Exploring the microbial communities associated with *Botrytis cinerea* during berry development in table grape with emphasis on potential biocontrol yeasts

P.C. Carmichael, N. Siyoum, L. Chidamba and L. Korsten

Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, South Africa.

Correspondence Lise Korsten, Department of Plant and Soil Sciences, University of Pretoria, South Africa. Phone: +27 12 420 3295. Fax: +27 12 420 4588. Electronic mail address: lise.korsten@up.ac.za.

Abstract

Table grapes harbour a wide diversity of microbes, some of which are potential biocontrol agents that may be responsible for the control of fungal pathogens on the phyllosphere. This study evaluated the diversity of microbial communities associated with naturally present *Botrytis cinerea* inoculum, with special emphasis on populations of potential biocontrol yeasts during berry development in table grapes. Samples were collected from two agro-ecological habitats in South Africa (Northern Province), characterised by low rainfall (site A) and high rainfall (site B). The phenological development samples included those at full bloom, pea size and mature berry stages. Within the group of yeasts known to be natural antagonists, *Aureobasidium*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* could be cultured, while pathogenic fungal genera from asymptomatic samples included *Cladosporium*, *Alternaria*, and *Aspergillus*. *Botrytis cinerea* could only be cultured at the harvest stage from symptomatic and asymptomatic berries. Overall, the study showed the highest prevalence of *Alternaria* (35.6%), *Cladosporium* (27.2%) and *Rhodoturula* (21.2%). In conclusion, the study reveals a diverse pathogenic and beneficial naturally known yeast genera in the presence of *B. cinerea*. Such

information and knowledge can be further explored to manipulate potential antagonistic populations to prevent establishment of pathogenic populations and secure dominance of antagonistic populations at the harvest stage.

Keywords: pathogenic, microbiome, phyllosphere, sequences, fungi, biocontrol agents.

Introduction

Table grape (*Vitis vinifera* L.) is a high value crop with a global production of about 21.9 million metric tonnes (USDA, 2017) recorded in 2016/17. However, table grapes are highly susceptible to a number of fungal diseases, with decay caused mainly by *Botrytis cinerea* Pers. (Dean et al. 2012; Rivera et al. 2013; Romanazzi et al. 2016). Early infection by *B. cinerea* occurs at full bloom, after which the pathogen goes into a quiescent phase until conducive environments prevail. Although, extensive research has been done and novel technologies explored over the years to limit postharvest decay, the pathogen still remains a priority concern to the industry. This is partially attributed to its latent behaviour and low isolation frequency at the preharvest stage as well as its ability to grow at low temperatures (0-2 ^{o}C) during storage (Dennis and Cohen, 1976), making it difficult to control.

Although several studies on fungal or bacterial populations of grape have been done (Bukulich et al. 2014; Pinto et al. 2014; Taylor et al. 2014; Setati et al. 2015), the studies were either based only on conventional culture methods and / or did not look into potential community interaction or shifts through the various phenological development stages. Moreover, the studies did not investigate the influence of natural populations of potential biocontrol yeasts on observed microbial dynamics and community structure including potential pathogenic fungi.

The identification and quantification of the epiphytic communities present during berry development provides knowledge and information that would improve understanding of the complex interactions that prevail between beneficial and pathogenic species. Such data may give practical implications in table grape disease management, particularly in establishing safer strategies for the prevention and control of economically important postharvest pathogens, including *B. cinerea* which greatly affect yield and postharvest quality of the produce (Van Boxstael et al. 2013). The objective of this study was to characterise the composition of fungal communities associated with *B. cinerea* positive samples previously detected at different phenological stages in table grapes. In addition, the diversity in potential biocontrol yeasts and other pathogenic populations was evaluated to compare co-existence during berry development. The study focused specifically on the abundances and diversity of yeast populations already known to be natural antagonists against postharvest pathogens, with particular interest to *Botrytis cinerea*. The groups of yeasts of interest included *Aureobasidium* (Rathnayake et al. 2018), *Cryptococcus* (Fu et al. 2015), *Rhodotorula* (Zapata et al. 2015) and *Sporobolomyces* (Ianiri et al. 2017).

Materials and Methods

Site and sample collection and analysis

Table grape samples (cv. Crimson Seedless) representing three phenological stages (full bloom, pea size and commercial maturity) were collected at preharvest from two commercial farms (site A and B) in the Northern Province situated in the north eastern part of South Africa. The two farms included in the study were located in geographically different agroecological environments, more than 100 km apart. Site A is situated at an elevation of 899 m above sea level, whereas site B is at 1123 m. Site B generally receives higher annual rainfall (350–700 mm) than site A (150–350 mm) under a normal rainfall pattern (Weather Burro, Pretoria). At

both sites, the samples were taken from 11-year-old Y-trellis vineyards under netting and drip irrigation. The farms are Global-GAP certified for over 10 years. Near-by crops found on site were citrus, water melon, natural forests and pasture grasses.

Sample collection and processing methods were described in Carmichael et al. 2018). For the current study, DNA samples previously confirmed as *B. cinerea* positive (Carmichael et al. 2018) were used. Samples were representatives of the three phenological stages used in the study, full bloom (*n*=6), pea size (*n*=6) and commercial mature stages (*n*=6). Each vine was sampled from three positions representing east, west, and inside canopy to harmonise the effect of micro-climate per sample. Amplification and sequencing for total fungal communities was done as previously described by Carmichael et al. (2017).

Data processing, taxa classification and identification

Sequence data from illumina platform were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA) as previously described (Carmichael et al. 2017). Diversity indices (Shannon, Choa1 and Observed richness) for each sample were calculated rarefied OTU table using the 'Visualization and Analysis of Microbial Populations Structure (VAMPS)' (http://vamps.mbl.edu) online pipeline. Krona plots (Ondov and Phillippy 2011) were used to show taxonomic probability mass. Venny 2.1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to define uniqueness of the OTUs representing the different samples. The accuracy of taxonomic assignments of sequences associated with each OTU within an identified fungal genus with at least 0.1% relative abundance (Abdelfattah et al. 2016b) was confirmed by extracting and comparing with validated barcode sequences.

Fungal pathogens and potential biocontrol yeasts

A total of 14 pathogenic fungi and 11 potential biocontrol yeast considered in this analysis are presented in Tables 1 and 2, respectively. Sequences were retrieved and compared with 18S rRNA gene sequences of these known pathogens using a distance matrix. The 18S rRNA method is restricted to fungal pathogens, which include yeasts. A Juke-Cantor distance of 0.03 (Schloss and Handelsman, 2006) was used as a measure of similarity at species level.

Table 1. Genus of pathogenic fungi considered for this study. Groups in bold font represent postharvest pathogens of concerns reported in table grapes

Genus	Pathogenic species	Reference
Alternaria	***Alternaria alternata**; Alternaria lewia*	Swart et al. 2017;
Phialophora	*Phialophora-type [anamorph]*	Ferreira et al. 2017
Cercospora	*Cercospora brachypus*	Kernaghan et al. 2017
Sphaceloma	*Sphaceloma ampelinum*	Poolsawat et al. 2010
Aspergillus	***Aspergillus niger***	Perrone et al. 2006
Botrytis	***Botrytis cinerea***	Holz et al. 2003
Cladosporium	***Cladosporium cladosporioides**; Cladosporium colombiae*	Briceño et al. 2008
Plasmopara	*Plasmopara viticola*	Liu et al. 2018
Erysiphe	*Erysiphe necata*	Dufour et al. 2011
Penicillium	***Penicillium expansum***	Lai et al. 2015
Fusarium	*Fusarium oxysporum*	Brum et al. 2012
Sclerotinia	*Sclerotinia*	Latorre et al. 2001

Table 2. Genus of potential biocontrol yeasts considered in the study and were previously reported effective against *Botrytis cinerea*

Genus	Species	Trade name
Candida	*Candida sake; Candida oleophila*	Nexy (for Candida oleophila)
Aureobasidium	*Aureobasidium pullulans*	Boni protect / Botector
Cryptococcus	*Cryptococcus albidus*	Yield Plus
Saccharomyces	*Saccharomyces cerevisiae*	-
Pichia	Not identified	-
Sporidiobolus	Not identified	-
Sporobolomyces	Not identified	-
Rhodotorula	Not identified	-
Bulleromyces	Not identified	-
Sporobolomyces	Not identified	-
Filobasidium	Not identified	-

Phylogenetic analysis and identification of pathogenic fungi and potential biocontrol yeasts

Pipeline Initial Process was used to sort and trim raw sequences on the Ribosomal Database Project II. Tag sequences were trimmed from sorted sequences, and those that could not be sorted or less than 150bp or had more than one identified base were removed. Classifications of pathogens were analysed for the presence of sequences affiliating within the identified pathogenic genera. The list of pathogenic fungi was based on literature review of genera previously reported to infect grapes (Table 1), and those of potential biocontrol (Table 2) agents, against *B. cinerea* and other postharvest pathogens of concern. Sequences were aligned using ClustalX for the respective genera together with those extracted from NCBI Genbank. In the final classification, the processed alignments were clustered using DNADist program in PHYLIP. Sequences that were within 0.03 Jukes-Cantor distance of either a known pathogenic fungi or biocontrol yeast against *B. cinerea* were classified as potential pathogens or biocontrol agents. All positions containing gaps and missing data were eliminated. Evolutionary analysis were conducted in MEGA7 (Tamura et al. 2013).

Statistical Analysis

Statistical analysis was done using General Linear Models procedure of Statistical Analysis Systems (SAS) version 9.4 (Institute Inc., Carry NC, USA).

Results

Sequencing results, diversity and richness

A total of 701,774 high quality sequences were recovered after quality evaluation (Table 3) and were assigned to 1,282 fungal OTUs. After collapsing biological replicates, the number of sequences in the grape samples varied between 80,374 and 135,419, while the number of OTUs ranged between 159 and 285. A rarefied analysis to an even depth of 1000 showed a higher

number of overall OTUs (674) at site B compared to site A (608). Of the total OTUs observed, 37.8% were revealed at the pea size stage followed by full bloom (31.5%) and mature stages (30.7%) respectively.

Table 3. Summary of analyses and metagenomic results of *Botrytis cinerea* positive samples from full bloom, pea size, and mature stages from two commercial table grape farm sites

Site	Phenological stage	Sequences	Total OTUs [a]	Observed Richness (S)	ACE	CHAO	Shannon-Weaver Diversity Index	Simpson Diversity Index	Inverse Simpson
Site A	Full bloom	80374	164	77	77.81	78.14	3.49	0.93	15.34
	Pea size	134836	285	82	92.67	96.88	2.68	0.81	5.18
	Mature	133375	159	84	84.34	85	3.94	0.97	32.14
Site B	Full bloom	135419	240	93	103.17	117.14	3.3	0.93	13.94
	Pea size	110548	200	70	76.22	79.53	2.2	0.74	3.92
	Mature	107222	234	100	107.06	114.7	3.65	0.95	19.65

[a] Total number of OTUs detected

Structure of fungal community associated with the presence of *Botrytis cinerea* in table grape developmental stages

The phylum *Ascomycota* dominated the data set (73.5% of total reads) irrespective of sites and phenological stages. This was followed by *Basidiomycota* at 26.5%. Other phyla included *Cryptomycota*, *Streptophyta*, and *Chytridiomycota*, at less than 2% abundance level. The pea size stage of site A had the highest level of *Ascomycota* (93.9%) while at site B, the highest dominance was observed at full bloom (86.3%). *Ascomycota* decreased with berry development from full bloom to mature stage in site B, while the opposite was observed in site A. In site B, a decrease by 39.5% from full bloom to mature stage was observed. *Basidiomycota* were highly dominant at the mature stage (53%) of site B, and site A, the full bloom stage had the highest dominance (47%). At site A, the *Basidiomycota* decreased drastically by almost eight-folds from full bloom to pea size stage, whereas in site B, an increasing trend from full bloom to mature stage was observed (Fig. 1). Of the least dominant phyla, *Cryptomycota* and *Chytridiomycota*, dominated at pea size stage of site A only with 34 and 15 of total reads, respectively.

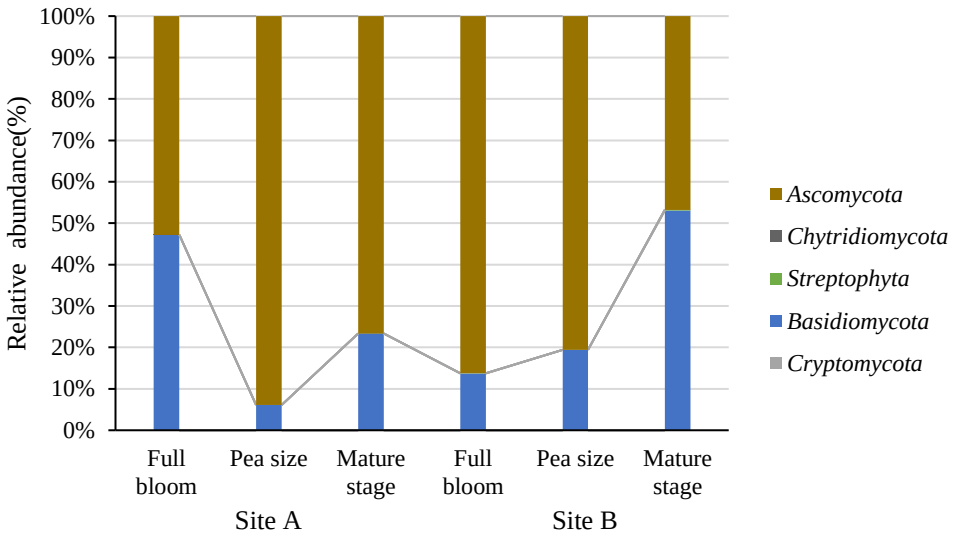

Fig. 1. Relative abundance of different fungal phyla, detected on *Botrytis cinerea* positive asymptomatic grape samples of the three phenological stages at two table grape farm sites (A and B)

At class level, the *Dothideomycetes* had the highest relative abundance in all data set (> 40%). This was followed by *Mycobotryomycetes*, ranging between 5 and 35 %. Pea size stage of site A showed the highest abundance of *Dothideomycetes*, representing more than 90% of the detected classes (Fig. 2). Although the class *Saccharomycetes* consisted of less than one percent of all total reads, 58% of the reads were from the full bloom stages of both site A and B. *Tremellomycetes*, a class of dimorphic fungi was highest in site B and increased significantly by 6.7% from full bloom (3.9%) to mature berry stage (10.6%).

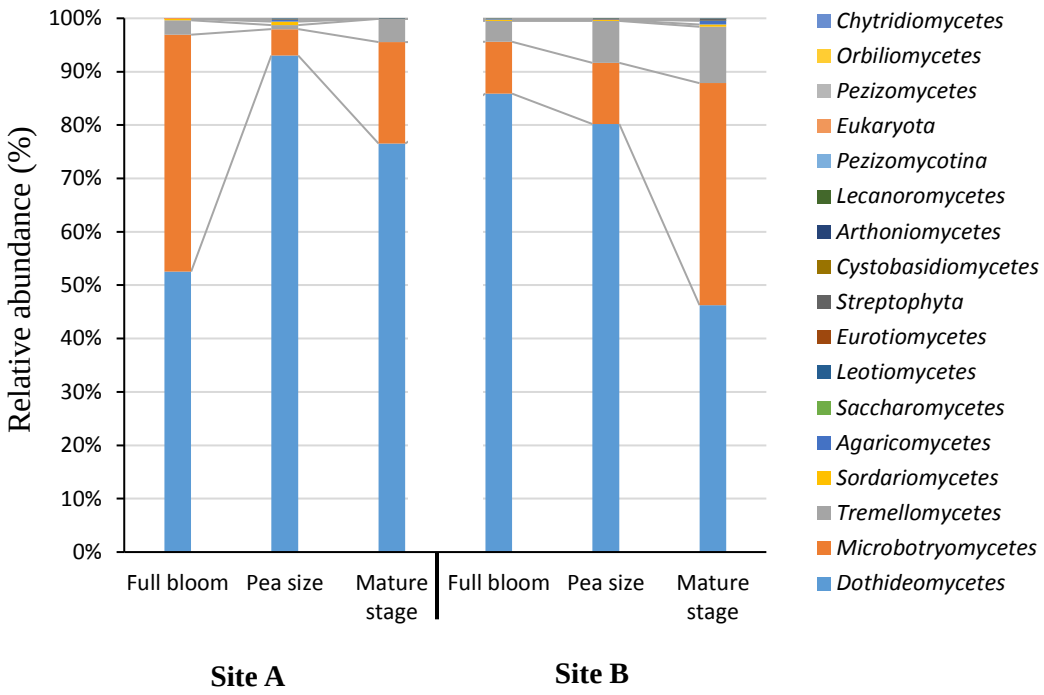

Fig. 2. Relative abundance of different fungal classes, detected on *Botrytis cinerea* positive asymptomatic grape samples of the three phenological stages at two table grape farm sites (A and B)

Of the 56 detected orders, *Pleosporales* (36.8%), *Capnodiales* (27.3%), *Sporidiobolales* (21.4%), *Dothideales* (7.1%) and *Filobasidiales* (3.9%) had a higher relative abundance. Other orders with abundance frequencies >1% were *Tremellales* (1.04%) and *Dothideomycetes* (1.18%) while the others had a dominance level <1%. The order *Pleosporales* was relatively the most abundant throughout all samples (Fig. 3), however, this varied between the sites. The pea size stage showed distinct differences in level of abundances of dominant orders. The highest relative abundance of *Pleosporales* (68.6%) was detected in site A, while *Capnodiales* (38%) was abundant at site B of this stage. Also, site B expressed the most dominance of *Pleosporales* during full bloom (46.3%), which later reduced to 15.7% and 15.4% at pea size and mature stages, respectively. A large variation was noted between individual samples. The order *Sporidiobolales* was widely abundant at full bloom of site A (43.9%) and mature stage of site B (41.3%), whereas <20% relative abundance was detected on other samples.

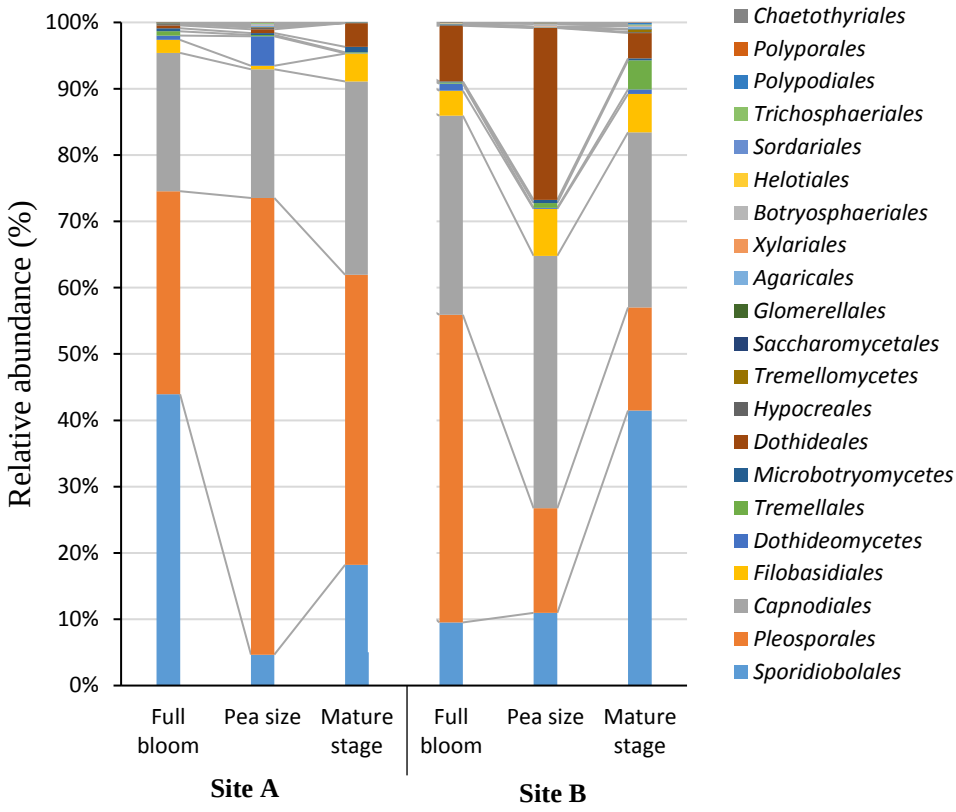

Fig. 3. Relative abundance of different fungal orders, detected on *Botrytis cinerea* positive asymptomatic grape samples of the three phenological stages at two table grape farm sites (A and B)

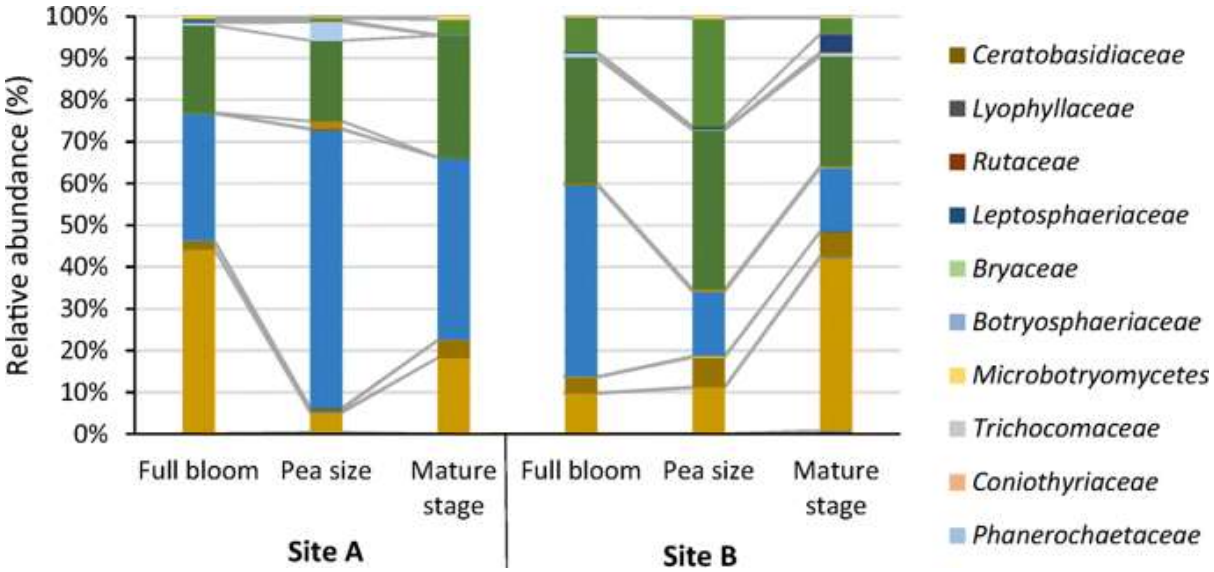

Fig. 4. Relative abundance of different fungal family, detected on *Botrytis cinerea* positive asymptomatic grape samples of the three phenological stages at two table grape farm sites (A and B)

A total of 116 families were found in the fungal populations. Of which the families *Sporidiobolales* (21.4%), *Filabasidiales* (3.8%), *Pleasporaceae* (36.1%), *Cladosporiaceae* (27.3%), *Aureobasidiaceae* (7.1%), and *Dothideomycetes* (1.2%), were the most abundant (Fig. 4). Other families included *Lycoperdaceae* (0.1%), *Tremellomycetes* (0.11%), *Nectriaceae* (0.11%), *Filobasidiaceae* (0.04%), *Didymellaceae* (0.5%), *Erythrobasidiaceae* (0.03%), *Microbotrymycetes* (0.4%) and had frequencies < 1%. Out of the 116 families detected, five and 23 were unique to full bloom and commercial mature stages of site A, respectively. There were no unique families identified to pea size stage of site A (Fig. 5). Thirty seven of the families in site A were shared between the three stages, and this constituted 46% of the total families detected. In site B, 11, two and eight families were detected exclusively to the investigated phenological stages (full bloom, pea size and commercial mature stages, respectively) (Fig. 5).

However, irrespective of the phenological stages, 18.2% of the families were unique to only site A, and this included *Erysiphaceae*, *Pleosporales*, *Glomerellaceae* and others, while 15.2% were distinct to site B. Exclusive families to site B included *Debaryomycetaceae*, *Agaricaceae*, *Pichiaceae*, and *Dothideales*.

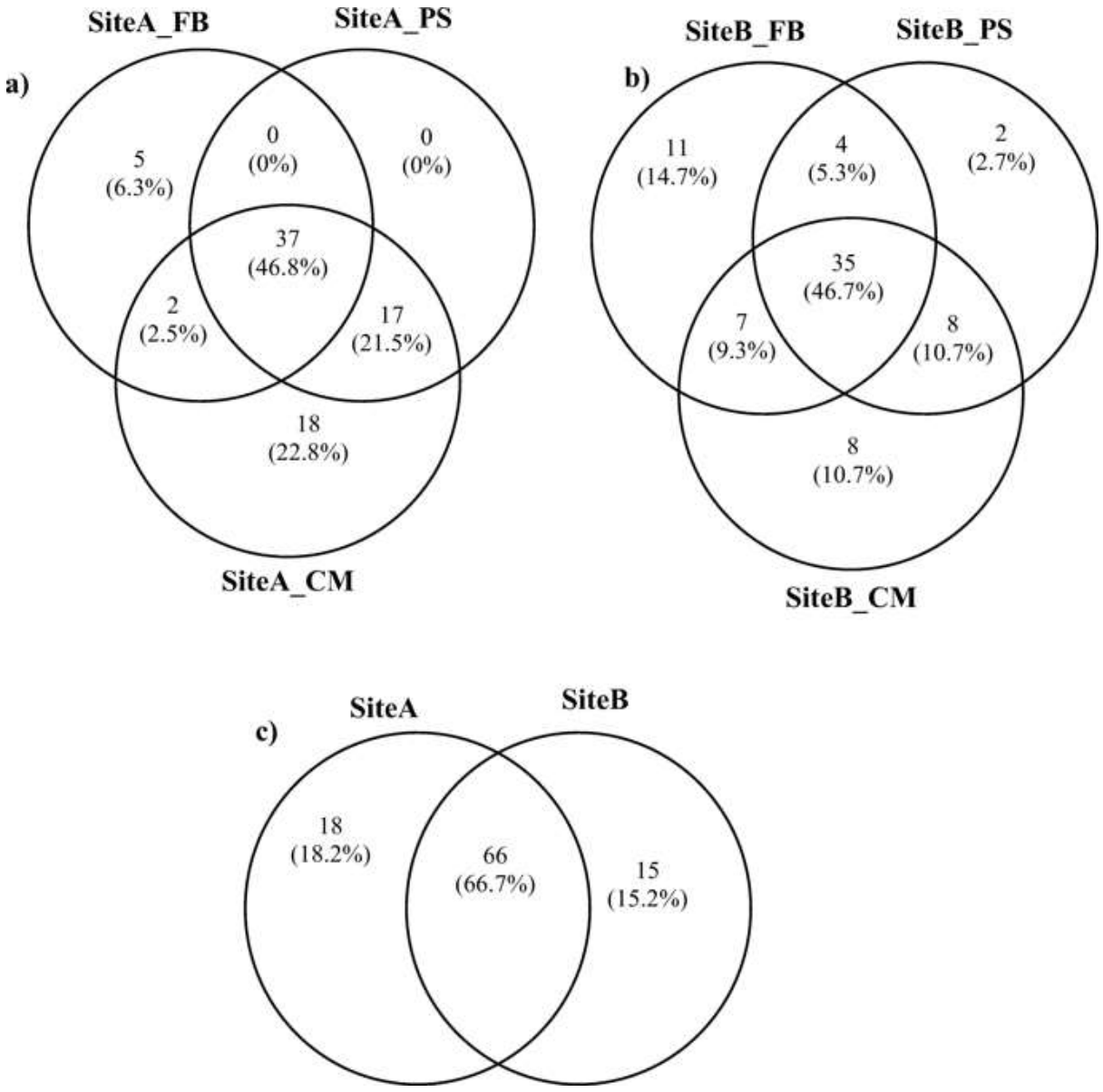

Fig. 5. Venn diagram showing the number of families unique and shared among the studied table grape phenological stages. Analysed samples include combinations of the three phenological stages, full bloom (FB), pea size (PS) and commercial mature stage (CM) independent of site A (a) and site B (b). An overall analysis of the represented families for the two sites irrespective of phenological stages is shown in 'c'

Analysis of yeast communities in *Botrytis cinerea* positive samples of table grapes

Yeast that constitute table grape microbial assemblage were grouped into two categories: (i) those previously reported with an antimicrobial potential against fungal pathogens, particularly *B. cinerea* (e.g *Aureobasidium pullulans*, *Cryptococcus* species, *Rhodotorula*, and *Candida* species), (ii) Spoilage or fermentative yeasts and were not previously associated with biocontrol activity (e.g S*accharomyces* spp.). These yeast communities accounted >50% and

<0.01% of the total yeast diversity overall irrespective of developmental stage. When stages were considered, the full bloom stage of site A (45%) and mature stage of site B (68%) showed the highest population of the potential biocontrol group followed by mature and pea size stages, respectively. The potential biocontrol yeasts were dominated by *Rhodotorula* (21.2%), *Aureobasidium* (7.1%), *Cryptococcus* (4.9%), *Epicoccum* (1.2%), *Sporobolomyces* (0.6%), while *Sporobolus* (0.03%), *Bulleromycetes* (0.06%), *Filobasidium* (0.3%), *Candida* (<0.01), *Kwoniella* (<0.01), and *Pichia* (<0.01%) were present in low levels.

The well-known commercially produced yeast biocontrol agents were also detected among the yeast genera of our samples. These included *Candida*, *Aureobasidium*, and *Cryptococcus.* The genus *Aureobasidium*, commercially known as Boni protector was the most dominant at site B, particularly during the pea size stage (25.9%). *Cryptococcus*, commonly traded as 'Yield Plus' increased with berry development from full bloom (3.9%) to mature stage (10.5%) by almost three-folds at site B, while in site A, only the mature stage had the most abundance (4.3%).

An overall comparison between positive and negative *B. cinerea* samples showed a higher abundance of *Basidiomycota* (25%) in the negative samples than the positive (14.2%) ones (Fig. 6). It is worth noting that the well-known potential biocontrol agent *Cryptococcus* against *B. cinerea* is a genus within the phylum *Basidiomycota*, and was most abundant in *B. cinerea* negative samples. Of the total genera detected in the study only 9.1% were exclusive to *B. cinerea* positive samples and 23.6% on negative samples (data not shown).

Distribution of pathogenic fungal taxa

The study revealed two pathogenic fungi with the most abundance in table grape samples at the two sites. The genus *Alternaria*, which has been reported as a pre- and postharvest pathogen of concern in table grapes, was the most overall dominant (35.6%). The pea size stage of site A had exceptionally high populations of up to 86,179 reads, accountable to 63.9% of the total reads (data not shown). This was followed by the full bloom stage of site A, with 61,444 reads contributing 45.4% of the total reads. Contrary, pea size stage at site B had lower abundance (16,944 reads) compared to site A. *Cladosporium*, a genus in the order *Capnodiales*, was the second mostly detected pathogenic fungi accounting for 192,402 overall total reads, of which 38% was from the pea size stage of site B, while its full bloom and mature stages were 30 and 26% respectively. At site A, a higher abundance of *Cladosporium* was detected at the mature stage, while full bloom and pea size stages had similar relative abundances contributing 20.8 and 19.3%, respectively. The least abundant pathogenic fungal genera included, *Fusarium* (782 reads), *Aspergillus* (16 reads) *Phoma* (619 reads), *Penicillium* (22 reads) *Exserohilum* (2,167 reads). *Colletotrichum* was also found in low frequency abundance (<0.001%) and only exclusive to site A full bloom and pea size stages, while *Cercospora* was only detected at full bloom site A. Overall fungal taxa that are potentially pathogenic to table grapes accounted for 69.8 and 56.8 % of the total population in site A and B, respectively.

Discussion

In this study we used illumina sequencing to investigate fungal microbial communities associated with asymptomatically *B. cinerea* positive table grape samples from full bloom, pea size and mature developmental stages. During fruit development, at preharvest, the grapes are exposed to several factors, which among others include varying climatic conditions (Jara et al. 2016), management practices (Abdelfattah et al. 2016b; Setati et al. 2015) and crop

physiological changes (Ottesen et al. 2013; Sternad Lemut et al. 2015). However, some of these factors have been found to either encourage or suppress prevalence of either pathogenic or non-pathogenic fungal species. Chemical sprays, for example, manipulate the epiphytic profiles. Recent work (Glenn et al. 2015) has shown that organically grown apples inhabit larger unique taxa of phyllosphere microbiota than apples under a conventional pest management practice. Also interrupted routine spray programmes were found to modify fungal profiles in strawberry plant organs (Abdelfattah et al. 2016b). Intensive cultivation and use of fungicides is associated with high prevalence of postharvest pathogens, particularly, *Botrytis* and *Cladosporium spp*. (Abdelfattah et al. 2016a). This was in agreement with previous work (Pinto et al. 2014; Singh et al. 2015), which confirmed that the use of chemicals modulate the overall genetic diversity in natural environments.

The full bloom stage comprises of the reproductive structure, made of several organs that provide habitat for microbial diversity (Andrews and Harris, 2000; Aleklett et al. 2014). Such a structure is significant for the biological success of the crop with respect to yield and quality, of which pathogenic microbes are likely to inhabit and impact on their reproductive output efficiency. The full bloom stage receives a diverse range of floral visitors which include pollinating and non-pollinating agents that may transfer microbial organisms to the flower (de Vega and Herrera, 2012; 2013). This may enhance richness of the microbial assemblage of this stage compared to the pea and mature stages. The variability in morphological structure compared to the two latter stages used in the study (pea and mature berry stages), have increased surface area for harbouring micro-organisms. Such variability may result in an imbalanced distribution of the epiphytic fungi between these stages, as observed in this study.

In agreement with previous work (Pinto et al. 2014), regardless of the phenological stage, *Ascomycota* was the most abundant phylum (75.1%) followed by *Basidiomycota* (24.8%), *Streptophyta* (<0.1%) and *Cryptomycota* (<0.1%). *Dothideomyetes* and *Microbotryomycetes* were the most dominant classes. Members of the *Ascomycota* phylum are known to cause economical postharvest losses (Dean et al. 2012) and have been isolated using culture based techniques and also detected using culture-independent based method (Abdelfattah et al. 2016a; Pinto et al. 2014). Moreover, representatives of this group were associated with fruit decay in other high value crops, such as apples (Shen et al. 2018), olives (Abdelfattah et al. 2015), strawberries (Abdelfattah et al. 2016b) and cherry (Tadych et al. 2012). Of the three stages investigated in the study, the mature berry stage had a higher incidence of phytopathogenic molds, which are likely to cause postharvest decay. These fungal genera included *Botrytis*, *Alternaria*, *Cladosporium*, *Ulocladium*, and *Aspergillus* have been previously isolated in table grape episphere (Pancher et al. 2012).

The most prevalent of the *Microbotryomycetes* was primarily associated by the high detection of *Rhodotorula* in the order *Sporidiobolales*, while the class *Dothideomycetes* was mainly represented by the genus *Alternaria* (order *Pleosporales*), which was abundantly detected in all samples. It is worth noting that the class *Dothideomycetes*, is one group in the *Ascomycota* mainly dominated by pathogenic fungal species (Wijayawardene et al. 2014).

In agreement with previous work (Abdelfattah et al. 2016b), the findings of this study showed that a high presence of potential biocontrol yeast populations is associated with a lower prevalence of pathogenic groups. Table grape developmental stages with low concentrations of *B. cinerea* had high abundances of *Rhodotorula*, *Aureobasidium* and *Cryptococcus*. This showed the importance of biocontrol populations in maintaining a 'healthy microbiome' in a

vineyard system (Setati et al. 2015), through suppression of pathogenic groups. Low levels of pathogenic populations, such as *Botrytis* are crucial during development of grapes inorder to reduce postharvest decay at postharvest.

Although our study was more focused on surface resident yeasts (epiphytes), the endophytic group was also identified. This could have been attributed to juice exudates through natural openings as the berry matures. Also coexistence may occur between these two groups, and most members of the grape yeasts are endophytic (Setati et al. 2015). Furthermore, other yeasts, such as the *A. pullulans*, a proven biocontrol agent against most common postharvest pathogen (Rathnayake et al. 2018), can occur as both epiphyte and endophyte (Martini et al. 2009). This yeast like fungi was also found in all the investigated table grape developmental stages.

Differences between the developmental stages observed included, fewer pathogenic communities observed at full bloom as compared to the mature stage. This could have been attributed to the production of volatiles by floral parts. Evidence of antimicrobial activity by petal volatiles has been reported (Aleklett et al. 2014). Tarpenoids and benzenoids compounds released by flowers are not only known for attracting smell, but have also shown some antimicrobial properties (Junker et al. 2011; Huang et al. 2012). The highest level of species richness was observed at the mature stages compared to the full bloom and pea size stages of both sites. This may have been attributed to the sugar rich environment associated with ripening fruit (Lievens et al. 2015), which promote survival of weak fungi that could not strive well under stressful conditions of limited energy source.

Pathogenic fungal populations of concern in preharvest environments were mainly characterised by high incidence of mainly two genera, *Alternaria* and *Cladosporium*, while

other fungi demonstrated very low abundances. Earlier reports in grape studies (Pinto et al. 2014; Setati et al. 2015) and strawberries (Abdelfattah et al. 2016b), have mostly reported high prevalence of *Alternaria*, using culture based methods (Johnson et al. 1992; Pancher et al. 2012). Abdelfattah et al. (2015) used next generation sequencing (metabarcoding approach), to revealed high incidence of *Dothideomyetes*, in the olive phyllosphere and carposphere, of which *Alternaria* is a member.

In conclusion, the findings of this study provide extensive information on fungal communities associated with presence of *B. cinerea* in the table grape natural environments. Several of the sequences detected were identified to species level, which made it possible to assume their function in the table grape phyllosphere. However, others could not be ascribed to a specific role, which could imply that their significance in table grape environments needs further investigation. Overall, this study showed the need of an in-depth assessment into fungal community relations under diverse table grape production environments, such as different cultural practices using several cultivars. The table grape fungal consortia in the different developmental stages had both pathogenic and beneficial microorganisms that are of significance towards improving table productivity. Cultural practices that favour beneficial communities could be further explored inorder to develop a sustainable healthy microbiome environment.

Results of the present study demonstrated a diverse variety of fungal communities that are known to be of pathogenic concern either at preharvest or postharvest conditions while others have been reported as potential biocontrol species against economically important pathogens, such as *B. cinerea*. These findings provide new information on key pathogenic microbial communities associated with table grape developmental stages and how they shift with

biocontrol populations. Such information is a significant contribution to the grapevine industry towards developing a target based management strategy to reduce postharvest pathogens. However, more detailed information on the influence of phytopathogenic inoculum pressure on the biocontrol populations as affected by cultivar and farming practice are needed. The findings of this study provide baseline information towards this future research.

Acknowledgements

This research was financially supported in part by the Department of Science and Technology in partnership with the Fresh Produce Exporters' Forum, Post-harvest Innovation Programme. Authors express their appreciation to the table grape growers for granting permission to conduct trials in their vineyards. The authors acknowledge Ms. Zama Zulu for her laboratory assistance.

Compliance with ethical standards

Ethical statement: This work did not involve any animal and / or human participants. The authors declare that they have no conflict of interest.

References

Abdelfattah, A., Wisniewski, M., Droby, S., & Schena, L. (2016a). Spatial and compositional variation in the fungal communities of organic and conventionally grown apple fruit at the consumer point-of-purchase. *Horticulture Research*, *3*, 16047.

Abdelfattah, A., Wisniewski, M., Li Destri Nicosia, M.G., Cacciola, S.O., & Schena, L. (2016b). Metagenomic analysis of fungal diversity on strawberry plants and the effect of management practices on the fungal community structure of aerial organs. *PLoS One*, *11*(8), e0160470.

Aleklett, K., Hart, M., & Shade, A. (2014). The microbial ecology of flowers: an emerging frontier in phyllosphere research. *Botany*, *92*(4), 253-266.

Andrews, J.H., & Harris, R.F. (2000). The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology*, *38*(1), 145–180.

Briceño, E.X., & Latorre, B.A. (2008). Characterization of *Cladosporium* rot in grapevines, a problem of growing importance in Chile. *Plant Disease*, *92*(12), 1635-1642.

Brum, M.C.P.D., Araujo, W.L.D., Maki, C.S., & Azevedo, J.L.D. (2012). Endophytic fungi from *Vitis labrusca* L. ('Niagara Rosada') and its potential for the biological control of *Fusarium oxysporum*. *Genetics and Molecular Research*, *11*(4), 4187-4197.

Bukulich, N.A., Thorngate, J.H., Richardson, P.M., & Mills, D.A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage and climate. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(1), E139-E148.

Carmichael, P.C., Siyoum, N., Chidamba, L., & Korsten, L. (2017). Characterization of fungal communities of developmental stages in table grape grown in the northern region of South Africa. *Journal of Applied Microbiology*, *123*(5), 1251-1262.

Carmichael, P.C., Siyoum, N., Jongman, M., & Korsten, L. (2018). Prevalence of *Botrytis cinerea* at different phenological stages of table grapes grown in the northern region of South Africa. *Scientia Horticulturae*, *239*, 57-63.

de Vega, C., & Herrera, C.M. (2012). Relationships among nectar-dwelling yeasts, flowers and ants: patterns and incidence on nectar traits. *Oikos*, *121*(11), 1878–1888.

de Vega, C., & Herrera, C.M. (2013). Microorganisms transported by ants induce changes in floral nectar composition of an ant-pollinated plant. *American Journal of Botany*, *100* (4), 792–800.

Dean, R., Van Kan, J, A, L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., et al. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, *13*(4), 414-430.

Dennis, C., & Cohen, E. (1976). The effect of temperature on strains of soft fruit spoilage fungi. *Annals of Applied Biology*, *82*(1), 51-56.

Dufour, M.C., Fontaine, S., Montarry, J., & Corio-Costet, M.F. (2011). Assessment of fungicide resistance and pathogen diversity in *Erysiphe necator* using quantitative real-time PCR assays. *Pest Management Science*, *67*(1), 60-69.

Ferreira, J.H.S., Van Wyk, P.S., & Venter, E. (2017). Slow dieback of grapevine: association of *Phialophora parasitica* with slow dieback of grapevines. *South African Journal of Enology and Viticulture*, *15*(1), 9-11.

Fu, D., Zeng, L., Zheng, X., & Yu, T. (2015). Effect of β-glucan on stress tolerances and biocontrol efficacy of *Cryptococcus laurentii* against *Penicillium expansum* in pear fruit. *BioControl*, *60*(5), 669-679.

Glenn, D.M., Bassett, C., & Dowd, S. (2015). Effect of pest management system on 'Empire'apple leaf phyllosphere populations. *Scientia Horticulturae, 183,* 58–65.

Holz, G., Gütschow, M., Coertze, S., & Calitz, F.J. (2003). Occurrence of *Botrytis cinerea* and subsequent disease expression at different positions on leaves and bunches of grape. *Plant Disease*, *87*(4), 351-358.

Huang, M., Sanchez-Moreiras, A.M., Abel, C., Sohrabi, R., Lee, S., Gershenzon, J., & Tholl, D. (2012). The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. *New Phytopathology*, *193* (4), 997–1008.

Ianiri, G., Pinedo, C., Fratianni, A., Panfili, G., & Castoria, R. (2017). Patulin degradation by the biocontrol yeast *Sporobolomyces* sp. is an inducible process. *Toxins*, *9*(2), 61.

Jara, C., Laurie, V.F., Mas, A., & Romero, J. (2016). Microbial terroir in Chilean valleys: diversity of non-conventional yeast. *Frontiers in microbiology*, *7*.

Johnson, G.I., Mead, A.J., Cooke, A.W., & Dean, J.R. (1992). Mango stem end rot pathogens-Fruit infection by endophytic colonisation of the inflorescence and pedicel. *Annals of Applied Biology*, *120*(2), 225-234.

Junker, R.R., Loewel, C., Gross, R., Dötterl, S., Keller, A., & Blüthgen, N. (2011). Composition of epiphytic bacterial communities differs on petals and leaves. *Plant Biology*, *13*(6), 918–924.

Kernaghan, G., Mayerhofer, M., & Griffin, A. (2017). Fungal endophytes of wild and hybrid *Vitis* leaves and their potential for vineyard biocontrol. *Canadian Journal of Microbiology*, *63*(7), 583-595.

Lai, T., Bai, X., Wang, Y., Zhou, J., Shi, N., & Zhou, T. (2015). Inhibitory effect of exogenous sodium bicarbonate on development and pathogenicity of postharvest disease *Penicillium expansum*. *Scientia Horticulturae*, *187*, 108-114.

Latorre, B.A., & Guerrero, M.J. (2001). First report of shoot blight of grapevine caused by *Sclerotinia sclerotiorum* in Chile. *Plant Disease*, *85*(10), 1122-1122.

Lievens, B., Hallsworth, J.E., Pozo, M.I., Belgacem, Z.B., Stevenson, A., Willems, K.A., & Jacquemyn, H. (2015). Microbiology of sugar-rich environments: diversity, ecology and system constraints. *Environmental Microbiology*, *17*(2), 278-298.

Liu, Y., Lan, X., Yin, L., Dry, I.B., Xiang, J., & Lu, J. (2018). In planta functional analysis and subcellular localization of the oomycete pathogen *Plasmopara viticola* candidate RXLR effector repertoire. *Frontiers in Plant Science*, *9*, 286.

Martini, M., Musetti, R., Grisan, S., Polizzotto, R., Borselli, S., Pavan, F., & Osler, R. (2009). DNA-dependent detection of the grapevine fungal endophytes *Aureobasidium pullulans* and *Epicoccum nigrum*. *Plant Disease*, *93*(10), 993-998.

Ottesen, A.R., Peña, A.G., White, J.R., Pettengill, J.B., Li, C., Allard, S., et al. (2013). Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). *BMC Microbiology*, *13*(1), 114.

Pancher, M., Ceol, M., Corneo, P.E., Longa, C.M.O., Yousaf, S., Pertot, I., et al. (2012). Fungal endophytic communities in grapevines (*Vitis vinifera* L.) respond to crop management. *Applied and Environmental Microbiology*, AEM-07655.

Perrone, G., Mulè, G., Susca, A., Battilani, P., Pietri, A., & Logrieco, A. (2006). Ochratoxin A production and amplified fragment length polymorphism analysis of *Aspergillus carbonarius*, *Aspergillus tubingensis*, and *Aspergillus niger* strains isolated from grapes in Italy. *Applied and Environmental Microbiology*, *72*(1), 680-685.

Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C., & Gomes, A.C. (2014). Unravelling the diversity of grapevine microbiome. *PLoS One*, *9*(1), e85622.

Poolsawat, O., Tharapreuksapong, A., Wongkaew, S., Reisch, B., & Tantasawat, P. (2010). Genetic diversity and pathogenicity analysis of *Sphaceloma ampelinum* causing grape anthracnose in Thailand. *Journal of Phytopathology*, *158*(11-12), 837-840.

Rathnayake, R.M.S.P., Savocchia, S., Schmidtke, L.M., Steel, C.C. 2018. Characterisation of *Aureobasidium pullulans* isolates from *Vitis vinifera* and potential biocontrol activity for the management of bitter rot of grapes. *European Journal of Plant Pathology,* 151 (3) 593-611.

Rivera, S. A., Zoffolli, J. P., & Latorre, B. A. (2013). Infection risk and critical period for the postharvest control of gray mold (*Botrytis cinerea*) on Blueberry in Chile. *Plant Disease*, *97*(8), 1069-1074.

Romanazzi, G., Smilanick, J.L., Feliziani, E., & Droby, S. (2016). Integrated management of postharvest gray mold on fruit crops. *Postharvest Biology and Technology*, *113*, 69-76.

Schloss, P.D., & Handelsman, J. (2006). Toward a census of bacteria in soil. *PLoS Computational Biology*, *2*(7), e92.

Setati, M.E., Jacobson, D., & Bauer, F.F. (2015). Sequence-based analysis of the *Vitis vinifera* L. cv Cabernet Sauvignon grape must mycobiome in three South African vineyards employing distinct agronomic systems. *Frontiers in Microbiology*, *6*.

Shen, Y., Nie, J., Dong, Y., Kuang, L., Li, Y., & Zhang, J. (2018). Compositional shifts in the surface fungal communities of apple fruits during cold storage. *Postharvest Biology and Technology*, *144*, 55-62.

Sternad Lemut, M., Sivilotti, P., Butinar, L., Laganis, J., & Vrhovsek, U. (2015). Pre-flowering leaf removal alters grape microbial population and offers good potential for a more

sustainable and cost-effective management of a Pinot Noir vineyard. *Australian Journal of Grape Wine Research*, *21*(3), 439-450.

Swart, A.E., Lennox, C.L., & Holz, G. (2017). Infection of table grape bunches by *Alternaria alternata*. *South African Journal of Enology and Viticulture*, *16*(1), 3-6.

Tadych, M., Bergen, M.S., Johnson-Cicalese, J., Polashock, J.J., Vorsa, N., & White, J.F. (2012). Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. *Fungal Diversity*, *54*(1), 101-116.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–9.

Taylor, M.W., Tsai, P., Anfang, N., Ross, H.A., & Goddard, M.R. (2014). Pyrosequencing reveals regional differences in fruit-associated fungal communities. *Environmental Microbiology*, *16*(9), 2848-2858.

United States Department of Agriculture (USDA). (2017). Production, Supply, Distribution online Database. Accessed online 24/08/ 2017 https://apps.fas.usda.gov/psdonline/app/index.html#/app/home

Van Boxstael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van de Perre, E., et al. (2013). Food safety issues in fresh produce: bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control*, *32*(1), 190-197.

Wijayawardene, N.N., Crous, P.W., Kirk, P.M., Hawksworth, D.L., Boonmee, S., Braun, U., et al. (2014). Naming and outline of *Dothideomycetes*–2014 including proposals for the protection or suppression of generic names. *Fungal Diversity*, *69*(1), 1–55.

Zapata, Y., Díaz, A., Grijalba, E., Rodríguez, F., Elad, Y., Cotes, A.M. 2015. Phyllosphere yeasts with potential for biological control of *Botrytis cinerea* in rose. In III International Symposium on Postharvest Pathology: Using Science to Increase Food Availability. 1144, 77-84.