresh produce were enumerated and the presence of foodborne pathogen was determined. *Escherichia coli* was detected from 15.2% of fresh produce samples (n=125), *Salmonella* spp. was detected from 6.4% and *Listeria* spp. from 3.2%. Average coliform counts on all products exceeded the log<sub>2</sub> CFU/g guideline levels, stated by the South African Department of Health for ready-to-eat products. In this study, it was found that leafy vegetables had higher prevalence of *Listeria* spp., *Salmonella* spp. and *E. coli* when compared to fruit and root vegetables. Cabbage samples had high prevalence of *Listeria* spp., while spinach was found to have significantly high prevalence of *E. coli* and *Salmonella* spp. Overall however, fresh produce purchased from the street-vending green grocers was found to have a low prevalence of *Listeria* spp., *Salmonella* spp. and *E. coli*. Future studies will focus on the characterisation of all foodborne pathogens isolated.

#### 46 Involvement of nitric oxide in the defense response of MeJA-induced disease resistance in strawberry fruit

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**Keywords** Strawberry; Methyl jasmonate; Nitric oxide; Postharvest disease; Induced resistance; *Botrytis cinerea*

#### ABSTRACT

Methyl jasmonate (MeJA) and nitric oxide (NO) are important signalling molecules in disease resistance in plants. To explore whether NO is involved in the defense response of MeJA-induced postharvest disease resistance in strawberry fruit, strawberry fruits (*Fragaria ananassa* Duch. cv. *Hongyan*) were treated with Methyl jasmonate (MeJA) and nitric oxide synthase (NOS) inhibitor (*L*-NNA). The effects of *L*-NNA on the resistance to *Botrytis cinerea*, NOS activity, NO level and defensive enzyme activity were explored. Our results showed that the MeJA treatment improved disease resistance and that the *L*-NNA treatment inhibited the activities of defence-related proteins, including phenylalanine ammonia-lyase (PAL), chitinase (CHI),  $\beta$ -1,3-glucanase (GLU) and polyphenol oxidase (PPO). Moreover, both treatments also delayed the increase in malondialdehyde (MDA), lipoxygenase (LOX) and H<sub>2</sub>O<sub>2</sub> contents, while superoxide (SOD) and peroxidase (POD) activities were greatly enhanced. Based on the data, treatment with MeJA produced the best effect. These results implied that NOS is a key enzyme regulating NO production in induced resistance. Therefore, NO is involved in the defense response of MeJA-induced disease resistance in strawberry fruit after harvest.



## **47 Survey of postharvest spoilage and shelf life of fresh vegetables from urban and rural markets in Bangladesh**

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**Keywords** spoilage, vegetable production, Bangladesh, shelf life

### **ABSTRACT**

Vegetable production in Bangladesh is variable in volume and methods utilized, depending on region and proximity to urban areas. The vegetable value chain is difficult to track and produce is pooled at each step, making postharvest spoilage studies a challenge and not thoroughly investigated. A survey of fresh vegetables was conducted in Dhaka and Mymensingh, Bangladesh to establish baseline data on average shelf life, spoilage pathogens, and exploration of possible methods to extend shelf life. Farms supplying market produce were visited to put data into perspective and also help identify weak points in the production system that could improve postharvest quality. Average shelf of fresh vegetables was poor, but considered normal among people frequenting the markets. Within the first five days from market purchase 20-100% of produce was completely spoiled and 90-100% of all produce was spoilage after 10 days. Postharvest quality varied among markets, though open-air markets or vegetables cart had significantly less spoilage in the first five days than produce purchased at modern, enclosed grocery stores. Refrigeration and cleaning with dilute sodium hypochlorite reduced spoilage dramatically, illustrating that common postharvest practices are rarely utilized for fresh produce in this region. Though limited in scope and duration, this study highlights the need for more extensive postharvest research in Bangladesh and South Asia as a whole in order to extend shelf life, resulting in greater food security.

## 48 Characterization and comparison of fungicide sensitivity of postharvest *Geotrichum candidum* isolates from the Eastern Shore of Virginia

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**Keywords** *Geotrichum candidum*, sour rot, tomato, postharvest, MLST, fungicide sensitivity

### ABSTRACT

A newly developed multilocus sequencing technique (MLST) and *in vitro* fungicide sensitivity trials were utilized to characterize local populations of *Geotrichum candidum* causing sour rot on tomato fruit grown on the Eastern Shore of Virginia. Thirty-seven pathogen isolates were collected from infected fruit at commercial tomato fields, a packing house, a cull pile, and boxed fruit. Six primer pairs were used for amplification and sequencing; *ala1*, *cdc19*, *erg10*, *gln4*, *pgi1*, and *pgm2*. Sequences were aligned using Clustal W, and neighbor-joining analysis was done to produce a phylogenetic tree. Field isolates showed genetic similarity and there was a general separation between those and post-harvest related isolates (packinghouse, cull pile, boxed fruit). One subgroup of isolates exhibited similarity to *Galactomyces reessii* at the *pg1* locus. *In vitro* fungicide sensitivity trials were conducted on potato dextrose agar amended with 8 fungicides and antimicrobial products. Active colonies were placed on amended plates at three concentrations of product and the diameters of colonies were compared to control plates. Propiconazole and tebuconazole inhibited 100% growth at all labeled rates, while other demethylation inhibitors partially reduced *G. candidum* growth. Characterization of *G. candidum* on the Eastern Shore of Virginia revealed a genetically diverse population for such an isolated and small location, and responded relatively uniformly to products used in tomato production and postharvest handling.

## 49 Effects of light on in vitro growth and sporulation of *Stagonosporopsis cucurbitacearum*

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**Keywords** Cucurbits, fruit rot, *Didymella bryoniae*, gummy stem blight, mycelial growth, sporulation

### ABSTRACT

The *Cucurbitaceae* are a large and diverse family containing several important commodity crops in many parts of the world, such as pumpkin (*Cucurbita spp.*), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) and watermelon (*Citrullus lanatus*). In the last decades, fruit rot caused by *Stagonosporopsis spp.* became a major disease in both field grown and greenhouse grown cucurbits. Yield losses due to *Stagonosporopsis* can show seasonal peaks up to 30%. Despite its economic importance, limited information is available about growth characteristics of *Stagonosporopsis cucurbitacearum*. A more profound understanding of the influence of ambient factors on growth and sporulation of the fungus is a first step towards the development of sustainable management strategies to prevent the disease. Therefore, the effect of different light compositions on *in vitro* growth and sporulation of *S. cucurbitacearum* were investigated on two different growth media, i.e. potato dextrose agar (PDA) and an inorganic minimal medium supplied with 20 g sucrose (MM). Results indicate that *Stagonosporopsis cucurbitacearum* varied in its ability to grow and sporulate under different light compositions. The type of lighting seems less important for growth than for sporulation, which is significantly reduced in the dark and under a blue/red LED regime. Moreover, this research clearly demonstrates the variable nature of the influence of light as clear interactions have been noticed between light and composition of the growth medium. UV confers a stimulus for both growth and sporulation for the fungus on PDA but on MM growth is not affected by UV treatment.

### 53 A post-harvest disease survey on *Punica granatum* fruit in South Africa

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**Keyw ords** *Punica granatum*, pos-harvest diseases, South Africa, pomegranate

#### ABSTRACT

Pomegranate (*Punica granatum*) as new crop in South Africa, is mainly produced as fresh commodity destined for export to northern hemisphere markets from February to June each year. Post-harvest rot is causing economic losses to producers and a disease survey was done to determine the scope of fungal pathogens involved in post-harvest decay of pomegranate fruit in South Africa. The survey was further refined by evaluating the effect of the current standard industry post-harvest treatment protocol of a chlorine (100 – 150 ppm) and fludioxonil (0.6 g/L at 35°C) drench on the amount and type of pathogens isolated. Fludioxonil is presently the only post-harvest chemical registered on pomegranates in South Africa. A significantly lower amount of fruit with decay symptoms was observed after fruit were treated with chlorine and fludioxonil and stored for 12 weeks at 7°C, and a quantitative evaluation indicated a reduction of up to 100% in some production units of post-harvest rot symptoms in treated fruit. A study done to determine the spectrum of fungal genera found, indicated the presence of a variety of fungi including *Penicillium* spp., *Alternaria* sp., and a *Pilidiella* sp. A comparison was made between isolates found on treated and non-treated fruit in order to determine the need for alternative or additional post-harvest treatments for isolates currently not efficiently controlled by the present standard protocol applied by South African pomegranate pack houses. *Cytospora punicae* was one of the diseases found to cause post-harvest fruit rot and was isolated from non-treated as well as treated fruit. This finding lead to a first description of this disease as a fruit pathogen on pomegranate.

## 55 Assessment of foodborne pathogen presence in the peach supply chain

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**Keywords** postharvest contamination; food safety; foodborne pathogens

### ABSTRACT

Contamination of fruit with foodborne pathogens can occur through various ways and at different points within the supply chain while the fruit move from farm to retail. Foodborne pathogens are able to attach to and colonise the peach fruit surface and survive the local and export supply chains. It is therefore important to implement preventative strategies and ensure effective food safety management systems. The aim of this study was to determine the presence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* in water, and on fruit and contact surfaces in the production and postharvest arena to determine the various potential sources of contamination. A case study model was used to assess the presence of foodborne pathogens on fruit, water and contact surfaces (n=428) from a peach farm in Limpopo Province over four seasons. No *Salmonella* Typhimurium was detected from samples. *E. coli* O157:H7 (n= 25; 5.8%), *L. monocytogenes* (n=4; 0.9%) and *S. aureus* (n=5; 1.4%) were detected on fruit and environmental samples. Livestock frequented water sources which lead to *E. coli* O157:H7 contamination. This conclusion was based on positive detection of foodborne pathogens from the water sources and subsequent removal of livestock which resulted in a definite decrease in pathogen detection. A number of *E. coli* O157:H7 and *S. aureus* were detected during the second year of monitoring from environmental samples and it was observed that the personal hygiene and facility sanitation was not adequately enforced. To prevent contamination, it is therefore important to ensure that water sources are potable and the food safety management system is functioning effectively.

## **56 Prevalence and characterization of multidrug resistant and extended-spectrum- $\beta$ -lactamase producing Enterobacteriaceae on fresh produce (spinach and tomatoes)**

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**Keywords** Antimicrobial resistance, food safety, foodborne pathogens, Enterobacteriaceae

### **ABSTRACT**

Extended-Spectrum-Beta-Lactamase (ESBL) producing Enterobacteriaceae are one of the six main antibiotic resistance related health risks in the world and are prevalent in human, animal and plant environments. Limited information is however available on the microbiological quality and safety of fresh produce that are supplied by formal and informal traders in South Africa. In this study, 150 spinach and tomato samples were obtained from five different retailers, street trading greengrocers and mobile trolley vendors respectively, in order to determine the microbiological quality and prevalence of multidrug-resistant ESBL-producing Enterobacteriaceae. Presumptive ESBL-producing Enterobacteriaceae isolate identities were confirmed using MALDI-TOF MS whilst antibiotic resistance was determined using phenotypic and genotypic analysis. Microbiological analysis indicated that 99% of the spinach samples and 95% of the tomato samples exceeded the minimum acceptable number ( $2.3 \log \text{CFU/g}$ ) of coliforms determined by the South African department of Health guidelines. ESBL-producing microorganisms were detected on the fresh produce and the antibiotic resistance profiles of the Enterobacteriaceae isolates showed that 74% were multidrug-resistant. The results of this study indicate the presence of multidrug-resistant pathogens on selected fresh produce in the postharvest environment in both the informal and formal sectors of South Africa.

## 57 Host-pathogen interaction of *Penicillium* species on stone fruit

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**Keywords** Postharvest Pathology, *Penicillium* spp., Stone Fruit

### ABSTRACT

Stone fruit are highly perishable and susceptible to several postharvest pathogens of which *Penicillium* spp. form part. *Penicillium expansum* can cause significant losses (>50 %) when fruit (prunes) are wounded. Little is known about other *Penicillium* spp. that could cause decay. Numerous studies have explored the host-pathogen interactions of *P. expansum* and *P. digitatum* on pome and citrus fruit but very little is known about these pathogens on stone fruit. This study aims to determine the pathogenicity and aggressiveness of *P. digitatum*, *P. expansum*, *P. crustosum*, and *P. solitum* on some stone fruit cultivars and explore the host-pathogen interactions of the most aggressive species at different host ripeness levels. *Penicillium digitatum* was the most aggressive species on most cultivars, followed by *P. expansum* and *P. crustosum*. Disease incidence varied for *P. digitatum* and *P. solitum*, but *P. expansum* and *P. crustosum* expressed high incidences over the cultivar range evaluated. Tissue colonized by *P. digitatum* and *P. expansum* was acidic and lower compared to uncolonized tissue of the same fruit. Both species seemed to accelerate ripening of host tissue ahead of colonization. *Penicillium expansum* was unaffected or less affected by fruit ripeness. *Penicillium digitatum* caused larger lesions and disease incidence increased as fruit became riper. To our knowledge this is the most comprehensive study on the pathogenicity and aggressiveness of *Penicillium* spp. on stone fruit. Rapid decay caused by *P. digitatum* highlighted the potential risk the species holds to the stone fruit industry, especially towards the end of the fresh produce supply chain when fruit tend to be riper. Risk is amplified when citrus is handled and stored in close proximity with stone fruit. Further work includes gene expression analyses of pH regulated genes from infected tissue samples. Future research should investigate the presence and impact of *P. digitatum* in the stone fruit supply chain.

## 65 Preharvest stroby multiple sprays induced resistance of muskmelon fruits at harvest and during storage

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**Keywords** Stroby; fruit; preharvest sprays; induced resistance; mechanism

### ABSTRACT

Stroby is a biological fungicide, which can effectively control the field disease of crops. In this study, Stroby at 400 ppm was sprayed four times during development of muskmelon fruit (*Cucumis melo* cv. Manao) at the young fruit period, fruit enlarging period, netting period and 48 h before harvesting. The effects of treatments were investigated on incidence of disease on fruit during storage, and the partial mechanisms of treatment were studied. The results showed that preharvest sprays effectively decreased the lesion diameter of fruit inoculated with *A. alternata*, *F. sulphureum* and *T. roseum*. The treatments significantly increased the activity and the gene expression of phenylalanine ammonia-lyase, 4-coumarate CoA ligase and cinnamate-4-hydroxylase at harvest and at the postharvest stage of the fruit. Stroby treatments promoted the accumulation of the lignin, total phenols and flavonoids at the harvest and postharvest stage of the fruit, and enhanced the activity and expression of chitinase and  $\beta$ -1,3-glucanase. The treatments suppressed the accumulation of  $O_2^-$  and  $H_2O_2$ , maintained membrane integrity, regulated the activity and expression of NADPH oxidase, superoxide dismutase, peroxidase and catalase to some degree. The treatment also increased activity and the expression of ascorbate peroxidase, glutathione reductase, dehydroascorbate reductase and monodehydroascorbate reductase, improved the accumulation of ascorbic acid and reduced glutathione, and inhibited the accumulation of dehydroascorbate and oxidized glutathione. It is therefore suggested that preharvest Stroby multiple sprays during fruit development induces resistance of muskmelon fruit by activating the phenylpropanoid pathway and reactive oxygen metabolism.



## 68 Exogenous polyamines improve disease resistance to black spot in apricot fruit

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**Keywords** exogenous polyamines, apricot fruit, defence-related enzymes reactive oxygen species

### ABSTRACT

Polyamines are low molecular weight organic polycations, positively charged at physiological pH, that are involved in different physiological processes, such as growth, development, and response to abiotic and biotic stresses in plants. However, relatively little work has been carried out on the role of these compounds in disease resistance of postharvest fruit and vegetables. Here the effect of postharvest exogenous polyamines treatment on black spot disease control and its mechanism involved were studied. The results showed that postharvest exogenous polyamine treatment significantly inhibited black spot development and increased the disease resistance of apricot fruit, the control activity had a polyamine type and concentration dependent manner. Among them, 1.5 mM spermine, 1.5 mM spermidine and 10 mM putrescine treatment significantly decreased the lesion diameters by 38.0%, 26.6% and 34.2% respectively compared to the control. Further studies showed that exogenous treatment increased  $O_2^-$  production rate and  $H_2O_2$  content in apricot tissue, the activities and gene express of the NADPH oxidase (NOX), such as superoxide dismutase (SOD), catalase (CAT), polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia-lyase (PAL) in fruit tissue were enhanced as well by exogenous polyamines treatment. Thus, polyamines may improve disease resistance of apricot fruit by accumulation reactive oxygen species (ROS) content, and inducing gene expression and activities of defence-related enzymes.

## 70 Formulation of *Bacillus amyloliquefaciens* CPA-8 using different systems: liquid, freeze-drying and fluid-bed spray-drying

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**Keywords** *Bacillus*, formulation, protectants, shelf life, *Monilinia* spp., biocontrol efficacy

### ABSTRACT

The efficacy of the biocontrol agent *Bacillus amyloliquefaciens* CPA-8 against brown rot caused by *Monilinia* spp. has been described and suggested as an effective alternative to chemical applications. To develop a commercial microorganism-based product, this work focuses on the assessment and comparison of three different formulation technologies: liquid formulation, freeze-drying and fluid-bed spray-drying. In order to meet the requirements of product quality, the effect of protectants on process survival, storage stability and antagonistic activity has been considered. For each process, CPA-8 cultures were concentrated and mixed with different protective substances such as MgSO<sub>4</sub>, sucrose and skimmed milk (SM). Results showed that CPA-8 freeze-dried cells without protectants or amended with SM suffered the highest survival losses (0.41-0.48 log unit). Moreover, the viability of the freeze-dried products assessed slightly decreased after four months of storage at both temperatures studied, 4 and 22 °C. Otherwise, liquid and fluid-bed spray-dried products were stable for four months at 4 °C and for 12 months at 22, 4 and -20 °C, respectively and no positive effect of the protectants could be observed. The most suitable CPA-8 products were then tested against *M. laxa* and *M. fructicola* in artificially wounded nectarines and in all cases the antagonistic activity was maintained similarly to fresh cells. The efficacy results revealed that the formulation process did not affect the biocontrol potential of CPA-8. This work led us to conclude that effective formulations with final concentrations ranging from 1.93x10<sup>9</sup> to 2.98x10<sup>9</sup> CFU ml<sup>-1</sup> and from 4.76x10<sup>9</sup> to 1.03x10<sup>10</sup> CFU g<sup>-1</sup> for liquid and dried products, respectively, have been obtained. Additionally, the suitability of the fluid-bed spray drying technology should be taken into account to develop a stable, effective and easily distributable CPA-8 product for practical applications to control brown rot in stone fruit.

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### 73 Postharvest diseases in pipfruit, new threats?

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**Keywords** apple, pear, fungal diseases, management

#### ABSTRACT

Postharvest diseases caused by fungi can cause large economic losses for growers. Retailers are requesting fruit with less residue than legally allowed and with as few active substances as possible. This causes changes in pre-harvest treatments and can contribute to a shift in the population of fungi present in orchards. Also climate is changing, new conditions give new opportunities for different sorts of fungi. Fungal diseases normally more common in Southern Europe are now increasingly common in the north. At pcfruit npo (Belgium) growers can deliver samples of fruit when problems occur. Past seasons more (new) fungal diseases are being diagnosed than in the past. More pear orchards are infected with *Stemphylium vesicarium* causing problems in the orchard as well as after storage. Infections of pears with *Phialophora malorum* caused great losses in 2015 and 2016. In those years *Tilletiopsis* spp., a disease that was rare became more common on apple. And last year fruits affected with *Botryosphaeria dothidea* were discovered. To observe changes in postharvest diseases, pcfruit npo collaborates with fruit growers and grading companies. Researchers assist in the grading process to count the number of affected fruits and to obtain samples. After isolation of the respective pathogens, in vitro and in vivo lab-tests were executed to check the efficacies of different fungicides.

**75 Developing a method to determine the resistance of stone fruit to *Monilinia* spp**  
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**Key words** *Monilinia fructicola*, *Prunus persica*, disease resistance, brown rot,  
phenotyping.

## ABSTRACT

Brown rot caused by *Monilinia* spp. is one of the most important diseases of stone fruit leading to generate postharvest losses of 80% in years of climate conditions favourable for pathogen, especially in late-ripening varieties. The current strategy to control postharvest diseases is based on the application of fungicides in the field. However, the appearance of fungicide-resistant strains, the difficulties to control the disease and registration issues are obstacles that need for research. Therefore, it is necessary to understand the virulence of the pathogen in the process of host infection to select varieties more resistant or tolerant to *Monilinia* spp. The aim of this study was to develop a reliable test to screen the level of resistance of different stone fruit varieties to this disease. The work carried out to develop this test has two approaches: a) from the side of the fruit, the effects of wounding, incubation time, ripeness and disinfection were investigated and b) from the side of the pathogen, the effects of conidial concentration and strain aggressiveness were determined. The results obtained indicate significant differences between wounded and non-wounded fruit, conidial concentration and incubation time. The effect of strain aggressiveness was also confirmed by evaluating the severity of the disease (lesion diameter) but, not for incidence. In general, for ripeness state no significant difference was observed between fruit at the same harvest date. Our results showed that disinfection of fruit prior to inoculation had an effect on the process of colonization. Data obtained has allowed to develop a robust phenotyping test including the most important factors involved in the process of screening for resistance to *Monilinia* spp. of commercial varieties as well as individuals. Moreover, the data obtained combined with QTL analysis will constitute a first step to understand the basis of the resistance of plant material. *Authors are grateful to Spanish Government for their financial support by a national project AGL2014-55287-C02-02 from Ministry of Economy, Industry and Competitiveness (MINECO) and to the Catalan Government (Generalitat de Catalunya) for the PhD grant 2016FL\_B 00442 (N. Baró-Montel) and for the funding received from CERCA Programme / Generalitat de Catalunya.*

## **77 Infection capacity of *Monilinia fructicola* on fruit under storage conditions and water dump postharvest processes**

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**Keywords** brown rot, stone fruit, postharvest processes, epidemiology, risk of infection

### **ABSTRACT**

Brown rot is the principal responsible of postharvest losses worldwide on stone fruit caused by *Monilinia* spp. During cold room storage or water dump processes, contaminated fruit with *Monilinia* spp. conidia could be infected during the postharvest period and develop decay. Study the capacity infection of *Monilinia* spp. during the postharvest management processes is crucially important to identify the moment where infection could occur. The aim of this study was to investigate the capacity of *Monilinia fructicola* to infect peaches and nectarines under storage cold conditions or when fruits are submerged in the water dump. Dry inoculum was prepared with sterilized sand and mixed with *M. fructicola* conidia. Then, each fruit was inoculated with 0.10 grams of sand using  $5 \times 10^6$  conidia/ fruit. Inoculated fruit were stored during several days under cold conditions. After 30 days of incubation, brown rot incidence was registered only in 3.3%. In relation to the water dump process, the results obtained for fruit artificially inoculated and incubated for 24 hours under cold conditions and then submerged in the water dump process showed that *M. fructicola* was not able to infect nectarines, and only 26.3 % of peaches. Our results showed that postharvest cold room storage conditions did not suppose a risk for *Monilinia* spp. infection on fruit. However, water dump process could provide optimal conditions to infect inoculated fruit, mainly on peaches although subsequent conditions of storage also influences the capacity of *M. fructicola* infection. This study provide new knowledge of the epidemiology of *Monilinia* spp. in postharvest, an area where low information is available. Ministry of Economy and Competitiveness (Government of Spain) with the project AGL2011-30472-C02-01, PhD grant BES-2012-059949 to Maria Bemat and CERCA Programme/Generalitat de Catalunya.

## **79 Nanopore sequencing of the genome of a soil isolate of *Metschnikowia pulcherrima***

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**Keywords** Nanopore sequencing, *Metschnikowia pulcherrima*

## ABSTRACT

Nanopore sequencing is a novel technology which allows sequencing of entire genomes using a relatively cheap, handheld device – Oxford Nanopore's MinION – powered by a state of the art personal computer. The technology generates long reads allowing for high quality genome assemblies while offering unmatched portability and simplicity of use. In the current study we sequenced the whole genome of a soil isolate of *Metschnikowia pulcherrima*, with proven *in vitro* efficacy in controlling post-harvest fungal pathogens. Using five flow cells, 338MB of high quality sequence data was obtained allowing a *de novo* assembly of the 16MB genome of *Metschnikowia pulcherrima* to 21x coverage. The *de novo* assembly generated consists of 20 contigs of a N50 contig length of 1.23MB and shows a 98.17% consensus identity to the reference genome. Furthermore, a polishing of the raw nanopore assembly using short and accurate Illumina reads resulted in an increase of this consensus identity to 99.45%. The data presented here shows that Nanopore sequencing allows for efficient and accurate sequencing of fungal genomes when combined with Illumina sequence data. While a *de novo* assembly shows promising results, the accuracy is not yet high enough. Nonetheless, we estimate that further improvements to the sequencing technology and better bioinformatic tools will allow such *de novo* sequencing of fungal genomes using only Nanopore sequencing data in the near future.

## 84 Effect of the Jintao kiwifruit floral remains on the incidence of stem end rot in different types of packaging during storage

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**Keywords** *Botrytis* spp., postharvest

### ABSTRACT

Packaging and storage at low temperature are critical practices to extend "Jintao" kiwifruit storage time while decreasing the metabolic rate and delaying the development of pathogens. In the industry, the fruits are brushed and then packed to be exported to destination. The objective of the study was to evaluate the effect of the presence of floral remains on the stem end rot caused by *Botrytis* spp in kiwi cv. Jintao from two orchards under different conditions of relative humidity. The inoculum level maintained on sepals and styles was quantified by cultivating these tissues on acidified potato dextrose agar plus Igepal for 7 days at 20 °C. The fruits were cured for 48 h, packed with or without the presence of floral remains, and stored without bag, in perforated (0.9%) or modified atmosphere bag (4,1 and 4,7% CO<sub>2</sub>) at 0°C. After 90 days, it was observed that the fruit brushed and packed without floral remains had a high prevalence of stem end rot, and this was even higher in fruits stored in a perforated bag (92% relative humidity) and modified atmosphere (98% relative humidity) than without bag. This study suggests that high relative humidity inside the packaging and the damage induced by removal of floral remains favours the expression of symptoms caused by *Botrytis* spp.

## 85 Bacterial dynamics and the prevalence of foodborne pathogens associated with the avocado fruit, *Persea americana* Mill

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### Key words

*Persea americana*; avocado; microbial dynamics; *Salmonella* spp.; *Escherichia coli*

## ABSTRACT

The volume of international food trade increases annually mainly due to increased consumption of ready-to-eat products and its convenience. The global demand for fresh produce has increased by 38% in the last decade, and due to the significant increase the awareness of microbial safety has become a priority. Fresh produce has received significant focus over the last few years with association to foodborne related outbreaks; this may be due to increasing demand of year-round availability and the increasing consumption globally. This study was aimed to monitor the microbial dynamics on the avocado carpoplane and pulp, comparing the microbial dynamics of the samples in season and out of season, determining the prevalence of foodborne pathogens on both the carpoplane and fruit pulp and determining the efficiency of fruit washing on the microbial load on the fruit carpoplane. In this study both conventional and molecular methods were used for the isolation and detection of bacteria. The 3M<sup>®</sup> Molecular Detection System and conventional culture based methods were used for the detection of *Salmonella* spp. and *Listeria* spp. Matrix assisted laser desorption ionization time-of-flight mass spectrometry was primarily used for the classification and identification of microorganisms. The results obtained during the avocado season indicated a decrease in microbial load on the cultural carpoplane after washing of the fruit, although an increase in the microbial concentration was observed in the fruit pulp after pre-processing washing. An increase in the coliform concentrations on both the carpoplane and pulp following pre-processing fruit washing was observed. Presence of *Escherichia coli* decreased in both the carpoplane and fruit pulp following pre-washing of the fruit, with *E. coli* isolated from 9.4% of total number of samples (n=180), equally from surface and pulp. However, *E. coli* were more frequently isolated from samples before washing (6.1%) than after washing and accounts (3.3%). *Salmonella* spp. presence accounted for 1.6% of the total number of samples (n=180), with 1.1% of *Salmonella* spp. present from the avocado pulp before pre-processing washing. During the season, no *Listeria* species were isolated.



## 86 The potential use of natural antimicrobial compounds to reduce decay of strawberries in the supply chain

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**Keywords** strawberry, limonene, grey mold, humidity, shelf-life

### ABSTRACT

Strawberries (*Fragaria x ananassa* Duch. cultivar "Sonata") are very perishable fruits with a short shelf-life. Strawberries are susceptible to mechanical injuries, desiccation, decay, and physiological disorders, and berries rapidly spoil by infection from *Botrytis cinerea*. Spoilage develops at higher rates and at variable temperatures if soft berries are packaged and surfaces are wet during handling in the supply chain. The aim of this study was to evaluate the potential use of limonene as a natural antimicrobial compound to reduce decay of strawberries after harvest. The experiment consisted in four different types of packaging for strawberries: i) unwrapped cardboard trays; ii) cardboard trays wrapped with plastic film; iii) dipped in water, placed in cardboard trays wrapped with plastic film; iv) dipped in water with 0.025% limonene, placed in cardboard trays wrapped with plastic film. The samples were stored in climate chambers for 2 days at 5 °C. Before analysis samples stayed in room temperature for 8 h to simulate retail conditions. Unwrapped strawberries shriveled and lost 3.5±0.6 % of the initial weight during 2 days (+8h) of storage at 5 °C, while other treatments 0.7±0.1 % of the initial weight. Wrapped berries maintained quality as berries were firm, glossy and with green sepals after storage. However strawberries must be relative dry on the surface at the moment of packaging, as a wet surface will increase the development of mold in the retail, especially softer types as this particular strawberry variety. Limonene reduced grey mold growth on strawberries that were packed wet. The result shows that the use of antimicrobial compounds for packaging of strawberries may be a promising tool especially if strawberries are packaged with slightly wet surfaces or temperature fluctuates in the supply chain as it will increase condensation of water on the surface. The study underlines that the quality of strawberries in the supply chain depends on the harvested raw material quality, the selected handling and packaging solution, and the duration and temperature in the supply chain, the cause of grey mould decay in plums, is of commercial importance in the South African export fruit industry.

## 87 Characterization of environmental yeasts for the control of *Botrytis cinerea* in table grapes

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**Keyw ords** Yeast, table grape, gray mold, *Botrytis*, post-harvest

### ABSTRACT

*Botrytis cinerea* is a phytopathogen fungus that infects a wide variety of fruits and vegetables, causing the gray mold disease. A target to this plant pathogen is the table grapes, causing significant economic losses to Chile and other export countries. Currently, chemical fungicides are used to control infection by *Botrytis*; however, there has been the emergence of resistant strains and side effects due to the intensive use of these compounds. An alternative to the use of chemical fungicides are biological control agents but commercially only *Trichoderma spp.* and *Bacillus subtilis* have been used at preharvest level. The purpose of this work was to characterize environmental microorganisms able to inhibit the growth of *Botrytis cinerea* on table grapes and to evaluate the main mechanism by which they exert this effect. A total of 19 yeast-like isolates were initially selected for their ability to grow at low temperature (4 °C). Three of these isolates (858AB, UCT138 and 1351A) caused significant inhibition of growth of *Botrytis cinerea* (strain B05.10) in table grape trials. These isolates were identified by sequencing the ITS region and corresponded to *Candida railenensis* (858AB and UCT138) and *Saccharomyces sp.* (1351A). When the control mechanism by which these isolates inhibited the growth of *Botrytis* was evaluated, the effect was determined to be mediated by the production of volatile metabolites. In this context, because these isolates can grow at a low temperature and do not need a direct contact with the pathogen in order to inhibit their growth, these microorganisms have an interesting potential as biological control agents to reduce the gray mold caused by *Botrytis* in post-harvest conditions of table grapes.

## 95 Detection and quantification of *Botrytis cinerea* on table grapes at preharvest using ddPCR

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**Keywords** DNA, rapid detection tool, inoculum build-up

### ABSTRACT

*Botrytis cinerea*, is one of the major causal agents of postharvest decay in table grapes contributing to 20% losses in fresh produce world-wide. The aim of this study was to detect and quantify *B. cinerea* on asymptomatic grape tissue at different phenological stages (full bloom, pea size and commercial maturity) using droplet digital polymerase chain reaction (ddPCR). Samples were collected from two commercial vineyards (sites A and B). *Botrytis cinerea* could be detected at different phenological stages using the optimised ddPCR method. Prevalence and concentration of *B. cinerea* differed between phenological stages and sites. The highest prevalence of *B. cinerea* (82.9%) was found at full bloom stage at site B followed by mature berry stage (44.4%) and pea size (35.2%). *Botrytis cinerea* prevalence in site A increased from 33.3% to 98.21% between full bloom and mature stage. Furthermore, the *B. cinerea* concentration varied between stages within the two sites. The concentration in site A showed a threefold increase from pea size (2.67 copies /  $\mu$ l of DNA) to mature berry stage (9.16 copies /  $\mu$ l of DNA), compared with the decline noted for similar growth stages at site B. This study demonstrates the potential of ddPCR as a rapid detection tool for *B. cinerea* in preharvest environments. This novel method will assist growers in more effective monitoring on inoculum build-up on asymptomatic grape tissues, and help limit the risk of postharvest decay.

**96 Post-harvest monitoring of *Botrytis cinerea* inoculum in table grapes: Towards the development of predictive model for *Botrytis* rot incidence and severity**

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**Co-author(s)** None

**Keywords** Packhouses, Detection, Quantification

**ABSTRACT**

A significant factor affecting table grape quality is post-harvest decay, caused by the fungus *Botrytis cinerea*. To assist in the management of *Botrytis* rot and to further reduce the impact of the disease, an economically viable method to monitor inoculum levels within a batch of grapes is essential. Westcape Biotech has developed a tool to detect and quantify *B. cinerea* in grape berries. To test the accuracy and applicability of the method to monitor *B. cinerea* infections in packhouses, a trial was conducted. *B. cinerea* inoculum levels were measured over 6 weeks in grape cartons artificially inoculated or uninoculated with *B. cinerea* spores. Furthermore cartons were either treated with fungicide or kept untreated. The results showed that the method was highly accurate and sensitive in detecting and quantifying *B. cinerea* under all conditions at all time-points analysed. The initial inoculum present within grape berries at harvest seems to be the most important factor determining the amount of inoculum present after 6 weeks of storage; the pattern of accumulation was the same regardless of the starting amount of inoculum. These findings suggest that the method is suitable to develop diagnostic tools and predictive models to determine the likelihood of *Botrytis* rot symptom development, which would greatly assist stakeholders in reducing the impact of the disease.

## 103 Sanitisation of fungicide drench solution and effects on green mould and sour rot control

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**Keywords** *Penicillium digitatum*, sanitisers, Residue levels

### ABSTRACT

Green mould (*Penicillium digitatum*) is the most important citrus postharvest disease, however, sour rot (*Galactomyces citri-aurantii*) becomes a serious decay concern after rainfall, and since the fungicide guazatine is restricted on certain export markets. Sanitisers can be added to drench solutions and are useful to reduce sour rot inoculum levels accumulating from dust on harvested fruit. The effect of two sanitisers were compared during *in vitro*, *in vivo* and commercial packhouse trials. Variables investigated were green mould and sour rot development, ability of the sanitiser to reduce microbial load (CFU mL<sup>-1</sup>) in the drench solution and fungicide persistence for effective green mould control. In commercial packhouse trials, wounded navel orange fruit were drenched with a thiabendazole (TBZ), pyrimethanil (PYR), guazatine (GZT) and 2,4-dichlorophenoxyacetic acid (2,4-D) drench mix, with either chlorine (Cl) or hydrogen peroxide/peracetic acid (PPA) added after every 50 bins during a drenching run of 150 fruit bins. Green mould infection was reduced from  $\geq 78.3\%$  to  $\geq 67.7\%$  following fungicide drench application. Infection and fungicide persistence were similar regardless of sanitiser treatment, although green mould infection levels increased significantly by bin 150 (10.6% vs. 5.2 – 6.0%). A range of sanitiser concentrations (0, 20, 40, 60 and 80  $\mu\text{g mL}^{-1}$  Cl or 0.00, 0.01, 0.10, 0.30 and 0.60% PPA) were combined with mixtures of TBZ, PYR and 2,4-D and *G. citri-aurantii* spores ( $\approx 3.175 \times 10^4$  spores mL<sup>-1</sup>) for 1, 3 and 60 min exposure, and plated out. The sanitisers did not affect fungicide concentration levels. Hydrogen peroxide or peracetic acid treatments completely reduced the sour rot inoculum (0.0 CFU mL<sup>-1</sup>) in mixtures after treatments for 1 to 3 min. at high pH levels (> 10). *In vivo* trials involved exposing 24 h *P. digitatum* inoculated and uninoculated wounded fruit to TBZ, PYR and 2,4-D and *G. citri-aurantii* spores (similar to *in vitro* trials) containing either 80  $\mu\text{g mL}^{-1}$  Cl or 0.3% PA with the addition of 0, 500 or 1000  $\mu\text{g mL}^{-1}$  kaolin, used to simulate dust accumulation during drenching. Residue levels, solution concentration and green mould control were similar between sanitiser and kaolin treatments. Hydrogen peroxide or peracetic acid treatments improved sour rot control on Valencia and Nadorcott mandarin fruit and improved green mould control on Nadorcott mandarin fruit. Exposure of the mixtures to 0.3% PPA for 3 min gave superior results to the Cl treatment at high pH levels. This study contributes to our understanding of the use of sanitisers to augment fungicide action.

## 105 Postharvest fungicide sensitivity of South African *Botrytis cinerea* isolates causing grey mould on pears

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**Keywords** calyx end rot, fungicide resistance, postharvest fruit rot

### ABSTRACT

Botryticides currently registered for postharvest use on pears are at risk for fungicide resistance development due to the nature of the pathogen and the site directed activity of the chemicals. Monitoring of fungicide sensitivity is a prerequisite for fungicide resistance management. *Botrytis cinerea* baseline fungicide sensitivities were investigated on fungal isolates from three orchards of pear trees previously exposed to botryticides. The isolates were tested for their sensitivity to benomyl, fludioxonil, iprodione, and pyrimethanil at discriminatory doses (N=45). Pyrimethanil was found to reduce fungal growth of *B. cinerea* by 50% (EC<sub>50</sub>) at a concentration of 0.18 mg L<sup>-1</sup>. Significant shifts towards resistance were identified in *B. cinerea* isolates towards pyrimethanil when tested at the discriminatory dose (29% resistant). At the discriminatory dose mycelial growth was inhibited in 93 % isolates with benomyl, 99.4 % with fludioxonil, and 95% with iprodione. Furthermore, a microtiter-assay was optimised, which promises to be a less labour- and time intensive method to monitor *B. cinerea* fungicide sensitivity. In 96 well cell culture clusters, spore suspensions of *B. cinerea* were exposed to a range of iprodione and pyrimethanil concentrations in the presence of rezazurin dye. The measured reduction of the rezazurin dye indicated the presence of cells with active respiration. Thus non-respiring fungal cells indicate toxicity and fungicide sensitivity could be measured by quantifying fungal biomass with the use of a microplate photometer. Results suggest that pyrimethanil sensitivity levels in *B. cinerea* have shifted towards resistance and that botryticides involving dicarboximides, or phenylpyrrole group fungicides could effectively control grey mould on pears.

## 115 *Penicillium* in postharvest fruit handling environments

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**Keywords** Air mycoflora, Decay, Supply chain, Presence, Prevalence, Fungal inoculum

### ABSTRACT

Fresh produce quality deteriorates during postharvest handling mainly due to microbial decay. Fresh fruit moves long distances in the supply chain from the farm to the point of sales especially when exported. Along the supply chain, fruit is stored in or passes through several facilities that may expose the product to different postharvest pathogen inoculum sources. In this study, such facilities including cold storages, fruit container before and after export, receival areas, re-pack areas, distribution centre, retail storage and display areas were sampled for presence and prevalence of fungal inoculum loads. *Penicillium* was the most common genus and the dominant species consisted of *P. glabrum* (23.40%), *P. chrysogenum* (15.13%), *P. crustosum* (14.16%), *P. brevicompactum* (8.96%) and *P. expansum* (8.39%). The highest and the lowest mycofloral counts were found in re-pack and cold storage facilities respectively. In addition, a more realistic threshold values were proposed for the air of fruit handling environments and re-pack facilities exceeded even these values.

## 116 Economic aspects of losses and waste: case study of the South African table grape supply chain

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**Keywords** export value chain, social and environmental consequences

### ABSTRACT

Globally 1.3 billion tons of good food is wasted per annum. Having regard for the economic, social and environmental consequences the management of food losses and waste is an obvious priority in pursuing a sustainable, sovereign global food system. A study was undertaken to explore the phenomenon of food losses and waste in the South African Table Grape Industry's export value chain. A mixed method approach was used to develop a framework to guide the identification and quantification of losses and waste within the particular chain. Practically the framework is a tool for stakeholders to guide policymaking and decision making at industry and operator level to manage losses and waste. Application of the framework to selected South African table grape export chains suggests that the bulk of the losses and waste historically occur at the production and intake stages of the chains. An approximate, 9.5% (R270.5m) of losses and waste occurred between the production and intake stages and 2.2% (R93.2m) and 3.8% (R0.4m) occurred between intakes and exports and between the importers to retail depot, respectively. Reducing losses and waste requires more thoughtful and customized alignment between stakeholders to enhance overall supply chain performance by managing losses and waste.



## 123 RE-PEAR: New sustainable and long term solution for the pear postharvest sector

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**Key words** Post-harvest coating, fungicides, superficial scald, antioxidant, postharvest pathogens.

### ABSTRACT

Pears are a highly perishable seasonal product which needs to be picked up before maturation. Once harvested, pears are subjected to a post-harvest treatment and stored in cold chambers under controlled atmosphere to be commercialized in optimal conditions throughout the year. However, long term storage at low temperatures may render the fruit to develop superficial scald, a physiological disorder caused by oxidative damage and

leading to the browning of the pear skin; moreover, during the process pear production can be significantly reduced due to decay, mainly due to fungi diseases. Until now, the control of superficial scald and postharvest disease has been carried out using different chemical synthetic products. However, public concerns about food safety and the use of chemical products have produced a change in the EU legislation leading to the proposal of a new Directive (2009/128/EC) banning the use of the most popular antioxidant, bactericide and fungicide chemical treatments for the pear sector. REPEAR project has been designed to overcome the current problems of the pear production sector after the changes in the EU legislation concerning pesticides.

The project aims to find an integrated solution based on:

- a) Preparation of Post-harvest Management Control Practice (PMCP) guidelines aimed at minimizing the risk of contamination of pears during the harvest and postharvest period;
- b) Development of an anti-scald and antimicrobial coating based on natural products for postharvest application on pears;
- c) Development of a novel air filtration system to minimize the presence of fungi spores and bacteria in pear conservation chambers.

By achieving the targeted objectives, REPEAR project will help the pear production sector to reduce the postharvest losses giving a solution according to EU legislation.

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## 124 Effect of postharvest treatments on development of latent CBS infections

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### ABSTRACT

Citrus black spot (CBS) is caused by *Phyllosticta citricarpa*, regarded as a quarantine pathogen in certain countries. CBS is present only in areas with warm summer rainfall climates in South Africa. It is of economic importance to this citrus industry, particularly since the European Union, a major export destination for South African citrus fruit, imposes a zero tolerance on the import of fruit with CBS lesions. Very effective, high cost preharvest fungicide programmes are therefore sprayed to control CBS. However, since infections on fruit remain latent until fruit maturity, infected but asymptomatic fruit might still be packed to these CBS sensitive markets. Latent infections may develop during the postharvest period and after packing (Kiely, 1948; Kotzé, 1981). Previous research has demonstrated that postharvest sanitation, fungicide, wax and cold storage treatments significantly reduced CBS lesion development, and the viability of spores produced from these lesions (Korf et al., 2001). Moreover, latent infection that did develop into CBS lesions had a very low reproductive ability, with less than 2.1% of these lesions developing asexual fruiting bodies. When keeping in mind the epidemiological requirements for pycnidiospore release, it can be concluded that harvested fruit is not an epidemiologically significant pathway for the spread of the CBS fungus, especially when the fruit has been subjected to standard packhouse treatments