Yeast diversity in the vineyard: how it is defined, measured and influenced by fungicides

K. M. SUMBY[1,2], N. S. CALIANI[1,2] and V. JIRANEK[1,2]

[1]Australian Research Council Training Centre for Innovative Wine Production, Urrbrae, SA 5064, Australia; [2] Department of Wine Science, The University of Adelaide, Urrbrae, SA 5064, Australia

Corresponding author: Professor Vladimir Jiranek,
email vladimir.jiranek@adelaide.edu.au

Keywords: *fungicide, mode of action, microbial ecology, grapes, yeast*

Abstract

This review focuses on the considerable amount of research directed at defining microbial diversity in the vineyard and the subsequent contribution to uninoculated fermentations, with an emphasis on the effect of fungicide applications. From this research it is clear that there are many factors affecting diversity in the vineyard including: sprays, climate, location and grape derived parameters. With their increasing affordability, Next Generation Sequencing methods to measure diversity in environmental samples are now being adopted for studies of the grapevine microbiome. We bring together the results of these studies, discuss how diversity is measured and consider the potential applications of current knowledge. An in-depth analysis of how fungicides affect yeast diversity in the vineyard and the mode of action of different fungicide groups are also discussed. Finally, we report on alternative treatments to maintain vineyard health and reduce fungicide applications in the future.

Introduction

Grapes and wine are not produced in a sterile environment and by extension of this we can infer that winemaking does not involve a microbial monoculture. Not only does the vineyard contain a range of yeast, bacteria, and fungi (Bokulich et al. 2014, Morrison-Whittle and Goddard 2018), but so do the wineries in which the grapes are fermented (Bokulich et al. 2013, Varela et al. 2017). During winemaking, grape must is converted to wine via an alcoholic fermentation (AF) carried out by one or more strains of yeast (Fleet 1990). If inoculated with yeast, typically *Saccharomyces cerevisiae* is used, or if un-inoculated a range of non-*Saccharomyces* yeasts usually begin the fermentation prior to the eventual dominance of *S. cerevisiae* (Beltran et al. 2002, Albergaria and Arneborg 2016). After the alcoholic or primary fermentation, a secondary fermentation by lactic acid bacteria (LAB), known as malolactic fermentation (MLF), is often undertaken, depending on the style of wine that the winemaker seeks to achieve (Sumby et al. 2019).

In the vineyard, the soil and rhizosphere microbiome make an important contribution to grapevine health and performance boosting yields (Belda et al. 2017a). Mechanisms include phosphorus solubilisation and, when vines are associated to a *Leguminosae* cover crop, nitrogen fixation (Misra et al. 2017, Schütz et al. 2018). Arbuscular mycorrhiza fungi can enhance vine resistance to drought by creating an extended root system for them (Dodd and Ruiz-Lozano 2012). Moreover, when beneficial soil microbiota dominate, soil-borne pathogens are less likely to thrive, enhancing overall plant health (Vukicevich et al. 2016). Leaves and grapes share common species with bark and soil indicating a possible role of soil in defining the whole plant microbiome (Martins et al. 2013). Additionally, the soil is a microbial pool that may also be the source for endophytes colonising internal tissues (Zarraonaindia et al. 2015). These can affect plant growth, and resistance to herbivores, pathogens, or environmental factors (Bakker et al. 2012). Last, phyllospheric microorganisms, those living on the aboveground tissues, including yeasts contribute to vineyard health because of their biocontrol abilities (Sipiczki 2006, Raspor et al. 2010, Nally et al. 2012, Carmichael et al. 2019).

The vineyard microbiome is not only important for vine health but will also contribute to fermentation and the final product (Liu et al. 2016, Padilla et al. 2016a, Belda et al. 2017a Morrison-Whittle and Goddard 2018). Therefore, the contribution of the microbiome is important for winemakers because consumers influence wine style preferences. For example, market trends show that consumers are more willing to pay for more complex wines, where aromas and flavours play a key role (Malherbe et al. 2013, Pagliarini et al. 2013, Danner et al. 2016), especially if they are produced under environmentally friendly and sustainable systems (Vecchio 2013). So-called 'natural wines', those produced without preservatives such as sulfur dioxide (SO_2), are also highly appreciated by some consumers. Young, Italian consumers demonstrated an increased willingness to pay for natural wine because of an interest in natural

products and label information (winemaking techniques, sensory attributes) (Galati et al. 2019). In addition, Italian and Spanish consumers that linked sulfites with headaches were willing to pay premium prices for wines labelled with 'No-added sulfites' (Amato et al. 2017), a trend borne out through interviews with American consumers (Costanigro et al. 2014). Thus in the context of a consumer move towards more complex wines and natural 'wild ferments', the non-*Saccharomyces* yeasts contained within a diverse microbiome will be desirable for increasing aromatic complexity (Capozzi et al 2015, Whitener et al. 2015, 2017, Padilla et al. 2016b, Varela et al. 2016, Lin et al. 2020) and potentially lower alcohol concentration wines (Contreras et al. 2014).

Both biotic and abiotic factors will affect yeast diversity in the vineyard and by default the overall microbiome. In this review, we examine the current literature with regard to the diversity of yeast on wine-grapes, discuss what diversity is and how it is measured, and take a deeper look at one factor that may influence diversity, fungicides. The mode of action of different fungicides and an overview of at times conflicting results is discussed. Finally, fungicide alternatives such as biocontrol agents are explored.

What is diversity and is it important?

Diversity in the context of grape and wine microbiology is seen as many different fungal and bacterial species existing together either in the soil or the vine phyllosphere, that is grape berries, leaves and bark (Gilbert et al. 2014, Perazzolli et al. 2014, Mezzasalma et al. 2017, Wei et al. 2018). Therefore, when discussing fungal diversity, this review is referring to species richness, that is the number of species present in a particular environment/microbiome. The microbiome of humans and plants has garnered increasing attention in recent years and it is generally accepted that the microbiome of a given system, be it human gut (Cénit et al. 2014, Yang et al. 2016) or plant (Compant et al. 2019), plays an important role in the overall health of the organism with which it is associated. It is also possible that microbial diversity is not only affected by its environment but responds to it and, in the case of wine, ultimately sculpts final wine aroma characteristics.

Much research attempts to define the unique terroir of a region, and whether microbial diversity of the vineyard is contributing to this. Whilst diversity is important, however, not all species are desirable. Certainly, some can be important for grapevine and berry health, potentially being used as indicators of good plant health. *Aureobasidium pullulans*, the predominant species on sound grape surfaces from conventional, organic, and biodynamic vineyards (Setati et al. 2012) has biocontrol effects against *Botrytis cinerea* (Bozoudi and Tsaltas 2018). Moreover, *A. pullulans* can stop growth of *Diplodia seriata*, a grapevine pathogenic fungus that causes significant economic losses every year (Pinto et al. 2018). Other

species cause undesirable effects if present during/after fermentation. For example, *Dekkera bruxellensis* and *Zygosaccharomyces bailii* are strictly considered spoilage yeasts (Loureiro and Malfeito-Ferreira 2003), with *S. cerevisiae* also causing spoilage by refermenting sweet wines. *D. bruxellensis* is common in rot-infected berries and may be transported to the winery (Loureiro and Malfeito-Ferreira 2003). In addition, insects such as *Drosophila*, can be a vector (Christiaens et al. 2014, Steensels et al. 2015). Being resistant to ethanol and carbon dioxide, *D. bruxellensis* can develop after fermentation has finished during wine maturation in oak barrels (Howell 2016). This yeast generates phenolic off flavours (POF), which cannot be readily removed from wine, and thereby produce high economic losses worldwide. A maximum threshold of 620 µg/L has been cited at which POF becomes unpleasant and spoils wine (Chatonnet et al. 1992, 1993, Loureiro and Malfeito-Ferreira 2003). Like *D. bruxellensis*, *Pichia guilliermondii* converts *p*-coumaric acid into 4-ethyl phenol but cannot grow in wine (Loureiro and Malfeito-Ferreira 2003).

Zygosaccharomyces bailii, a well-recognised spoiler of wine, is often isolated from wine fermentations (Kuanyshev et al. 2017). It has a high osmotolerance (Martorell et al. 2007) and thrives under high ethanol concentration, low pH, and elevated sulfur dioxide (Thomas and Davenport 1985). This species can colonise finished sweet wines and through refermentation produce CO_2 that risks the bottle exploding (Leyva et al. 1999, Zuehlke et al. 2013). *Zygosaccharomyces bailii* grows only in sour-rot infected berries in the vineyard (Loureiro and Malfeito-Ferreira 2003, Zuehlke et al. 2013), but has been isolated from winery equipment.

Diversity in the vineyard will carry into winemaking where autochthonous yeasts aid in the creation of unique wines (Liu et al. 2016, Padilla et al. 2016a, Belda et al. 2017a, Morrison-Whittle and Goddard 2018, Gupta et al. 2019, Liu et al. 2019a). Avoidance of sulfur dioxide or starter cultures allows the impact of these yeasts to be greater, even though they typically do not persist until the end of fermentation (Wang et al. 2016). Wines from such uninoculated fermentations are seen as a way of maintaining or expressing the 'microbial terroir' (microbial biogeography) of the region (Belda et al. 2017b, Liu et al. 2019b). Representing up to 99% of species richness and diversity, non-*Saccharomyces* yeast originating from grapes are therefore of particular interest (Carrau et al. 2015). Those often found in uninoculated fermentations include, *Kluyveromyces marxianus*, *Pichia kluyveri*, *Torulaspora delbrueckii*, *Lachancea thermotolerans*, *Kazachstania* spp., *Starmerella bacillaris*, and *Metschnikowia pulcherrima*. Many contribute positively to wine quality by modifying aroma and/or mouthfeel and, in some cases, result in a reduction in ethanol concentration (Anfang et al. 2009, Gobbi et al. 2013, Jolly et al. 2014, Loira et al. 2015, Varela et al. 2017, Whitener et al. 2017, Benito 2018, Hranilovic et al. 2018, Rollero et al. 2018, Ruiz et al. 2018, Lin et al. 2020). In addition to species diversity, non-*Saccharomyces* present a high level of strain variability that can also be exploited in obtaining unique products (Capozzi et al. 2015).

Whilst the above studies report that the microflora present in a given fermentation is extremely important for the quality of the final product, one question that remains is how much diversity is needed in order to create unique wines. It is also suggested that the ratio of *Saccharomyces* to non-*Saccharomyces* may be the main driver of sensory quality of the finished wine (Capozzi et al. 2015). For example, when Chardonnay was fermented using two different techniques, uninoculated vs co-inoculated with *Hanseniaspora vineae*, aromatic profiles were richer when compared to the fermentation by a monoculture of a conventional yeast (Carrau et al. 2015). Esters and higher alcohols are the main compounds resulting from yeast secondary metabolite production during fermentation (Rapp and Versini 1995, Romano et al. 2003, Sumby et al. 2010). Chardonnay ferments using a *M. pulcherrima* starter followed by *S. cerevisiae* (sequential inoculation) produced wines with a higher total concentration of esters and higher alcohols (Contreras et al. 2014). *Kazachstania spp*. used in sequentially inoculated Viognier ferments yielded elevated phenylethyl and isoamyl acetate/alcohol concentration (Lin et al 2020). *Pichia fermentans* used as a pure culture or sequentially with *S. cerevisiae*, increased acetaldehyde and higher alcohols in Macabeo wines (Clemente-Jimenez et al. 2005). The maximum acetaldehyde concentration was 350 mg/L, which exceeds the 125 mg/L where negative bruised-apple characters are seen (The Australian Wine Research Institute 2020a). Other non-*Saccharomyces* species reported to show higher ester concentration in Bobal musts are *Hanseniaspora guilliermondii* and *Pichia anomala*, inoculated as a 10:1 mixed culture with *S. cerevisiae* (Rojas et al. 2003). Thus even though autochthonous yeasts may require an *S. cerevisiae* inoculum to complete fermentation, they offer a useful palette of interesting properties, something that is also being explored for biofuel and distillate production, cheese making, and in biocontrol (Varela and Borneman 2017).

Yet to be fully investigated is the creation of artificial diversity of that seen in juices to recreate uninoculated fermentation. Such creations offer the potential to eliminate undesirable yeast and influences, whilst tailoring the beneficial ones. In addition, while species interactions are important in the wine itself (Bartle et al. 2019), the nature and significance of such interactions before winemaking on the grapes has not been defined. Microbial diversity is considered to be the regional imprint of the place where grapes are grown (Knight et al. 2015, Liu et al. 2019b), whereas cultivar and farming practices can also have a driving effect (Martins et al. 2012, Bokulich et al. 2014, Gilbert et al. 2014, Wang et al. 2015). In this context, application of chemicals in the vineyard will shift populations of these naturally occurring yeast species, depending on their sensitivity to treatments, and thereby influence wine sensory attributes.

Factors that influence fungal diversity on grapes

One motivator for detailed studies of the grapevine-associated microbiome is that they may lead to the identification of autochthonous strains of oenological value, which enhance regional characteristics in wine (Lin et al. 2020). Certainly, it is hypothesised that the grapevine-associated microbiome, or even the main subset of it, could be used to identify a vineyard, thereby linking wine characteristics specifically to the 'terroir' or environment (e.g. Knight et al. 2015). There are several factors that are thought to influence this microbiome including: grape cultivar, insect activity, berry physiology, species-species interactions, geographical location, climate, soil, terrain and farming and harvesting procedures including herbicide and fungicide spray use and canopy management (Figure 1) (Barata et al. 2012, Capozzi et al. 2015, Kioroglou et al. 2019). To date, however, several reports on grapevine microflora do not detail any spray regime utilised in the tested vineyard.

As discussed above many individual factors affect fungal diversity in the vineyard. The study of vineyard diversity is compounded by the likely interaction of the above factors with each other (Figure 1). The grapes themselves naturally provide a variable environment with differences in: skin thickness (i.e. microbial access to nutrients), bunch architecture, berry phenolic substances and flavonoids. Grape temperature also affects the types of species present on grapes, with *Hanseniaspora* spp. more commonly found in warm climates and *Kloeckera* spp. in cooler climates (Villa and Longo 1996). Add to these geographical features, such as location, altitude, sunlight hours, and rainfall, and a complex matrix of effects is quickly developed. It has been shown that when rain falls around harvest *Metschnikowia* and *Hanseniaspora* communities tend to increase, potentially due to the high RH (Jara et al. 2016), whereas basidiomycete yeast numbers decline (Perazzolli et al. 2014). Such studies did not consider, however, the likelihood that wet conditions also demand an increased use of fungicides, which themselves may influence yeast diversity and numbers. Drumonde-Neves and co-workers (2016) suggested that abandoned vineyards, which do not receive fungicide applications, provide a means of teasing apart fungicide vs rain effects, wherein less-abundant yeast populations would result more specifically from heavy rainfall patterns.

During ripening and depending on vintage, climatic conditions, and any chemical treatments used, microbial diversity generally drops (Pinto et al. 2014). The predominance of specific yeasts is linked to the phenological phase of the vines. Generally, the poor fermenters *Cryptococcus* and *Rhodotorula* exist during the early stages of berry development, but as berries ripen, *Hanseniaspora, Candida,* and *Metschnikowia* ascomycetes appear on the berry surface (Rosini et al. 1982, Combina et al. 2005, Raspor et al. 2006). But farming practices including fungicide and herbicide sprays, and canopy management can have a large effect on fungal populations, and potentially mask other influences (Martins et al. 2012, Bokulich et al. 2014, Gilbert et al. 2014, Wang et al. 2015, Morrison-Whittle et al. 2017, Chou et al. 2018, Mandl et al. 2018, Carneiro et al. 2019, Vincent and Lasnier 2020). As discussed above, rainfall

and its timing may alter fungal populations, but rainfall will also impact berry physiology, itself an important determinant of fungal populations, and potentially also wash sprays from grapevines. Thus, the study of the grapevine fungal microbiome has many unanswered questions and further careful investigation that looks at vineyard condition more holistically is warranted.

Methods to measure diversity

Microbial diversity can be explored on different tissues (barks, leaves, grapes), and soils, at a specific developmental stage or as a continuum through time to have a better understanding of fungal community behaviour (Martins et al. 2013, Bokulich et al. 2014, Gilbert et al. 2014, Belda et al. 2017b, Morrison-Whittle et al. 2017, Liu et al. 2019a). Choosing the right approach for data acquisition is key to research success, as is defining the most suitable statistical analysis for it. The first step towards untangling the possible interactive effects and complexity of the grapevine microbiome is the establishment of consistent methods to not only measure diversity, but statistically analyse these data. Species diversity itself has two separate components: (i) the number of species present (species richness); and (ii) their relative abundance (termed dominance or evenness). Therefore, whilst vineyard microbial diversity is of great interest to the wine industry, quantifying species diversity of different vineyards is complicated as it requires careful planning and a multidisciplinary approach. Many authors used both culture dependent and independent methods (Milanović et al. 2013, Martins et al. 2104, Cordero-Bueso et al. 2014, Grangeteau et al. 2017b, Escribano-Viana et al. 2018, Agarbati et al. 2019a, Agarbati et al. 2019b, Cachón et al. 2019, Anguita-Maeso et al. 2020) with a range of statistical techniques being applied (Figure 2, Table 1). Genome analyses start with DNA extraction from the samples followed by quantitation and purification ahead of downstream analysis, perhaps by quantitative polymerase chain reaction (qPCR), denaturing gradient gel electrophoresis (DGGE) or next generation sequencing (NGS). Culture-independent studies are especially valuable when dealing with non-culturable or difficult-to-cultivate microorganisms (Zapka et al. 2017). Moreover, the utilisation of multi-omics techniques makes it possible to quantify these communities, at both a taxonomic and functional level (Zhang et al. 2010, Franzosa et al. 2015, Bokulich et al. 2016, Malla et al. 2018).

Several issues need to be addressed in such work, including; statistical sampling methods, the arbitrary nature of delineating an ecological community, and the difficulty of positively identifying all of the species present. Next generation sequencing technology has an advantage over culture-dependent techniques in that slower growing or less abundant species have a greater chance of being detected. Identification of the species present is, however, only the first step. From that information it is necessary to investigate the proportionality and role of

each species. As a result, many different measures (or indices) of biodiversity have been developed. Microbiome diversity is traditionally assessed by means of a large list of metrics that account for the richness (S), reported as the number of operational taxonomic units (OTUs) or amplicon sequence variants (ASVs), and evenness (how homogeneously distributed these species are) at different scales (α, β, γ diversities) (Whittaker 1972).

Alpha diversity (α) measures richness and evenness at the ecosystem scale (i.e. within a sample). Traditional metrics include, Shannon diversity index (H) (Shannon 1948), Simpson diversity Index (D) (Simpson 1949), Simpson's evenness (E, (DeBenedectis 1973, Morris et al. 2014) and Berger-Parker dominance (BP, Berger and Parker 1970). H includes both richness and evenness, so it measures how uniformly microbial taxa are distributed amongst samples. It is based on a logarithmic calculation and ranges between 1.5 and 3.5, increasing when richness and evenness increase (Shannon 1948). D focuses on species evenness and is calculated from the sum of squared proportions. It ranges between 0 and 1, the higher the value of D, the lower the diversity (Simpson 1949). E can be calculated by taking Simpson's index (D) and expressing it as a proportion of the maximum value that D could assume if individuals in the community were completely evenly distributed, in which case the use of this index might not be useful as there is a mathematical correlation between both (DeBenedectis 1973, Morris et al. 2014). Finally, Berger-Parker incorporates proportional abundance of species to the diversity analysis, estimating dominance of the most abundant species (Berger and Parker 1970, Morris et al. 2014). If phylogenetic distances are also considered, the phylogenetic diversity index must be included in the analysis (Lozupone and Knight 2008). Additionally, it is necessary to take into account that identification of fungi by sequencing based on internal transcribed spacer (ITS) () is more accurate compared to 18S rRNA sequencing approaches (Halwachs et al. 2017). However, because of the high intraspecific variability of ITS, read alignment can be problematic and, therefore, phylogenetic trees derived from it may not be definitive (Fouquier et al. 2016, Halwachs et al. 2017).

Beta diversity (β) establishes how different two environments (samples) are. The main metrics employed are: Bray–Curtis dissimilarity index, which takes the number of OTUs measured in two samples, compares them and depending on how different they are, ranges between 0 (exact same species at the same abundance) and 1 (completely different species and their abundances) (Bray and Curtis 1957). The Jaccard distance is based on species presence or absence, and ranges between 0 and 1 (Jaccard 1912). UniFrac adds information on phylogenetic relationships between organisms (Lozupone and Knight 2005, Lozupone et al. 2011). This measurement can include only the distance between OTUs (unweighted), or weight branches by abundance information (weighted). Gamma diversity can be considered as an overall measurement of how different a set of samples is, and it considers both α and β diversities.

All of the changes in the yeast species present on grapes must be taken with the preverbal 'grain of salt'. Whilst it is rarely acknowledged, count data generated by NGS exist as compositions for which the abundance of each component (i.e. ITS gene sequences and OTUs) is only coherently interpretable relative to other components within that sample (Quinn 2018). This is because the number of counts recorded for each sample is constrained by an arbitrary total sum (i.e. library size). Therefore, without normalisation or transformation, many conventional analyses, including distance measures, correlation coefficients and multivariate statistical models cannot be used. Whilst several studies report differences observed with NGS in yeast diversity based on various chemical (e.g. sprayed and unsprayed grapes) and environmental factors (Chou et al. 2018, Agarbati et al. 2019a), many do not consider the actual abundance of species (i.e. CFU/mL). It may be that changes in the balance of particular genera/species are going unnoticed by being masked by changes in other genera/species. With this in mind, it is important to note that changes in the presence of a single species might also be explained by correlated changes in all of the other species.

Therefore, if alpha and beta diversity indices are used in isolation a seemingly static image of microbial assemblages might be observed, where no information on underlying relationships between these communities is provided. New data techniques are needed to analyse the complexity of the information and provide a more complete overview of diversity. In addition, there are cases where the complexity of the analysis leads to the need for alternative or complementary data interpretation (Morris et al. 2015, Morton et al. 2017). Using these metrics, a path model of 'hypothesised relationships between organism/traits' was reported by Morris and collaborators (2014). They investigated how the relationships between different traits, in this case aboveground arthropods, arbuscular mycorrhizal fungi, land use intensity and *Plantago lanceolata* chemical and molecular diversity, affected diversity indices (species richness (S), Shannon's diversity (H'), Simpson's diversity (D1), Simpson's dominance (D2), Simpson's evenness (E), and Berger–Parker dominance (BP)). This study served as a community interactions model, from which it could be concluded which traits and relationships had positive or negatives effects, which were significant, and which were not. This highlights the need of considering the system globally, and where obtaining additional data can become vital for understanding microbial community evolution (Figure 2).

More recently it has been recommended that looking at the balance of species might be more useful than examining changes in the proportion of species (Morton et al. 2017). Taking the approach of focusing on balance in a system and the transition of dominance between these species might avoid the error of inferring absolute decreases or increases in their abundance (Morton et al. 2017). The concept of log-ratio balance, which turns out to be more dynamic, has been introduced as a novel approach for microbial diversity understanding (Morton et al. 2017, Kioroglou et al. 2019). The former group relied on balance to 'infer

meaningful properties of subcommunities, rather than properties of individual species', thereby helping to separate niches and underpin the types of relationships being held between these taxa. Kioroglou and co-workers (2019) found how specific genera changes affected the fungal community structure, which was not obvious when traditional OTUs/relative abundance type data analysis was undertaken. The authors use the species data to build a bifurcating tree (where the tree reflects a series of branching processes in which one lineage splits into two descendant lineages) relating microbial taxa to each other by using the criteria of interest. Balances can be calculated on the internal nodes of the tree from the geometric means of the corresponding subtrees (Morton et al. 2017). All NGS abundance data are compositional because sequencers sample only a portion of the total input material. The benefit of analysing the data this way is that due to their scale invariant nature balance trees correct for differences in sequencing depth without requiring rarefaction, therefore avoiding many of the limitations associated with this procedure. Additionally, balances are sub compositionally coherent, which means that changes in non-overlapping subcommunities do not impact each other. These examples break our paradigm of having to measure diversity in a particular and strict way. There is no one valid approach. Indeed, it might be that some cases require a mix of methods to get the most informative results.

Alternate indicators also take in account the phylogenetic association between microbial communities (Lozupone and Knight 2008). Simpler analysis, such as those only based on alpha and/or beta diversity, might use any of the available parameters without altering results too much, but in more complex situations, parameters must be chosen carefully. Moreover, special considerations such as a rarefaction step should be considered in some cases. Bias can arise from sampling size or, in sequencing data, from library sizes altering final results and their interpretation (Willis 2019). It is important to remember that the presence of a particular species does not describe either their function or if they are viable/active. Some questions that we could look to answer with more in-depth approaches include: are environmental factors having an effect that masks the true trend? What is the effect of interactions between more resistant fungi and those undergoing recovery? What is also needed in this space is for researchers to deposit diversity data into a central database following publication to enable other researchers to mine for information. Whilst traditionally sequencing data such as purified ITS sequences from single colony isolates, and whole genome sequences are regularly deposited into databases such as NCBI, there currently appears to be no requirement to do so with diversity profiling sequences. Examples of such databases that do contain this information include: Human Microbiome Project Data Portal (https://portal.hmpdacc.org/), MicrobiomeDB (https://microbiomedb.org/mbio/app/), Human Oral Microbiome Database, (http://www.homd.org/index.php), and Genomic Features of Bacterial Adaptation to Plants (http://labs.bio.unc.edu/Dangl/Resources/gfobap_website/).

Access to the sequencing data of others would increase the ability of the research community to compare studies and generate a more holistic view of particular microbiomes. Having discussed the importance of diversity and how it is measured the following sections take an in-depth look at the role that fungicide applications play on yeast diversity.

Fungicides and grapevine protection

Grapevines often need to be treated with fungicides in order to prevent or cure the wide range of fungal and oomycete-caused diseases affecting soil, vine and grape health. The first attempts at human intervention and active protection of wine grapes began in 1847 in England, and 3 years later in France (Lamy 1992). Powdery mildew (*Erysiphe necator*) destroyed most of the 1854 harvest, reaching a record low level of 2.82 hL/ha (Chevet et al. 2011). Due to the high economic importance of controlling this disease, the French Government and the Société d'Encouragement pour l'Industrie Nationale (Society for National Industry Encouragement) organised a competition in 1855 to inspire the rise of treatment ideas (Lamy 1992); with sulfur treatments being successful. Some years later, when downy mildew (*Plasmopara viticola*, an oomycete) was responsible for a new production crisis (Gianessi and Williams 2011), the discovery of the 'Bordeaux mixture' (copper, lime, and water) in Médoc (France) by Alexis Millardet and Ulysse Gayon gave growers hope (Roudié 1997). Consequently, copper and sulfur-based products have been marketed and used for more than 150 years.

Unfortunately, many other diseases threaten crops every year, and chemical companies have developed a vast list of different active ingredients (Fungicide Resistance Action Committee 2020), to help growers fight economic and quality loses due to fungal infection. Many of these new compounds are used at lower doses, which is beneficial from an environmental point of view. Whilst some of them have succeeded and persist in the market, others whose efficacy was destroyed because of resistance mechanisms were simply abandoned or reformulated (Morton and Staub 2008). This is often the case with products with a single action mechanism or in the same family (Sharpe et al. 2017).

Different vineyard management approaches are used in the field: conventional, organic, and biodynamic. No matter the approach taken, by the end of the season, all have released chemicals into the environment, potentially polluting water bodies and soils (Komárek et al. 2009). Additionally, this represents a high economic cost to growers. Conventional systems use a range of products as either preventive treatments or as curatives. Some fungicides are systemic, which means they enter the vine tissue and move around the plant, stopping fungal growth in all tissues. Organic management systems rely only on copper and sulfur, however, formulations containing live microbes can be also applied in certain cases (Pylak et al. 2019). The main difference between organic and biodynamic systems is the incorporation of the 'moon

cycle' and special composting techniques (preparations) by the latter (Diver 1999). Consumers consider organic practices to be more environmentally friendly, sustainable, and healthy, and thus are willing to pay extra for organic wines (Vecchio 2013). As a result, growers feel encouraged to certify their organic vineyards, and/or sometimes also apply biodynamic procedures. Australia is the country with the most extensive organic-certified surface, being 4.5% of the world vineyard surface managed organically (Castellini et al. 2017).

Regardless of management approach, fungicide overuse is undesirable because it can lead to pest resistance development (Hahn 2014), environmental pollution (Zubrod et al. 2019), human health issues and economic loss to growers (Pimentel and Burgess 2012). One way to combat this is to grow/develop plants that are naturally resistant. To that end, breeding has recently resulted in new grape cultivars with resistance to fungal and oomycete pathogens (Holzapfel et al. 2020). Until these are in more common use, however, fungicide applications remain necessary during the growing season. Many fungicides will have a wide spectrum of activity, which is useful to prevent resistance, however, this will also potentially affect non-target microorganisms and thus their application has the potential to affect the vineyard microbiome. Additionally, fungicides are often applied several times throughout the season in order to control a range of pathogens including, but not limited to, powdery and downy mildew. It is generally recognised that fungicide treatments have the potential to influence both the health and natural balance of the grapevine microbiome and, as a consequence, wine quality especially from uninoculated fermentations.

Effect of fungicide application on yeast diversity

Whilst attention has been directed towards the effect of fungicide sprays on bacteria and fungi (including yeast) using both culture-dependent and independent approaches, there are many contradictory reports of the actual effect (Table 1). The effect that the type of vineyard protection has on yeast populations is summarised in Tables 1 and 2. Contradictory reports may be due to several factors, including the wide range of vineyard protection practices, such as conventional, organic, biodynamic, integrated, differences in cultivar or location, sampling differences, experimental design (i.e. treatments in the vineyard prior to the experiment), method used to detect yeast species and downstream analysis of the effect on biodiversity (Figure 2). This is likely the basis for inconsistencies such as Milanović et al. (2013) finding the yeast-like fungus *A. pullulans* linked to conventional vineyards, whilst Martins et al. (2014) found the same species more frequently associated with organic vineyards. Both research teams, however, reached the conclusion that copper-based fungicides had a detrimental effect on fungi. In contrast, some authors suggested that anti-fungal sprays do not have a significant effect on yeast communities (Čadež et al. 2010). Other authors have also reported lower yeast biodiversity in vineyards using organic management and a shift in yeast populations towards *A. pullulans* (Comitini and Ciani 2008). Organic production has also shown a greater richness of minor species (Cachón et al. 2019).

While *Aureobasidium* sp. is one of the most commonly reported fungal species on grapes, many reports fail to mention enough detail, for example, about farming practices and climate (e.g. see Wei et al. 2018) making it more difficult to interpret what is influencing diversity differences. It is not even possible to speculate that *A. pullulans* is present on all organic grapes as this is true for some studies (Martins et al. 2014, Cachón et al. 2019, Rantsiou et al. 2020) but not others (Milanovic et al. 2013). This may just be because it was not reported or was deliberately not selected based on the method used to look at diversity, for example culture-dependent methods (Cordero-Bueso et al. 2014). Additionally, it has been hypothesised that the presence of some filamentous fungi might inhibit certain yeast genera (Grangeteau et al. 2017b). This is most likely to be a problem when filamentous fungi become resistant to the fungicides applied allowing them to outcompete other yeast and fungi.

It has been reported that synthetic fungicides inhibit fermentative yeast species to a greater degree than oxidative species. For example, *S. cerevisiae* is quite sensitive to these fungicides but *Cryptococcus* spp. and *Rhodotorula* spp. less so (Villa and Longo 1996, Oliva et al. 2020). Agarbati and coworkers (2019b) found that *H. uvarum* was abundant in Montelpuciano and Verdicchio samples, but fungicide treatments influenced its relative abundance. Organic treatments enhanced oxidative colonization by *Cryptococcus* spp., whereas conventional treatments had the same effect on *A. pullulans.* A wider survey is still needed in order to be definitive. For example, Cordero-Bueso et al. (2014) reported that while *S.*

cerevisiae had the highest resistance to sulfur (along with other fermenting species) it was the most sensitive to penconazole (FRAC 3). It is therefore important to understand the role that fungicide application plays in the larger picture of a vineyard microbiome.

A consistent observation is that within a given vineyard, when multiple protection systems are studied, the fungal microbiome is affected by the farming system used (Milanovic et al. 2013, Cordero-Bueso et al. 2014, Cachón et al. 2019). What is still unclear is whether inconsistencies between studies arise because most are single time-point, that is just a snapshot in time. Longitudinal studies have the potential to help unravel these effects. One study measuring diversity over three seasons reported that grapes sprayed only twice with sulfur showed an increase in diversity over 3 years compared to those that were sprayed multiple times, those with no treatment and those treated with penconazole (FRAC 3) (Cordero-Bueso et al. 2014). It is possible that sulfur sprays are selecting for fermenting yeast over other fungi. The limitation of this study is the analysis techniques used; random selection of up to 30 colonies from YPD agar plates. Bias may have been unintendedly introduced due to the differential ability of isolates to grow on this laboratory medium. Of interest would be a longitudinal study (up to 5–10 years) in multiple locations with care taken to avoid spray drift and utilising a metagenomics approach to achieve a more holistic view of the vineyard microbiome with different treatments. Finally, it is also possible that unreported insecticides, miticides and herbicides are indirectly adding to the differences in conventional vineyards by affecting insect vectors or their habitats.

Recent reports investigating the impact of chemical and biological fungicides on grape microbial diversity have used a wide range of techniques making it difficult to compare between studies. For example, Escribano-Viana et al (2018) utilised both culture-dependent and non-culture-based (PCR-DGGE) techniques, however, whilst they report the species present the relative abundance of those species was not reported. The two species common to all treatments were *A. pullulans* and *H. osmophila* and whilst the authors reported that the microbial community was not significantly modified after fungicide application, no information was given on spray regimes in previous years, which could potentially affect results due to lower background diversity (Escribano-Viana et al. 2018).

There is also a large amount of variation in relative abundance of yeast species between replicates within the same treatment (Agarbati et al. 2019b) and the cause of this variation deserves further investigation. For example, are differences due to sampling strategies, berry heterogeneity, or sequencing errors? While it is clear that fungicides will have an effect on the fungal microflora, this is yet to be comprehensively defined. There is need for a rigorous study looking at these effects. As expected, the use of NGS technology consistently identifies more species that culture-dependent techniques, however, the most abundant species are usually detected by both methods (Agarbati et al. 2019b).

Mode of action of fungicides and how these might affect pathways in yeast

Commercial fungicides belong to several classes and affect several cellular functions. Fungicides can be described by their mode of action (MOA), or their chemical class and with a large range of products on the market this can be confusing. The available fungicides are too numerous to list in this review, however, there are a several online resources available to help navigate the choice. These sites include the Pesticide Properties DataBase (Lewis et al. 2016), viticulture spray guides from The Department of Primary Industries in Australia (Department of Primary Industries and Regional Development 2020), and The Australian Wine Research Institute (The Australian Wine Research Institute 2020b).

To avoid the development of fungicide resistance, it is necessary to know how a particular fungicide works. Most agrochemicals (fungicides, herbicides, insecticides and miticides) are assigned an 'activity group' based on their mode of action and this FRAC (Fungicide Resistance Action Committee 2020) code now appears on the product label. The mode of action of a given fungicide will vary depending on the chemical class to which it belongs (Figure 3). It is often recommended that fungicides with different modes of action are alternated to reduce the risk of fungi becoming resistant. Often when resistance does develop to one chemical in a group, fungi are resistant to other chemicals in the same group.

The mode of action can be described in general or specific terms, that is a fungicide with broad-spectrum activity is effective against a large variety of pathogenic fungi. Examples of broad-spectrum fungicides include the multisite inhibitor group, M for example captan (M4), sulfur (M2) and mancozeb (M3). Other fungicides have a narrow spectrum of activity, for example Mefenoxam (FRAC 4), which is effective only against downy mildew and must be used in mixtures (The Australian Wine Research Institute 2020b). The problem with fungicide use, however, is that indirect non-target effects are likely and difficult to predict. Microorganisms exist in a community, often either functionally or nutritionally connected with each other. Therefore, a decrease in the population of sensitive yeast may affect the structure of the whole community. The mode of action of several fungicide groups and how they might affect autochthonous fungi populations in the vineyard is reported in Figure 3.

Although fungicides are sprayed on grapevines to reduce unwanted fungi and oomycetes such as powdery mildew and downy mildew, there are many ways fungicides might also affect yeast present on the grapevines. Fungicides can be divided into two groups: those that are permitted in organic production or synthetic fungicides which cannot. The main fungicides used in organic production are sulfur and copper. Copper is a broad-spectrum antifungal and works by causing plasma membrane damage (Ohsumi et al. 1988, Avery et al. 1996), whereas the MOA of sulfur as a fungicide is not fully understood. Sulfur may inhibit spore germination and mycelium growth in filamentous fungi and part of its MOA is also likely

529 related to oxidation of sulfhydryl groups in mitochondrial respiratory enzymes (Williams and
530 Cooper 2004, Fungicide Resistance Action Committee 2020). Sulfur residues can be toxic to
531 autochthonous yeast found on grapes, however, sulfur is not thought to be toxic to strains of *S.*
532 *cerevisiae* (Boudreau et al. 2017). It has also been suggested that there might be a synergistic
533 effect of copper and sulfur, and of the non-*Saccharomyces* yeast tested so far, only *A. pullulans*
534 is able to withstand both products (Grangeteau et al. 2017a). The many synthetic fungicides on
535 the market can be grouped based on their chemistry and MOA (Figure 3).
536 The potential MOA of a fungicide is currently classed into 11 categories. These include
537 those that target nucleic acid metabolism, cytoskeleton and motor proteins, respiration, amino
538 acid and protein synthesis, signal transduction, lipid synthesis or transport, sterol biosynthesis,
539 cell wall biosynthesis and cell membrane integrity. Additionally, there are several fungicides
540 whose mode of action remains unknown (MOA = U), while a newer group that utilises the
541 plants natural defence mechanisms are in group P (Fungicide Resistance Action Committee
542 2020). The main target of many fungicides is the cell wall, which has a characteristic structure
543 in fungi being composed mainly of glucans, chitin and glycoproteins (Figure 3). The cell wall
544 is arranged in different layers where the innermost layer is a more conserved structure on which
545 the remaining layers are deposited and can vary between different species of fungi.
546 Several fungicides have been studied for their effect on various fungi. Many that have
547 been in long-term use have multisite activity (M) indicating that these molecules affect several
548 different fungal structural components and or metabolic pathways (Lukens 1971). For example,
549 dithiocarbamates (e.g. mancozeb, thiram, ziram; FRAC M3) interfere with membrane
550 organisation and embedded transport systems. This induces intracellular acidification and
551 oxidative stress leading to inactivation of cellular thiol groups (Dias et al. 2010), and possibly
552 apoptosis (Scariot et al. 2016). Other studies indicate that mancozeb interferes with respiration
553 and is therefore more inhibitory towards respiring rather than fermenting yeast (Casalone et al.
554 2010). Another fungicide group in long-term use is phthalimides (e.g. captan, captafol, folpet;
555 FRAC M4), which interferes with cellular respiration and glycolysis. Captan is a broad-
556 spectrum fungicide that can affect non-target microorganisms including wine yeast (Scariot et
557 al. 2016).
558 Fungicides with more site-specific actions targeting certain functions of the fungal cell
559 include: dicarboximides, which interfere with membrane function (FRAC 2) and ergosterol
560 biosynthesis inhibitors (FRAC 3, including triazole-based fungicides). Triazole-based
561 fungicides contain compounds that are demethylation and sterol biosynthesis inhibitors
562 (Trzaskos et al, 1989), inhibiting three steps in ergosterol biosynthesis (Figure 3). More
563 recently, Katragkou et al. (2016) showed that the biosynthesis of amino acids, including
564 glycine, proline, tryptophan, asparagine, aminoisobutanoate (thiamine catabolism product), and
565 products of purine metabolism, represented by guanine, were decreased in the presence of

fluconazole, suggesting that the mode of action of theses fungicides is possibly more complex than so far reported. Non-*Saccharomyces* yeast are also susceptible to these fungicides with *Metschnikowia* spp. reported to be susceptible to the azole antifungals (FRAC 3), ketoconazole, epoxiconazole and to a lesser extent imazalil (Álvarez-Pérez et al. 2016). It is hypothesised that the lower sensitivity to the latter compound may be linked to its extensive use in agriculture since the 1970s (Álvarez-Pérez et al. 2016).

One way to look at how fungicides affect yeast is to investigate their metabolism in the presence of various fungicides. To this end, the BacTiter-Glo microbial cell viability assay, which determines the number of viable microbial cells by quantifying the ATP present has been useful (Kosel et al. 2019). Several authors have also investigated how fungicides might affect gene expression and/or aroma compounds in the final wine. Effects will obviously depend on the fungicide tested, for example pyrimethanil (FRAC 9) has been shown to alter gene expression in a manner dependent on the dosage tested. Genes differentially expressed include those involved in biosynthesis of arginine and sulfur amino acid metabolism, energy conservation, antioxidant response and multi-drug transport (Gil et al. 2014). Whereas tetraconazole (FRAC 3) was reported to alter the activity of enzymes involved in methionine and ergosterol biosynthesis (Sieiro-Sampedro et al. 2020). Interestingly, there were also differences when purified antifungal agent was tested alongside the commercial product. It is possible that other components of the commercial products are affecting biosynthesis (Sieiro-Sampedro et al. 2020), something that will need to be taken into account in future analysis of fungicide effects.

It is important to note that other plant protection procedures, such as herbicides and insecticides, also have the ability to affect the vineyard microbiome, either directly or indirectly. This is outside the scope of this review and the reader is referred to recent publications that address this (Chou et al. 2018, Mandl et al. 2018, Carneiro et al. 2019, Vincent and Lasnier 2020).

In vitro fungicide resistance of non-*Saccharomyces* yeasts

Researchers have begun to examine the effect of fungicides on desired non-*Saccharomyces* yeast. So far, it appears that fungicides reduce the viability of desirable non-*Saccharomyces* yeast and could potentially promote the growth of spoilage yeast (Kosel et al. 2019). This is obviously an undesirable side-effect of crop protection and in the case of winemaking it may be one with a negative impact on wine aroma by favouring spoilage organisms. Agarbati et al. (2019a) suggested that *A. pullulans* and *Cryptococcus* spp. are favoured by conventional and organic treatments, respectively, as neither of them were significant on untreated samples. This is hypothesised to be due to reduced competition from susceptible yeasts and their ability to detoxify $CuSO_4$ (Schmid et al. 2011). Resistance of *A. pullulans* to both copper and sulfur

603 reveals this organism can oxidise inorganic sulfur into sulfate ions (Killham et al. 1981).
604 Additionally, *A. pullulans* can differentiate into melanin pigmented chlamydospores and hyphal
605 filaments, which allows for biosorption of copper (Gadd and Griffiths 1980a, Gadd 1983, Gadd
606 and de Rome 1988, Fogarty et al. 1996). Resistant strains take up less metal than sensitive
607 strains (Gadd and Griffiths 1980b). Copper had an inhibitory effect on the cultivable yeast
608 population from two Bordeaux vineyards, and was found at higher levels in conventionally
609 managed vineyards (Martins et al. 2014). With regards to *S. cerevisiae*, Adamo et al. (2012)
610 reported a sevenfold amplification of *CUP1* expression in a copper resistant isolate. This
611 suggests that *CUP1*, encoding a copper-binding protein, plays a role in protecting *S. cerevisiae*
612 cells against copper toxicity. This type of resistance could also be engineered in *Pichia pastoris*
613 (Koller et al. 2000) and *Kluyveromyces lactis* (Macreadie et al. 1991).
614 A recent study testing 21 isolates of *Aureobasidium pullulans*, nine *Hanseniaspora*
615 *guilliermondii*, 13 *Hanseniaspora uvarum*, 63 *Metschnikowia* spp., eight *Pichia*
616 *membranifaciens*, 41 *Starmella bacillaris* and one isolate of *S. cerevisiae* for their sensitivity
617 to copper and sulfur reported a high degree of intraspecies variability (Grangeteau et al. 2017a).
618 In this study, *A pullulans* and *St. bacillaris* were the most resistant to copper and *A. pullulans*,
619 *H. guilliermondii* and *Metschnikowia* spp. to sulfur. Only isolates of *A. pullulans* had high
620 resistance to both antifungal agents (Grangeteau et al, 2017a). In the vineyard, copper and sulfur
621 are often applied simultaneously or sequentially and future work should therefore include
622 analysis of the combined effect of the two products and the mechanisms behind intraspecies
623 variation in resistance. Authors who have reported fungal abundance on untreated grapes report
624 that *A. pullulans* is only a minor part of the whole yeast population under those conditions
625 (Agarbati et al 2019a).
626 It is possible that some yeasts are dominant in sprayed vineyards because of an increase
627 in the resistance of the particular population to a fungicide that may have been applied over
628 several years. Yeast fungicide resistance was tested in a recent study which investigated the
629 minimal inhibitory concentration of different fungicide treatments (iprodione, pyrimethanil,
630 and fludioxonil + cyprodinil) for 109 grape associated yeasts. Species such as *S. cerevisiae*,
631 *Naganishia adeliensis*, *Papiliotrema flavescens*, *Meyerozyma guilliermondii*, *P.*
632 *membranifaciens* and *Pseudozyma prolifica* were not susceptible to any of the tested fungicides
633 (Kosel et al. 2019). Both viability and growth of many beneficial isolates, however, were
634 inhibited by fungicides at a residue concentration below the maximum permitted residue limits
635 including isolates of *M. pulcherrima* (three strains, iprodione; three strains, both iprodione and
636 fludioxonil + cyprodinil), *P. kluyveri* (four strains iprodione and pyrimethanil), and *H. uvarum*
637 (seven strains pyrimethanil and fludioxonil + cyprodinil). Furthermore, isolates of spoilage
638 yeasts, for example *D. bruxellensis*, were found to be tolerant of a concentration of fungicides
639 greater than that recommended for application by the suppliers (Kosel et al. 2019). Further

work is therefore required to model fungicide application and diversity in the vineyard to improve our understanding of the impact of sprays towards yeast.

The effect of fungicide residues on fermentation

The level of fungicide in must will of course be related to the nature and amount of the specific fungicide applied in the vineyard. For example, a comprehensive review of the concentration of fungicides in wines and must throughout the 1990s reported that azoxystrobin (FRAC 11) and pyrimethanil (FRAC 9) residues in the must were equivalent to that on the grapes (Cabras and Angioni 2000). In all other cases, residues in the must were lower than on the grapes, and in some cases no residues [myclobutanil (FRAC 3) and tetraconazole (FRAC 3)] were present in the must (Cabras and Angioni 2000). As for non-*Saccharomyces*, a wide range of methods have been utilised to study the effect of fungicide residues. For example, Conner (1983) used the paper-disc agar diffusion technique to demonstrate that fungicides varied markedly in their toxicity to *Saccharomyces* wine yeasts. This, however, is not likely to be an accurate reflection of the impacts in fermentation, since juice/wine have features that could influence the effect of fungicides, including a low pH, increasing concentration of ethanol and other stresses. It can be hypothesised that anything that also impacts the target of the fungicide, such as the cell wall, may enhance the sensitivity of the yeast to the fungicide. Such interactions may well be additive, if not synergistic and thereby affect fermentation progress.

Interestingly, when Noguerol-Pato et al. (2014) tested ten new generation fungicides added to must at the maximum permitted residue level, they found that the prior filtration of the must had a strong influence on fermentative activity. Specifically, when *S. cerevisiae* was grown in red pasteurised must enriched with sugar and addition of various fungicides, only three fungicides showed an effect. These were ametoctradin (FRAC 45, MOA = C), dimethomorph (FRAC 40, MOA = H) and mepanipyrim (FRAC 09, MOA = D) (see Figure 3 for MOA definitions and FRAC codes). The same was observed in filtered Tempranillo must, but when the must was tested unfiltered, no effect was observed (Noguerol-Pato et al. 2014). Calhelha et al. (2006) also reported that while fungicides had a negative effect on in vitro yeast growth, laboratory-scale red wine fermentations spiked with benomyl (FRAC 1) and dichlofluanid (FRAC M6) had a limited effect on wine chemical and sensory properties when compared to a control without fungicides. These findings imply that the complex matrix that is unfiltered grape must offers protection to yeast, perhaps by absorption of fungicide residues onto particles in the must. Other factors such as a difference in pH can also impact fungicide inhibition, with low pH values enhancing fungicide disassociation and thereby producing different MIC values compared to tests at a neutral pH (Scariot et al. 2016).

The presence of antifungal residues and grape must can seriously affect progress of alcoholic fermentation (Bizaj et al. 2014). For example, the fungicides pyrimethanil (FRAC 9)

and fenhexamid (FRAC 17) negatively affect fermentation kinetics in a strain dependent manner, when various industrial strains were inoculated into spiked grape juice (Bizaj et al. 2014). Additionally, both the aromatic and basic composition of the resulting wines were affected, albeit it differently, depending on the fungicide added, but with no increase in desirable compounds and an increase in undesirable ones (Bizaj et al. 2014). Yeast cells treated with captan exhibited altered membrane integrity, reduction of thiol compounds and an increase in intracellular reactive oxygen species. Concentration of 2.5 μmol/L of captan completely inhibit fermentation with a dose-dependent delay when a lower concentration was tested (Scariot et al. 2016, 2017).

More recently Sieiro-Sampedro et al. (2020) reported that when tetraconazole (FRAC 3) was added into Garnacha wine, to mimic residual fungicide concentration, the volatile profile ranged between 23and 145% of the control, mainly due to changes in ethyl esters derived from medium-chain fatty acids. Proteomic analysis of the yeast was also carried out and when fungal residues were present there were changes in the abundance of enzymes involved in the methionine and ergosterol biosynthesis pathways (Sieiro-Sampedro et al. 2020).

Therefore, in impacting yeast populations on grapes, in the fermentation as well as the metabolism of these, fungicides have the potential to not only interfere with fermentation progress but also the final aroma profile. The formation, release or degradation of sensorially important compounds could conceivably be altered either through a metabolic response to fungicides or because of the altered yeast population profile and metabolic interactions that result. Certainly, it is well established that different combinations and proportions of yeast result in a different wine composition, including aroma compounds (e.g. Capozzi et al. 2015, Padilla et al. 2016b). This is a potentially important determinant of winemaking outcome that is perhaps not often considered before the application of fungicide sprays to the vineyard.

Alternative treatments

Due to high potential yield losses, agriculture relies on the application of chemical treatments to protect against bacteria, fungi, viruses, weeds and insects. Unfortunately, a large proportion of these products do not arrive at their site of action and a large quantity of spray is needed to ensure coverage. For example, pesticide losses ranging between 63–74% were reported when testing different nozzle and pressure levels in lemon and tangerine orchards (Soheilifard et al. 2020). This potentially causes pollution of soil, water bodies and air, as well as economic losses to growers. Additionally, these chemical compounds can negatively impact human health, affect non-target microorganisms and may also encourage pest/disease resistance (Worrall et al. 2018). It is therefore imperative to find new, safer ways of protecting crops. This next section

describes and evaluates alternative ways to control pathogenic fungi in the vineyard, with a view towards finding less harsh treatments that enable us to nurture the grapevine microbiome.

Is there a vineyard version of probiotics?

Previous studies in banana trees and maize (Marcano et al. 2016, Youseif 2018) show that the application of plant growth promoters (PGP) as probiotics in grapevines is feasible but is yet to be investigated. In order to achieve this, we hypothesise that it would be necessary to isolate and purify bacterial probiotics from the roots and rhizosphere of healthy grapevines for this technique. The soil and rhizosphere microbiome contain species that are cultivar specific due to the chemical composition of root exudates varying among plant genotypes, thereby representing a selective force defining host-microbiome interaction (Bakker et al. 2012). In addition, soil physical properties such as particle size and chemical characteristics, such as pH, nutrient, water and oxygen concentration and texture, also affect microbial community development and activity (Gilbert et al. 2014). We suggest that research should prioritise local and culturable PGP strains to guarantee growth and reproduction of these PGP following transplant. Also, those PGP species that look promising in in vitro screenings, need to be tested in the field before further conclusions about their efficacy can be made.

Insect vectors to improve diversity

Vineyards are complex agroecosystems with intricate relationships occurring between all their components even if grapevines are the predominant species. Throughout this review we have emphasised how important yeast diversity is to ensure vineyard health. Yeast diversity must be considered as a part of the overall vineyard biodiversity. Plant, insects and microbes will enhance vine adaptation and resilience (Retallack 2011). In previous sections we have discussed how yeast diversity could be affected by fungicides. It is also important, however, to consider the potential unwanted side effects of other pesticides such as insecticides, which should be mindfully chosen as they can affect beneficial insects as well. Yeasts rely on insects to move around the vineyard and also from the vineyard to the winery (Madden et al. 2018, Liu et al. 2019b). In addition, practices such as adding vegetal species, native or exotic, around the vineyard (vineyard scaping), planting flowering species that beneficial insects feed from, and creating shelter for them can improve not only pest management, but yeast diversity (Retallack 2011). Finally, the use of grasses and *Leguminosae* species as cover crops improves the carbon/nitrogen relationship in the soil, enhancing soil structure and microflora diversity around the roots (Vukicevich et al. 2016).

Bioprotectants

749 Instead of using of fungicides to control undesirable fungi it is possible that antagonistic yeast
750 and/or bacterial species could be propagated in the vineyard and used as 'bioprotectants' (Bleve
751 et al. 2006). Indeed, many isolates that are sensitive to residue levels of fungicides could
752 potentially be used against the species that the fungicides are ultimately designed to eradicate.
753 The introduction of bacteria such as the lactic acid bacterium *Lactobacillus plantarum* (Gobbi
754 et al. 2020) or yeast strains with biocontrol activity is a 'hot-topic' and a promising alternative
755 to traditional methods. Indeed, grape-derived epiphytic yeast species such as *Issatchenkia*
756 *terricola* have antagonistic activity towards *Aspergillus* spp. in vitro (Bleve et al. 2006). Raspor
757 et al. (2010) tested several wine yeast species for their potential against the growth of *Botrytis*
758 *cinerea* with *A. pullulans* and *M. pulcherrima* having the highest potential biocontrol activity.
759 A more recent study comparing a range of available biological controls against *Botrytis* bunch
760 rot, (*Bacillus subtilis*, *B. amyloliquefaciens*, *A. pullulans*, *Ulocladium oudemansii*, and *Candida*
761 *sake*) along with six experimental bacterial and two fungal biological control strains found that
762 treatments based on *C. sake* and *B. subtilis* QST713, achieved the highest reduction in disease
763 severity rates (45 and 54%, respectively), but the effect was dependant on the season (year
764 tested) and the grape cultivar (Calvo-Garrido et al. 2019).
765 *Aureobasidium pullulans* is also a good biocontrol agent able to limit the development of
766 *B. cinerea*, *Rhizopus stolonifer*, *Aspergillus niger* and *A. carbonarius* on grape berries (Schena
767 et al. 1999, 2003, Dimakopoulou et al. 2008). These capacities combined with a resistance to
768 copper and sulfur (used in organic viticulture) make *A. pullulans* an interesting choice as an
769 organic biological control agent. The *A. pullulans* strain Fito_F278 significantly reduced the
770 mycelium growth of the botryosphaeria dieback agent *Diplodia seriata*, via direct antagonism
771 under in vitro conditions (Pinto et al. 2018). No significant reduction, however, of disease
772 lesions and relative frequency were found in cutting plants, reinforcing that the antagonistic
773 activities of this strain are dependent on a direct interaction with the phytopathogen (Pinto et
774 al. 2018). Thus, further studies using direct application treatments are required. However, in
775 order to measure the impact and interaction of these strains on the resident microbial
776 community, studies based on analyses of the microbiome in vineyards are necessary. Many
777 epiphytic yeast isolates that have been tested in vitro have shown antagonistic activity towards
778 a range of grape pathogens (Bleve et al. 2006, Cordero-Bueso et al. 2017). But when tested in
779 vivo (outdoor conditions) they are so far ineffective (Perazzolli et al. 2014). Therefore, there is
780 still much work to be done to maximise this antagonistic activity in the field.
781 Looking to the future, many agrochemical manufacturers are using techniques such as
782 RNAi or CRISPR-Cas9 to generate microbes able to produce biopesticides (Borel 2017). Even
783 so, more work is needed as the success of these products depends on producing large quantities
784 of organisms with a long-shelf life. In addition, since microbiome changes are expected
785 amongst regions, crops or climatic conditions, variable that may also alter the efficacy of

candidate agrochemicals, manufacturers are faced with significant challenge given their preference to produce a treatment with a standard or limited formulation(s) (Parnell et al. 2016, Schütz et al. 2018). Moreover, when we introduce microbes into a foreign environment, there will be competition between the indigenous and the inoculated microflora (Ambrosini et al. 2016), which also needs to be taken into consideration. Last, there are some incompatibilities between inputs (e.g. fertilisers) or agricultural practices (e.g. tillage), whose application may alter soil microbial communities (Lupwayi et al. 2010).

Other novel anti-fungal treatments

Other potential biological controls include; clay for powdery mildew treatments (Sholberg et al. 2006), vegetable or mineral oil, or potassium silicate or more recently, nanoparticles. Nanoparticles can act either as protectants themselves (gold, silver, chitosan, copper, titanium dioxide) or be the carriers for other compounds (Worrall et al. 2018). Practically speaking they have improved efficacy, over a longer period of time, which potentially translates to reduced need to spray. For example, nanoparticle-delivered tebuconazole had a similar decay amount when used at 10% of the original dose in wood treatments (Liu et al. 2002). Additionally, decreased phytotoxicity of carbendazim when loaded onto nanoparticles, improved germination rates and root growth in cucumber, tomato and corn (Kumar et al. 2017). Pyrimethanil is a specific fungicide for the treatment of *Botrytis* on grapes (Bayer Crop Science Australia 2020), but if applied after 80% capfall, traces will still be detectable in finished wines, which can lead to export issues (The Australian Wine Research Institute 2020b). Zhao et al. (2017) working with pyrimethanil loaded onto mesoporous silica nanoparticles (MSN) concluded that this formulation minimised the risk of the fungicide accumulating in cucumber. If applied to grapevines, a similar strategy may improve both grape health and the safety of finished wines for human consumption. Another pathogen-specific nanoparticle delivery system has also been shown to be effective with azoxystrobin, pyraclostrobin, tebuconazole, and boscalid, to treat *Phaeomoniella chlamydospora* and *P. minimum* (ESCA) in *Vitis vinifera* cv. Portugieser. But despite showing great promise, the effect of this technology on indigenous microflora is to yet be defined, therefore demanding further research.

Enhancing plant immune systems

A newer approach to plant protection is being developed by seeking to enhance the response to pathogens by means of 'antibodies' collected from symptomatically affected plants. This method, utilising NDM (Natural Defence Messengers), transforms a sensitive plant into a resistant one (Gabel 2019) and depends on the plant's phenology and its sensitivity to infections. This process begins with an infected plant from which material is collected and antibodies are extracted from affected tissues using organic solvents (Gabel 2019). In contrast

with the human immune system in which defence responses are specific to a particular microbe, the effects of priming in plants are broad-spectrum, protecting the plant against a wide range of diseases and insect pests. For a review and more information on the potential use of the grapevine defence response (by the use of elicitors) as an alternative to fungicide treatment please see Delaunois et al. (2014). Again, further research is needed to improve our understanding of the molecular mechanisms behind NDM but priming a plants natural defence mechanism could well be a valuable tool in sustainable agriculture.

Conclusion

This review has highlighted the complex nature of investigating yeast diversity in the vineyard. The factors that determine diversity inevitably form a complex matrix of interactions and we have chosen to focus on fungicides. In doing so we have highlighted the large amount of work that has been undertaken in this area of research. Unfortunately, due to the inherently complex nature of investigating yeast diversity under many variable conditions, it is difficult to achieve standardised methods. Perhaps a microbiome database where sequences from such studies are deposited along with as much available information about sprays, climate and geographical location as possible would enable other researchers to make comparisons between studies.

The microbial population on the grapes will have an effect on both inoculated and uninoculated fermentations. It is likely, however, that many vignerons and winemakers do not consider the impacts of fungicides on their fermentations. There are several different modes of action for each fungicide, and yeast may have differing ability to adapt to each MOA, for example *Saccharomyces* yeast are more resistant to copper than non-*Saccharomyces* yeast. Finally, as we have reported there are several novel crop protection strategies being studied to help protect crops and increase diversity. Alternative treatments, such as plant growth promoters and bioprotectants, show great progress. It may also be after more longitudinal studies that we discover better management techniques to enhance microbial diversity whilst still protecting the vineyard.

Acknowledgements

Financial support for this work was provided by The Australian Research Council Training Centre for Innovative Wine Production [www.ARCwinecentre.org.au; IC170100008], which is funded by the Australian Government with additional support from Wine Australia and industry partners. The University of Adelaide is a member of the Wine Innovation Cluster (http://www.thewaite.org.waite-partners/wine-innovation-cluster/).

References

Abdelfattah, A., Sanzani, S.M., Wisniewski, M., Berg, G., Cacciola, S.O. and Schena, L. (2019) Revealing cues for fungal interplay in the plant–air interface in vineyards. Frontiers in Plant Science **10**, 922. Adamo, G.M., Lotti, M., Tamás, M.J. and Brocca, S. (2012) Amplification of the *CUP1* gene is associated with evolution of copper tolerance in *Saccharomyces cerevisiae*. Microbiology **158**, 2325–2335.

Agarbati, A., Canonico, L., Ciani, M. and Comitini, F. (2019a) The impact of fungicide treatments on yeast biota of Verdicchio and Montepulciano grape varieties. PLOS ONE **14**(6), e0217385.

Agarbati, A., Canonico, L., Mancabelli, L., Milani, C., Ventura, M., Ciani, M. and Comitini, F. (2019b) The influence of fungicide treatments on mycobiota of grapes and its evolution during fermentation evaluated by metagenomic and culture-dependent methods. Microorganisms 7, 114.

Albergaria, H. and Arneborg, N. (2016) Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes: role of physiological fitness and microbial interactions. Applied Microbiology and Biotechnology **100**, 2035–2046.

Álvarez-Pérez, S., de Vega, C., Pozo, M.I., Lenaerts, M., Van Assche, A., Herrera, C. M., Jacquemyn, H. and Lievens, B. (2016) Nectar yeasts of the *Metschnikowia* clade are highly susceptible to azole antifungals widely used in medicine and agriculture. FEMS Yeast Research **16**(1), fov115. doi: 10.1093/femsyr/fov115.

Amato, M., Ballco, P., López-Galán, B., De Magistris, T. and Verneau F. (2017) Exploring consumers' perception and willingness to pay for "Non-Added Sulphite" wines through experimental auctions: a case study in Italy and Spain. Wine Economics and Policy **6**, 146-154.

Ambrosini, A., de Souza, R. and Passaglia, L.M.P. (2016) Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. Plant and Soil **400**, 193-207.

Anfang, N., Brajkovich, M. and Goddard, M.R. (2009) Co-fermentation with *Pichia kluyveri* increases varietal thiol concentrations in Sauvignon Blanc. Australian Journal of Grape and Wine Research **15**, 1–8.

Anguita-Maeso, M., Olivares-García, C., Haro, C., Imperial, J., Navas-Cortés, J.A. and Landa, B.B. (2020) Culture-dependent and culture-independent characterization of the olive xylem microbiota: effect of sap extraction methods. Frontiers in Plant Science **10**, 1708.

Avery, S.V, Howlett, N.G. and Radice, S. (1996) Copper toxicity towards *Saccharomyces cerevisiae*: dependence on plasma membrane fatty acid composition. Applied and Environmental Microbiology, **62**, 3960-3966.

Bakker, M.G., Manter, D.K., Sheflin, A.M., Weir, T.L. and Vivanco, J.M. (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant and Soil **360**, 1-13.

Barata, A., Malfeito-Ferreira, M. and Loureiro, V. (2012) The microbial ecology of wine grape berries. International Journal of Food Microbiology **153**, 243–259.

Bartle, L., Sumby, K., Sundstrom, J. and Jiranek, V. (2019) The microbial challenge of winemaking: yeast-bacteria compatibility. FEMS Yeast Research **19**(4), foz040.

Bayer Crop Science Australia (2020) Scala® 400 SC Fungicide. Accessible at: https://www.crop.bayer.com.au/find-crop-solutions/by-product/fungicides/scala-400-sc-fungicide.

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascués, E., Marquina, D., Santos, A. and Moreno-Arribas M. (2017a) Microbial contribution to wine aroma and its intended use for wine quality improvement. Molecules **22**, 189.

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A. and Acedo, A. (2017b) From vineyard soil to wine fermentation: microbiome approximations to explain the "terroir" concept. Frontiers in Microbiology **8**, 821.

Beltran, G., Torija, M.J., Novo, M., Ferrer, N., Poblet, M., Guillamón, J.M., Rozès, N. and Mas, A. (2002) Analysis of yeast populations during alcoholic fermentation: a six year follow-up study. Systematic and Applied Microbiology **25**, 287–293.

Benito, S. (2018) The impacts of *Lachancea thermotolerans* yeast strains on winemaking. Applied Microbiology and Biotechnology **102**, 6775-6790.

Berger, W.H. and Parker, F.L. (1970) Diversity of planktonic foraminifera in deep-sea sediments. Science **168**, 1345-1347.

Bizaj, E., Curtin, C., Čadež, N. and Raspor, P. (2014) Interactions between industrial yeasts and chemical contaminants in grape juice affect wine composition profile. Food Technology and Biotechnology **52**, 222–231.

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A. and Visconti, A. (2006) Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. International Journal of Food Microbiology **108**, 204–209.

Bokulich, N.A., Collins, T.S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S.E. and Mills, D.A. (2016) Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. mBio **7**(3), e00631-16.

Bokulich, N.A., Ohta, M., Richardson, P.M., Mills, D.A. and Mormile, M.R. (2013) Monitoring seasonal changes in winery-resident microbiota. PloS One. **8**, e66437.

Bokulich, N.A., Thorngate, J.H., Richardson, P.M. and 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.

Borel, B. (2017) When the pesticides run out. Nature **543**, 302-304.

Boudreau, T.F.T., Peck, G.M., O'Keefe, S.F. and Stewart A.C. (2017) The interactive effect of fungicide residues and yeast assimilable nitrogen on fermentation kinetics and hydrogen sulfide production during cider fermentation. Journal of the Science of Food and Agriculture **97**, 693-704.

Bozoudi, D. and Tsaltas D. (2018) The multiple and versatile roles of *Aureobasidium pullulans* in the vitivinicultural sector. Fermentation **4**, 85.

Bray, J.R. and Curtis, J.T. (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs **27**, 326-349.

Cabras, P. and Angioni, A. (2000) Pesticide residues in grapes, wine, and their processing products. Journal of Agricultural and Food Chemistry **48**, 967–973.

Calhelha, R.C., Andrade, J.V, Ferreira, I.C. and Estevinho, L.M. (2006) Toxicity effects of fungicide residues on the wine-producing process. Food Microbiology **23**, 393–398.

Calvo-Garrido, C., Roudet, J., Aveline, N., Davidou, L., Dupin, S. and Fermaud, M. (2019) Microbial antagonism toward *Botrytis* bunch rot of grapes in multiple field tests using one *Bacillus ginsengihumi* strain and formulated biological control products. Frontiers in Plant Science **10**, 105.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld J. and Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335–336.

Capozzi, V., Garofalo, C., Chiriatti, M.A., Grieco, F. and Spano, G. (2015) Microbial terroir and food innovation: the case of yeast biodiversity in wine. Microbiological Research **181**, 75–83.

Carmichael, P.C., Siyoum, N., Chidamba, L. and Korsten, L. (2019) Exploring the microbial communities associated with *Botrytis cinerea* during berry development in table grape with emphasis on potential biocontrol yeasts. European Journal of Plant Pathology **154**, 919-930.

Carneiro, H.C.S., Ribeiro, N.Q., Bastos, R.W. and Santos, D.A. (2019) Effect of non-antifungal agrochemicals on the pathogenic fungus *Cryptococcus gattii*. Medical Mycology **58**, 47–53.

Carrau, F., Gaggero, C. and Aguilar, P.S. (2015) Yeast diversity and native vigor for flavor phenotypes. Trends in Biotechnology **33**, 148-154.

969 Casalone, E., Bonelli, E. and Polsinelli, M. (2010) Effects of mancozeb and other
970 dithiocarbamate fungicides on *Saccharomyces cerevisiae*: the role of mitochondrial
971 petite mutants in dithiocarbamate tolerance. Folia Microbiologica **55**, 593–597.
972 Castellini, A., Mauracher, C. and Troiano, S. (2017) An overview of the biodynamic wine
973 sector. International Journal of Wine Research **9**, 1-11.
974 Cachón, D.C., Crego, E.R, González, N.N. and Camba, P.B. (2019) Yeast diversity on grapes
975 from Galicia, NW Spain: biogeographical patterns and the influence of the farming
976 system: Macrowine 2018. OENO One **53**(3), 573-587.
977 Čadež, N., Zupan, J. and Raspor, P. (2010) The effect of fungicides on yeast communities
978 associated with grape berries. FEMS Yeast Research **10**, 619–630.
979 Cénit, M.C., Matzaraki, V., Tigchelaar, E.F. and Zhernakova, A. (2014) Rapidly expanding
980 knowledge on the role of the gut microbiome in health and disease. Biochimica et
981 Biophysica Acta (BBA) - Molecular Basis of Disease **1842**, 1981-1992.
982 Chatonnet, P., Boidron, J.-N. and Dubourdieu D. (1993) Influence des conditions d'élevage et
983 de sulfitage des vins rouges en barriques sur leur teneur en acide acétique et en ethyl-
984 phenols. OENO One **27,** 277-298.
985 Chatonnet, P., Dubourdieu, D., Boidron, J.N. and Pons M. (1992) The origin of ethylphenols
986 in wines. Journal of the Science of Food and Agriculture **60**, 165-178.
987 Chevet, J.-M., Lecocq, S. and Visser, M. (2011) Grapevine phenology, wine production and
988 prices: Pauillac (1800-2009). The American Economic Review **101**, 142-146.
989 Chou M.-Y., Heuvel J.V., Bell T.H., Panke-Buisse K. and Kao-Kniffin J. (2018) Vineyard
990 under-vine floor management alters soil microbial composition, while the fruit
991 microbiome shows no corresponding shifts. Scientific reports **8**, 11039.
992 Christiaens, J.F., Franco, L.M., Cools, T.L., De Meester, L., Michiels, J., Wenseleers, T.,
993 Hassan, B.A., Yaksi, E. and Verstrepen, K.J. (2014) The fungal aroma gene *ATF1*
994 promotes dispersal of yeast cells through insect vectors. Cell Reports **9**, 425-432.
995 Clemente-Jimenez, J., Mingorance-Cazorla, L., Martínez-Rodríguez, S., Las Heras-Vázquez,
996 F. and Rodríguez-Vico F. (2005) Influence of sequential yeast mixtures on wine
997 fermentation. International Journal of Food Microbiology **98,** 301-308.
998 Combina, M., Mercado, L., Borgo, P., Elia, A., Jofre, V., Ganga, A., Martinez, C. and Catania,
999 C. (2005) Yeasts associated to Malbec grape berries from Mendoza, Argentina. Journal
1000 of Applied Microbiology **98**, 1055-1061.
1001 Comitini, F. and Ciani, M. (2008) Influence of fungicide treatments on the occurrence of yeast
1002 flora associated with wine grapes. Annals of Microbiology, **58**, 489–493.
1003 Compant, S., Samad, A., Faist, H. and Sessitsch, A. (2019) A review on the plant microbiome:
1004 ecology, functions, and emerging trends in microbial application. Journal of Advanced
1005 Research **19**, 29-37.

1006 Conner, A. J. (1983) The comparative toxicity of vineyard pesticides to wine yeasts. American
1007 Journal of Enology and Viticulture **34**, 278-279.
1008 Contreras, A., Hidalgo, C., Henschke, P., Chambers, P., Curtin, C. and Varela C. (2014)
1009 Evaluation of non-*Saccharomyces* yeasts for the reduction of alcohol content in wine.
1010 Applied and Environmental Microbiology **80**, 1670-1678.
1011 Cordero-Bueso, G., Arroyo, T. and Valero, E. (2014) A long term field study of the effect of
1012 fungicides penconazole and sulfur on yeasts in the vineyard. International Journal of
1013 Food Microbiology **189**, 189–194.
1014 Cordero-Bueso, G., Arroyo, T., Serrano, A., Tello, J., Aporta, I., Vélez, M.D. and Valero, E.
1015 (2011) Influence of the farming system and vine variety on yeast communities
1016 associated with grape berries. International Journal of Food Microbiology **145**, 132–
1017 139.
1018 Cordero-Bueso, G., Mangieri, N., Maghradze, D., Foschino, R., Valdetara, F., Cantoral, J.M.
1019 and Vigentini, I. (2017) Wild grape-associated yeasts as promising biocontrol agents
1020 against *Vitis vinifera* fungal pathogens. Frontiers in Microbiology **8**, 2025. doi:
1021 10.3389/fmicb.2017.02025
1022 Costanigro, M., Appleby, C. and Menke S.D. (2014) The wine headache: consumer perceptions
1023 of sulfites and willingness to pay for non-sulfited wines. Food Quality and Preference
1024 **31**, 81-89.
1025 Danner, L., Ristic, R., Johnson, T.E., Meiselman, H.L., Hoek, A.C., Jeffery D.W. and Bastian,
1026 S.E.P. (2016) Context and wine quality effects on consumers' mood, emotions, liking
1027 and willingness to pay for Australian Shiraz wines. Food Research International **89**,
1028 254-265.
1029 DeBenedictis, P.A. (1973) On the correlations between certain diversity Indices. The American
1030 Naturalist **107**, 295-302. doi: 10.1086/282831.
1031 Delaunois B., Farace G., Jeandet P., Clément, C., Baillieul, F., Dorey, S. and Cordelier, S.
1032 (2014) Elicitors as alternative strategy to pesticides in grapevine? Current knowledge
1033 on their mode of action from controlled conditions to vineyard. Environmental Science
1034 and Pollution Research International, **21**, 4837-4846.
1035 Department of Primary Industries and Regional Development (2020) 2019/2020 Viticultural
1036 spray guide. Wine grapes and table grapes. Accessible
1037 at:https://www.agric.wa.gov.au/table-grapes/viticulture-spray-guide-western-australia
1038 Dias, P.J., Teixeira, M.C., Telo, J.P. and Sá-Correia, I. (2010) Insights into the mechanisms of
1039 toxicity and tolerance to the agricultural fungicide mancozeb in yeast, as suggested by
1040 a chemogenomic approach. OMICS: A Journal of Integrative Biology, **14**, 211-227.
1041 Dimakopoulou, M., Tjamos, S., Antoniou, P., Pietri, A., Battilani, P., Avramidis, N., Markakis,
1042 E. and Tjamos, E. (2008) Phyllosphere grapevine yeast *Aureobasidium pullulans*

reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece. Biological Control **46**, 158-165.

Diver, S. (1999) Biodynamic farming and compost preparation. Alternative farming systems guide (ATTRA-National Sustainable Agriculture Information Service: Butte, MT, USA). https://www.demeter-usa.org/downloads/Demeter-Science-Biodynamic-Farming-&-Compost.pdf

Dodd, I.C. and Ruiz-Lozano J.M. (2012) Microbial enhancement of crop resource use efficiency. Current Opinion in Biotechnology **23**, 236-242.

Drumonde-Neves, J., Franco-Duarte, R., Lima, T., Schuller, D. and Pais, C. (2016) Yeast biodiversity in vineyard environments is increased by human intervention. PLoS ONE **11**, e0160579.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods **10**, 996–998.

Escribano-Viana, R., López-Alfaro, I., López, R., Santamaría, P., Gutiérrez, A.R. and González-Arenzana, L. (2018) Impact of chemical and biological fungicides applied to grapevine on grape biofilm, must, and wine microbial diversity. Frontiers in Microbiology **9**, 59. doi.org/10.3389/fmicb.2018.00059

Fleet, G.H. (1990) Growth of yeasts during wine fermentations. Journal of Wine Research, **1**, 211–223.

Fogarty, R.V and Tobin, J.M. (1996) Fungal melanins and their interactions with metals. Enzyme and Microbial Technology **19**, 311–317.

Fouquier, J., Rideout, J.R., Bolyen, E., Chase, J., Shiffer, A., McDonald, D., Knight, R., Caporaso, J.G. and Kelley, S.T. (2016) Ghost-tree: creating hybrid-gene phylogenetic trees for diversity analyses. Microbiome **4**, 11.

Franzosa, E.A., Hsu, T., Sirota-Madi, A., Shafquat, A., Abu-Ali, G., Morgan, X.C. and Huttenhower, C. (2015) Sequencing and beyond: integrating molecular 'omics' for microbial community profiling. Nature reviews. Microbiology **13**, 360-372.

Fungicide Resistance Action Committee (2020) FRAC code list 2020: fungal control agents sorted by cross resistance pattern and mode of action (including FRAC Code numbering) (Fungicide Resistance Action Committee: Basel, Switzerland). Accessible at: https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2020-final.pdf?sfvrsn=8301499a_2

Gabel, B. (2019) New concept of vine grape protection–knowledge-based approach & high tech. BIO Web of Conferences **15**, 01020. doi:10.1051/bioconf/20191501020

Gadd, G.M. (1983). The use of solid medium to study effects of cadmium, copper and zinc on yeasts and yeast-like fungi: applicability and limitations. Journal of Applied Bacteriology **54**,57–62.

1080 Gadd G.M. and de Rome L. (1988) Biosorption of copper by fungal melanin. Applied
1081 Microbiology and Biotechnology **29**, 610-617.
1082 Gadd, G.M. and Griffiths, A.J. (1980a) Effect of copper on morphology of *Aureobasidium*
1083 *pullulans*. Transactions of the British Mycological Society **74**, 387–392.
1084 Gadd, G.M. and Griffiths, A.J. (1980b). Influence of pH on toxicity and uptake of copper in
1085 *Aureobasidium pullulans*. Transactions of the British Mycological Society **75**, 91–96.
1086 Galati, A., Schifani, G., Crescimanno, M. and Migliore G. (2019) "Natural wine" consumers
1087 and interest in label information: an analysis of willingness to pay in a new Italian wine
1088 market segment. Journal of Cleaner Production **227,** 405-413.
1089 Gianessi, L. and Williams, A. (2011) Fungicides have protected european wine grapes for 150
1090 years (CropLife Foundation - Crop Protection Research Institute: Washington DC,
1091 USA). https://croplife.org/wp-content/uploads/pdf_files/Fungicides-Have-Protected-
1092 European-Wine-Grapes-for-150-Years.pdf
1093 Gil, F.N., Becker, J.D. and Viegas, C.A. (2014) Potential mechanisms underlying response to
1094 effects of the fungicide pyrimethanil from gene expression profiling in *Saccharomyces*
1095 *cerevisiae*. Journal of Agricultural and Food Chemistry **62**, 5237–5247.
1096 Gilbert, J.A., van der Lelie, D. and Zarraonaindia, I. (2014) Microbial terroir for wine grapes.
1097 Proceedings of the National Academy of Sciences of the United States of America **111**,
1098 5-6.
1099 Gobbi, A., Kyrkou, I., Filippi, E., Ellegaard-Jensen, L. and Hansen, L.H. (2020) Seasonal
1100 epiphytic microbial dynamics on grapevine leaves under biocontrol and copper
1101 fungicide treatments. Scientific Reports **10**, 681.
1102 Gobbi, M., Comitini, F., Domizio, P., Romani, C., Lencioni, L., Mannazzu, I., and Ciani, M.
1103 (2013) *Lachancea thermotolerans* and *Saccharomyces cerevisiae* in simultaneous and
1104 sequential co-fermentation: a strategy to enhance acidity and improve the overall
1105 quality of wine. Food Microbiology **33**, 271–281.
1106 Grangeteau, C., David, V., Hervé, A., Guilloux-Benatier, M. and Rousseaux, S. (2017a) The
1107 sensitivity of yeasts and yeasts-like fungi to copper and sulfur could explain lower yeast
1108 biodiversity in organic vineyards. FEMS Yeast Research **17**(8), doi:
1109 10.1093/femsyr/fox092.
1110 Grangeteau, C., Roullier-Gall, C., Rousseaux, S., Gougeon, R.D., Schmitt-Kopplin, P.,
1111 Alexandre, H. and Guilloux-Benatier, M. (2017b) Wine microbiology is driven by
1112 vineyard and winery anthropogenic factors. Microbial Biotechnology **10**, 354–370.
1113 Gupta, V.V.S.R., Bramley, R.G., Greenfield, P., Yu, J. and Herderich, M.J. (2019) Vineyard
1114 soil microbiome composition related to rotundone concentration in Australian cool
1115 climate 'peppery' Shiraz grapes. Frontiers in Microbiology **10**, 1607.

1116 Hahn, M. (2014) The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as
1117 a case study. Journal of Chemical Biology **7,** 133-141.
1118 Halwachs, B., Madhusudhan, N., Krause, R., Nilsson, R.H., Moissl-Eichinger, C., Högenauer,
1119 C., Thallinger, G.G., and Gorkiewicz, G. (2017) Critical issues in mycobiota analysis.
1120 Frontiers in Microbiology **8**, 180.
1121 Holzapfel, B.P., Rossouw, G., Dry, I., and Thomas, M. (2020) Productivity and wine
1122 assessment of new disease resistant cultivars in Australia. Acta Horticulturae **1274**, 79-
1123 88.
1124 Howell, K. (2016) Spoilage: yeast spoilage of food and beverages. Caballero, B., Finglas, P.M..
1125 and Toldrá, F., eds. Encyclopedia of food and health (Academic Press: Oxford
1126 England) pp. 113-117.
1127 Hranilovic, A., Gambetta, J.M., Schmidtke, L., Boss, P.K., Grbin, P.R., Masneuf-Pomarede, I.,
1128 Bely, M., Albertin, W. and Jiranek, V. (2018) Oenological traits of *Lachancea*
1129 *thermotolerans* show signs of domestication and allopatric differentiation. Scientific
1130 Reports, **8**(1), 14812. Jaccard, P. (1912) The distribution of the flora in the Alpine zone.
1131 New Phytologist **11**, 37-50.
1132 Jara, C., Laurie, V.F., Mas, A. and Romero J. (2016) Microbial terroir in Chilean valleys:
1133 diversity of non-conventional yeast. Frontiers in Microbiology **7**, 663.
1134 Jolly, N.P., Varela, C. and Pretorius, I.S. (2014) Not your ordinary yeast: non-*Saccharomyces*
1135 yeasts in wine production uncovered. FEMS Yeast Research, **14**, 215-237.
1136 Katragkou, A., Alexander, E.L., Eoh, H., Raheem, S.K., Roilides, E. and Walsh, T.J. (2015)
1137 Effects of fluconazole on the metabolomic profile of *Candida albicans*. Journal of
1138 Antimicrobial Chemotherapy **71**, 635–640.
1139 Killham, K., Lindley, N.D. and Wainwright, M. (1981) Inorganic sulfur oxidation by
1140 *Aureobasidium pullulans*. Applied and Environmental Microbiology **42**, 629– 631.
1141 Kioroglou, D., Kraeva-Deloire, E., Schmidtke, M.L., Mas, A. and Portillo, C.M. (2019)
1142 Geographical origin has a greater impact on grape berry fungal community than grape
1143 variety and maturation state. Microorganisms **7**, 669. Knight, S., Klaere, S., Fedrizzi,
1144 B. and Goddard, M.R. (2015) Regional microbial signatures positively correlate with
1145 differential wine phenotypes: evidence for a microbial aspect to terroir. Scientific
1146 Reports **5**(1), 14233. Koller, A., Valesco, J. and Subramani S. (2000) The *CUP1*
1147 promoter of *Saccharomyces cerevisiae* is inducible by copper in *Pichia pastoris*. Yeast
1148 **16**, 651-656.
1149 Komárek, M., Cadková, E., Chrastný, V., Bordas, F. and Bollinger, J.-C. (2009) Contamination
1150 of vineyard soils with fungicides: a review of environmental and toxicological aspects.
1151 Environment International **36**, 138-151.

Kosel, J., Raspor, P. and Čadež, N. (2019) Maximum residue limit of fungicides inhibits the viability and growth of desirable non-*Saccharomyces* wine yeasts. Australian Journal of Grape and Wine Research **25**, 43–52.

Kuanyshev, N., Adamo, G.M., Porro, D. and Branduardi P. (2017) The spoilage yeast *Zygosaccharomyces bailii*: foe or friend? Yeast **34**, 359-370.

Kumar, S., Kumar, D. and Dilbaghi N. (2017) Preparation, characterization, and bio-efficacy evaluation of controlled release carbendazim-loaded polymeric nanoparticles. Environmental Science and Pollution Research **24**, 926-937.

Lamy, D. (1992) Miles Joseph Berkeley, Camille Montagne and *Oidium tuckeri* Berk. Bulletin de la Société Botanique de France. Lettres Botaniques **139**, 203-218.

Lewis, K.A., Tzilivakis, J., Warner, D. and Green, A. (2016) An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment: An International Journal **22**, 1050-1064. Leyva, J.S., Manrique, M., Prats, L., Loureiro-Dias, M.C. and Peinado, J.M. (1999) Regulation of fermentative CO_2 production by the food spoilage yeast *Zygosaccharomyces bailii*. Enzyme and Microbial Technology **24**, 270-275.

Libis, V., Antonovsky, N., Zhang, M., Shang, Z., Montiel, D., Maniko, J., Ternei, M.A., Calle, P.Y., Lemetre, C., Owen, J.G. and Brady, S.F. (2019) Uncovering the biosynthetic potential of rare metagenomic DNA using co-occurrence network analysis of targeted sequences. Nature Communications, **10**(1), 3848.

Lin, M.M.-H., Boss, P.K., Walker, M.E., Sumby, K.M., Grbin, P.R. and Jiranek, V. (2020) Evaluation of indigenous non-*Saccharomyces* yeasts isolated from a South Australian vineyard for their potential as wine starter cultures. International Journal of Food Microbiology **312,** 108373.

Liu, Y., Laks, P. and Heiden P. (2002) Controlled release of biocides in solid wood. II. Efficacy against *Trametes versicolor* and *Gloeophyllum trabeum* wood decay fungi. Journal of Applied Polymer Science **86**, 608-614.

Liu, P.T., Lu, L., Duan, C.Q. and Yan, G.L. (2016) The contribution of indigenous non-*Saccharomyces* wine yeast to improved aromatic quality of Cabernet Sauvignon wines by spontaneous fermentation. LWT-Food Science and Technology **71**, 356-363.

Liu, D., Zhang, P., Chen, D. and Howell K. (2019b) From the vineyard to the winery: how microbial ecology drives regional distinctiveness of wine. Frontiers in Microbiology **10**, 2679.

Liu, D., Chen, Q., Zhang, P., Chen, D., and Howell, K.S. (2019a) Vineyard ecosystems are structured and distinguished by fungal communities impacting the flavour and quality of wine. BioRxiv 2019.2012.2027.881656. doi: 10.1101/2019.12.27.881656.

Loira, I., Morata, A., Comuzzo, P., Callejo, M.J., González, C., Calderón, F. and Suárez-Lepe, J.A. (2015) Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality. Food Research International **76**, 325–333.

López-García, A., Pineda-Quiroga, C., Atxaerandio, R., Pérez, A., Hernández, I., García-Rodríguez, A. and González-Recio, O. (2018). Comparison of Mothur and QIIME for the analysis of rumen microbiota composition based on 16S rRNA amplicon sequences. Frontiers in Microbiology **9**, 3010. Loureiro, V. and Malfeito-Ferreira M. (2003) Spoilage yeasts in the wine industry. International Journal of Food Microbiology **86**, 23-50.

Lozupone, C. and Knight, R. (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology **71**, 8228-8235.

Lozupone, C.A. and Knight, R. (2008) Species divergence and the measurement of microbial diversity. FEMS microbiology reviews **32**, 557-578.

Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J. and Knight, R. (2011) UniFrac: an effective distance metric for microbial community comparison. The ISME Journal **5**, 169-172.

Lucaciu, R., Pelikan, C., Gerner, S.M., Zioutis, C., Köstlbacher, S., Marx, H., Herbold, C.W., Schmidt, H. and Rattei, T. (2019) A bioinformatics guide to plant microbiome analysis. Frontiers in Plant Science **10**, 1313. doi.org/10.3389/fpls.2019.01313

Lukens, R.J. (1971) Chemistry of fungicidal action (Springer-Verlag: Berlin, Germany)

Lupwayi, N.Z., Brandt, S.A., Harker, K.N., O'Donovan, J.T., Clayton, G.W. and Turkington, T.K. (2010) Contrasting soil microbial responses to fertilizers and herbicides in a canola–barley rotation. Soil Biology and Biochemistry **42**, 1997-2004.

Macreadie, I.G., Horaitis, O., Vaughan, P.R. and Des Clark-Walker, G. (1991) Constitutive expression of the *Saccharomyces cerevisiae CUP1* gene in *Kluyveromyces lactis*. Yeast **7**, 127-135.

Madden, A.A., Epps, M.J., Fukami, T., Irwin, R.E., Sheppard, J., Sorger, D.M. and Dunn, R.R. (2018) The ecology of insect-yeast relationships and its relevance to human industry. Proceedings of the Royal Society B: Biological Sciences **285**, 20172733.

Malherbe, S., Menichelli, E., Du Toit, M., Tredoux, A., Muller, N., Næs, T. and Nieuwoudt, H. (2013) The relationships between consumer liking, sensory and chemical attributes of *Vitis vinifera* L. cv. Pinotage wines elaborated with different *Oenococcus oeni* starter cultures. Journal of the Science of Food and Agriculture **93**, 2829-2840.

Malla, M.A., Dubey, A., Yadav, S., Kumar, A., Hashem, A. and Abd_Allah, E.F. (2018) Understanding and designing the strategies for the microbe-mediated remediation of

environmental contaminants using omics approaches. Frontiers in Microbiology **9**, 1132. doi:10.3389/fmicb.2018.01132.

Mandl, K., Cantelmo, C., Gruber, E., Faber, F., Friedrich, B. and Zaller, J.G. (2018) Effects of glyphosate-, glufosinate- and flazasulfuron-based herbicides on soil microorganisms in a vineyard. Bulletin of Environmental Contamination and Toxicology **101**, 562-569.

Marcano, I.-E., Díaz-Alcántara, C.-A., Urbano, B. and González-Andrés F. (2016) Assessment of bacterial populations associated with banana tree roots and development of successful plant probiotics for banana crop. Soil Biology and Biochemistry **99**, 1-20.

Martins, G., Miot-Sertier, C., Lauga, B., Claisse, O., Lonvaud-Funel, A., Soulas, G. and Masneuf-Pomarède, I. (2012) Grape berry bacterial microbiota: impact of the ripening process and the farming system. International Journal of Food Microbiology **158**, 93-100.

Martins, G., Lauga, B., Miot-Sertier, C., Mercier, A., Lonvaud, A., Soulas, M.L., Soulas, G. and Masneuf-Pomarede, I. (2013) Characterization of epiphytic bacterial communities from grapes, leaves, bark and soil of grapevine plants grown, and their relations. PLoS ONE **8**, e73013.

Martins, G., Vallance, J., Mercier, A., Albertin, W., Stamatopoulos, P., Rey, P., Lonvaud, A. and Masneuf-Pomarède, I. (2014) Influence of the farming system on the epiphytic yeasts and yeast-like fungi colonizing grape berries during the ripening process. International Journal of Food Microbiology **177**, 21-28.

Martorell, P., Stratford, M., Steels, H., Fernández-Espinar, M.T. and Querol A. (2007) Physiological characterization of spoilage strains of *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* isolated from high sugar environments. International Journal of Food Microbiology **114**, 234-242.

Mezzasalma, V., Sandionigi, A., Bruni, I., Bruno, A., Lovicu, G., Casiraghi, M. and Labra, M. (2017) Grape microbiome as a reliable and persistent signature of field origin and environmental conditions in Cannonau wine production. PLoS ONE 12, e0184615.

Milanović, V., Comitini, F. and Ciani, M. (2013) Grape berry yeast communities: Influence of fungicide treatments. International Journal of Food Microbiology **161**, 240–246.

Misra, S., Pandey, S., Dixit, V., Mishra, S.K., Khan, M.H., Agarwal, L. and Chauhan P.S. (2017) Soil microbiome for enhanced crop productivity. Kalia, V., Shouche, Y., Purohit, H. and Rahi, P., eds. Mining of microbial wealth and metaGenomics (Springer: Singapore) pp. 227-247.

Morris, E.K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T.S., Meiners, T., Müller, C., Obermaier, E., Prati, D., Socher, S.A., Sonnemann, I., Wäschke, N., Wubet, T., Wurst, S. and Rillig, M.C. (2014) Choosing and using diversity indices: insights for

ecological applications from the German Biodiversity Exploratories. Ecology and Evolution **4**, 3514-3524.

Morrison-Whittle, P. and Goddard, M.R. (2018) From vineyard to winery: a source map of microbial diversity driving wine fermentation. Environmental Microbiology **20**, 75-84.

Morrison-Whittle, P., Lee, S.A. and Goddard, M.R. (2017) Fungal communities are differentially affected by conventional and biodynamic agricultural management approaches in vineyard ecosystems. Agriculture, Ecosystems and Environment **246**, 306–313.

Morton, J.T., Sanders, J., Quinn, R.A., McDonald, D., Gonzalez, A., Vázquez-Baeza, Y., Navas-Molina, J.A., Song, S.J., Metcalf, J.L., Hyde, E.R., Lladser, M., Dorrestein, P.C. and Knight, R. (2017) Balance trees reveal microbial niche differentiation. MSystems **2**(1), e00162-16.

Morton, V. and Staub, T. (2008) A short history of fungicides. Online, APSnet Features **10,** 2008-0308.

Nally, M.C., Pesce, V.M., Maturano, Y.P., Muñoz, C.J., Combina, M., Toro, M.E., De Figueroa, L.C. and Vazquez F. (2012) Biocontrol of *Botrytis cinerea* in table grapes by non-pathogenic indigenous *Saccharomyces cerevisiae* yeasts isolated from viticultural environments in Argentina. Postharvest Biology and Technology **64**, 40-48.

Nerva, L., Pagliarani, C., Pugliese, M., Monchiero, M., Gonthier, S., Gullino, M.L., Gambino, G. and Chitarra, W. (2019) Grapevine phyllosphere community analysis in response to elicitor application against powdery mildew. Microorganisms 7(12), 662. doi: 10.3390/microorganisms7120662

Noguerol-Pato, R., Torrado-Agrasar, A., González-Barreiro, C., Cancho-Grande, B. and Simal-Gándara, J. (2014) Influence of new generation fungicides on *Saccharomyces cerevisiae* growth, grape must fermentation and aroma biosynthesis. Food Chemistry **146**, 234–241.

Ohsumi, Y., Kitamoto, K., and Anraku, Y. (1988) Changes induced in the permeability barrier of the yeast plasma membrane by cupric ion. Journal of Bacteriology, **170**, 2676–2682.

Oliva, J., Girón, F., Cayuela, J.M., Mulero, J., Zafrilla, P. and Cámara, M.Á. (2020) Effect of fungicides on the yeast population during spontaneous fermentation in the vinification of Monastrell grapes. LWT - Food Science and Technology **131**, 109816.

Padilla, B., Gil, J.V. and Manzanares, P. (2016b) Past and future of non-*Saccharomyces* yeasts: from spoilage microorganisms to biotechnological tools for improving wine aroma complexity. Frontiers in Microbiology 7, 411. doi:10.3389/fmicb.2016.00411

Padilla, B., García-Fernández, D., González, B., Izidoro, I., Esteve-Zarzoso, B., Beltran, G., and Mas, A. (2016a) Yeast biodiversity from DOQ Priorat uninoculated fermentations. Frontiers in Microbiology 7, 930. doi: 10.3389/fmicb.2016.00930.

Pagliarini, E., Laureati, M. and Gaeta D. (2013) Sensory descriptors, hedonic perception and consumer's attitudes to Sangiovese red wine deriving from organically and conventionally grown grapes. Frontiers in Psychology **4**, 896.

Parnell, J.J., Berka, R., Young, H.A., Sturino, J.M., Kang, Y., Barnhart, D.M. and DiLeo M.V. (2016) From the lab to the farm: an industrial perspective of plant beneficial microorganisms. Frontiers in Plant Science 7, 1110.

Pascual-García, A., Bonhoeffer, S. and Bell, T. (2020) Metabolically cohesive microbial consortia and ecosystem functioning. Philosophical Transactions of the Royal Society B: Biological Sciences **375**, 20190245. doi.org/10.1098/rstb.2019.0245

Pauvert, C., Fort, T., Calonnec, A., Faivre d'Arcier, J., Chancerel, E., Massot, M., Chiquet, J., Robin, S., Bohan, D.A., Vallance, J. and Vacher, C. (2020) Microbial association networks give relevant insights into plant pathobiomes. BioRxiv 2020.02.21.958033. doi:10.1101/2020.02.21.958033

Perazzolli, M., Antonielli, L., Storari, M., Puopolo, G., Pancher, M., Giovannini, O., Pindo, M. and Pertot, I. (2014) Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. Applied and Environmental Microbiology **80**, 3585–3596.

Pimentel, D. and Burgess, M. (2012) Small amounts of pesticides reaching target insects. Environment, Development and Sustainability **14**, 1-2.

Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C.C. and Gomes, A. (2014) Unravelling the diversity of grapevine microbiome. PLOS ONE **9**(1), e85622. Pinto, C., Custódio, V., Nunes, M., Songy, A., Rabenoelina, F., Courteaux, B., Clément, C., Gomes, A.C. and Fontaine, F. (2018) Understand the potential role of *Aureobasidium pullulans*, a resident microorganism from grapevine, to prevent the infection caused by *Diplodia seriata*. Frontiers in Microbiology **9**, 3047.

Pylak M., Oszust K. and Frąc M. (2019) Review report on the role of bioproducts, biopreparations, biostimulants and microbial inoculants in organic production of fruit. Reviews in Environmental Science and Bio/Technology **18**, 597-616.

Quinn, T. P. (2018) Visualizing balances of compositional data: a new alternative to balance dendrograms. F1000Research **7**, 1278.

Rantsiou, K., Giacosa, S., Pugliese, M., Englezos, V., Ferrocino, I., Río Segade, S., Monchiero, M., Gribaudo, I., Gambino, G., Gullino, M.L. and Rolle, L. (2020) Impact of chemical and alternative fungicides applied to grapevine cv Nebbiolo on microbial ecology and chemical-physical grape characteristics at harvest. Frontiers in Plant Science **11**, 700.

Rapp, A. and Versini G. (1995) Influence of nitrogen compounds in grapes on aroma compounds of wines. Charalambous G., ed. Developments in food science, Food

Flavors: Generation, Analysis and Process Influence. Proceedings of the 8th International Flavor Conference6-8 July 1994, Cos, Greece (Elsevier, Amsterdam, The Netherlands) **37**, pp. 1659-1694. doi:10.1016/S0167-4501(06)80257-8.

Raspor, P., Miklič Milek, D., Avbelj, M. and Čadež, N. (2010). Biocontrol of grey mould disease on grape caused by *Botrytis cinerea* with autochthonous wine yeasts. Food Technology and Biotechnology **48**, 336–343.

Raspor, P., Milek, D.M., Polanc, J., Smole Možina, S. and Čadež, N. (2006) Yeasts isolated from three varieties of grapes cultivated in different locations of the Dolenjska vine-growing region, Slovenia. International Journal of Food Microbiology **109**, 97-102.

Retallack, M. (2011) Vineyard biodiversity and insect interactions - establishing and monitoring insectariums, (Retallack Viticulture: Crafers, SA). https://www.wineaustralia.com/getmedia/fba82be2-ae5d-4b18-a59e-1129854199ac/Rmjr0811VineyardBiodiversityandInsectInteractionsBookletFINAL

Rojas, V., Gil, J.V., Piñaga, F. and Manzanares, P. (2003) Acetate ester formation in wine by mixed cultures in laboratory fermentations. International Journal of Food Microbiology **86**, 181-188.

Rollero, S., Zietsman, A.J.J., Buffetto, F., Schückel, J., Ortiz-Julien, A. and Divol, B. (2018) *Kluyveromyces marxianus* secretes a pectinase in Shiraz grape must that impacts technological properties and aroma profile of wine. Journal of Agricultural and Food Chemistry **66(**, 11739–11747.

Romano, P., Fiore, C., Paraggio, M., Caruso, M. and Capece A. (2003) Function of yeast species and strains in wine flavour. International Journal of Food Microbiology **86**, 169-180.

Rosini, G., Federici, F. and Martini, A. (1982) Yeast flora of grape berries during ripening. Microbial Ecology **8**, 83-89.

Roudié, P. (1997) Bordeaux, un modèle pour la viti-viniculture mondiale? Les Cahiers d'Outre-Mer **50**, 403-422.

Ruiz, J., Belda, I., Beisert, B., Navascués, E., Marquina, D., Calderón, F., Rauhut, D., Santos, A. and Benito, S. (2018) Analytical impact of *Metschnikowia pulcherrima* in the volatile profile of Verdejo white wines. Applied Microbiology and Biotechnology, **102**, 8501–8509.

Scariot, F.J., Jahn, L.M., Delamare, A.P.L. and Echeverrigaray, S. (2016) The effect of the fungicide captan on *Saccharomyces cerevisiae* and wine fermentation. BIO Web of Conferences 7, 02027. doi:10.1051/bioconf/20160702027

1368 Scariot, F.J., Jahn, L., Delamare, A.P.L. and Echeverrigaray, S. (2017) Necrotic and apoptotic
1369 cell death induced by captan on *Saccharomyces cerevisiae*. World Journal of
1370 Microbiology and Biotechnology **33(8)**, 159.
1371 Scariot, F.J., Jahn, L.M., Maianti, J.P., Delamare, A.P.L. and Echeverrigaray, S. (2016) The
1372 fungicide mancozeb induces metacaspase-dependent apoptotic cell death in
1373 *Saccharomyces cerevisiae* BY4741. Apoptosis **21**, 866–872.
1374 Schena, L., Nigro, F., Pentimone, I., Ligorio, A. and Ippolito, A. (2003) Control of postharvest
1375 rots of sweet cherries and table grapes with endophytic isolates of *Aureobasidium*
1376 *pullulans*. Postharvest Biology and Technology **30**, 209-220.
1377 Schena, L., Ippolito, A., Zahavi, T., Cohen, L., Nigro, F. and Droby, S. (1999) Genetic diversity
1378 and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots.
1379 Postharvest Biology and Technology **17**, 189-199.
1380 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,
1381 Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B.,
1382 Thallinger, G.G., Van Horn, D.J. and Weber, C. F. (2009). Introducing mothur: open-
1383 source, platform-independent, community-supported software for describing and
1384 comparing microbial communities. Applied and Environmental Microbiology **75**,
1385 7537-7541.
1386 Schmid, F., Moser, G., Müller, H. and Berg, G. (2011) Functional and structural microbial
1387 diversity in organic and conventional viticulture: organic farming benefits natural
1388 biocontrol agents. Applied and Environmental Microbiology 77, 2188-2191.
1389 Schütz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P. and Mathimaran N.
1390 (2018) Improving crop yield and nutrient use efficiency via biofertilization—a global
1391 meta-analysis. Frontiers in Plant Science **8**, 2204.
1392 Setati, M.E., Jacobson, D., Andong, U.-C. and Bauer, F.F. (2012) The vineyard yeast
1393 microbiome, a mixed model microbial map. PLoS ONE **7**, e52609.
1394 Shannon, C.E. (1948) A mathematical theory of communication. Bell System Technical Journal
1395 **27**, 379-423.
1396 Sharpe, C., Passmore, G., Trend, B., James, A., McCarthy, A., Fennessy, R., Fisher, D.,
1397 Learmonth, S., Collie, H., Mathews, A. and Gordon, C. (2017) 2017/18 Viticulture
1398 spray guide - wine grapes and table grapes (Department of Primary Industries and
1399 Regional Development - Government of Western Australia: Perth, WA, Australia)
1400 https://www.agric.wa.gov.au/sites/gateway/files/Viticulture%20Spray%20Guide.pdf
1401 Sholberg, P., Harlton, C., Boulé, J. and Haag, P. (2006) Fungicide and clay treatments for
1402 control of powdery mildew influence wine grape microflora. HortScience **41**, 176–182.
1403 Sieiro-Sampedro, T., Briz-Cid, N., Pose-Juan, E., Figueiredo-González, M., González-
1404 Barreiro, C., Simal-Gándara, J., Cancho-Grande, B. and Rial-Otero, R. (2020).

Tetraconazole alters the methionine and ergosterol biosynthesis pathways in *Saccharomyces* yeasts promoting changes on volatile derived compounds. Food Research International **130**, 108930.

Simpson, E.H. (1949) Measurement of diversity. Nature **163**, 688.

Sipiczki, M. (2006) *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. Applied and Environmental Microbiology **72**, 6716-6724.

Sirén, K., Mak, S.S.T., Fischer, U., Hansen, L.H., and Gilbert, M.T.P. (2019) Multi-omics and potential applications in wine production. Current Opinion in Biotechnology **56**, 172-178.

Soheilifard, F., Marzban, A., Ghaseminejad Raini, M., Taki, M. and van Zelm, R., (2020) Chemical footprint of pesticides used in citrus orchards based on canopy deposition and off-target losses. Science of The Total Environment **732**, 139118.

Steensels, J., Daenen, L., Malcorps, P., Derdelinckx, G., Verachtert, H. and Verstrepen K.J. (2015) *Brettanomyces* yeasts - From spoilage organisms to valuable contributors to industrial fermentations. International Journal of Food Microbiology **206**, 24-38.

Sumby, K.M., Bartle, L., Grbin, P.R. and Jiranek, V. (2019) Measures to improve wine malolactic fermentation. Applied Microbiology and Biotechnology **103**, 2033-2051.

Sumby, K.M., Grbin, P.R., and Jiranek, V. (2010) Microbial modulation of aromatic esters in wine: Current knowledge and future prospects. Food Chemistry **121**, 1-16.

The Australian Wine Research Institute (2020a) Wine flavours, faults and taints. Accessible at: https://www.awri.com.au/industry_support/winemaking_resources/sensory_assessment/recognition-of-wine-faults-and-taints/wine_faults/

The Australian Wine Research Institute (2020b) Agrochemicals registered for use in Australian viticulture. An essential reference when growing grapes for export wine 20/21. Accessible at: https://www.awri.com.au/wp-content/uploads/agrochemical_booklet.pdf

Thomas, D. and Davenport R. (1985) *Zygosaccharomyces bailii*—a profile of characteristics and spoilage activities. Food Microbiology **2**, 157-169.

Trzaskos, J.M. and Henry, M.J. (1989) Comparative effects of the azole-based fungicide flusilazole on yeast and mammalian lanosterol 14 alpha-methyl demethylase. Antimicrobial Agents and Chemotherapy **33**, 1228-1231.

Varela, C. and Borneman, A.R. (2017). Yeasts found in vineyards and wineries. Yeast **34**, 111-128.

Varela, C., Sengler, F., Salomon, M. and Curtin, C. (2016) Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chemistry **209**, 57-64.

1442 Varela, C., Barker, A., Tran, T., Borneman, A. and Curtin, C. (2017) Sensory profile and
1443 volatile aroma composition of reduced alcohol Merlot wines fermented with
1444 *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. International Journal of Food
1445 Microbiology **252**, 1–9.
1446 Vecchio, R. (2013) Determinants of willingness-to-pay for sustainable wine: evidence from
1447 experimental auctions. Wine Economics and Policy **2**, 85-92.
1448 Villa, T.G. and Longo E. (1996) Microbiology of grape surfaces and its importance for wine
1449 quality. Morris, C.E., Nicot, P.C. and Nguyen-The, C., eds. Aerial plant surface
1450 microbiology (Springer US: Boston, MA, USA) pp. 209-220. Vincent, C. and Lasnier
1451 J. (2020) Sustainable arthropod management in Quebec vineyards. Agriculture **10**, 91.
1452 Vorholt, J.A., Vogel, C., Carlström, C.I. and Müller, D.B. (2017) Establishing causality:
1453 opportunities of synthetic communities for plant microbiome research. Cell Host and
1454 Microbe **22**, 142–155.
1455 Vukicevich, E., Lowery, T., Bowen, P., Úrbez-Torres, J.R. and Hart M. (2016) Cover crops to
1456 increase soil microbial diversity and mitigate decline in perennial agriculture. A
1457 review. Agronomy for Sustainable Development **36**, 48.
1458 Wang, C., García-Fernández, D., Mas, A. and Esteve-Zarzoso, B. (2015) Fungal diversity in
1459 grape must and wine fermentation assessed by massive sequencing, quantitative PCR
1460 and DGGE. Frontiers in Microbiology **6**, 1156.
1461 Wang, C., Mas, A. and Esteve-Zarzoso, B. (2016) The Interaction between *Saccharomyces*
1462 *cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and
1463 strain specific. Frontiers in Microbiology **7**, 502.
1464 Whitener, M.E.B., Carlin, S., Jacobson, D., Weighill, D., Divol, B., Conterno, L., Du Toit, M.
1465 and Vrhovsek, U. (2015) Early fermentation volatile metabolite profile of non-
1466 *Saccharomyces* yeasts in red and white grape must: a targeted approach. LWT - Food
1467 Science and Technology **64**, 412-422
1468 Whitener, M.B., Stanstrup, J., Carlin, S., Divol, B., Du Toit, M. and Vrhovsek, U. (2017) Effect
1469 of non-*Saccharomyces* yeasts on the volatile chemical profile of Shiraz
1470 wine. Australian Journal of Grape and Wine Research **23**, 179-192.
1471 Whittaker, R.H. (1972) Evolution and measurement of species diversity. Taxon **21**, 213-251.
1472 Wei, Y., Wu, Y., Yan, Y., Zou, W., Xue, J., Ma, W., Wang, W., Tian, G. and Wang, L. (2018)
1473 High-throughput sequencing of microbial community diversity in soil, grapes, leaves,
1474 grape juice and wine of grapevine from China. PLOS ONE **13**(3), e0193097. Williams,
1475 J. S. and Cooper, R. M. (2004) The oldest fungicide and newest phytoalexin – a
1476 reappraisal of the fungitoxicity of elemental sulphur. Plant Pathology, **53**, 263–279.

Willis, A.D. (2019) Rarefaction, alpha diversity, and statistics. Frontiers in Microbiology **10**, 2407. Worrall E.A., Hamid A., Mody K.T., Mitter N. and Pappu HR (2018) Nanotechnology for plant disease management. Agronomy **8**, 285.

Yang, I., Corwin, E.J., Brennan, P.A., Jordan, S., Murphy, J.R. and Dunlop, A. (2016) The infant microbiome: implications for infant health and neurocognitive development. Nursing Research **65**, 76-88.

Youseif, S.H. (2018) Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. Annals of Agricultural Sciences **63**, 25-35.

Zarraonaindia, I., Owens, S.M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S. Bokulich, N.A., Mills, D.A., Martin, G., Taghavi, S., Van Der Lelie, D., Gilbert, J.A. and Jansson J.K. (2015) The soil microbiome influences grapevine-associated microbiota. mBio **6**, e02527-02514.

Zapka, C., Leff, J., Henley, J., Tittl, J., De Nardo, E., Butler, M., Griggs, R., Fierer, N., Edmonds-Wilson, S. (2017) Comparison of standard culture-based method to culture-independent method for evaluation of hygiene effects on the hand microbiome. mBio **8**, e00093-00017.

Zhang, S., Chen, X., Zhong, Q., Zhuang, X. and Bai, Z. (2019). microbial community analyses associated with nine varieties of wine grape carposphere based on high-throughput sequencing. Microorganisms 7(12):668

Zhang, W., Li, F. and Nie, L. (2010) Integrating multiple 'omics' analysis for microbial biology: application and methodologies. Microbiology **156**, 287-301.

Zhao, P., Cao, L., Ma, D., Zhou, Z., Huang, Q. and Pan, C. (2017) Synthesis of pyrimethanil-loaded mesoporous silica nanoparticles and its distribution and dissipation in cucumber plants. Molecules **22**, 817. doi:10.3390/molecules22050817.

Zubrod, J.P., Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J.J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R. and Schäfer, R.B. (2019) Fungicides: an overlooked pesticide class? Environmental Science & Technology **53**, 3347-3365.

Zuehlke, J., Petrova, B. and Edwards, C. (2013) Advances in the control of wine spoilage by *Zygosaccharomyces* and *Dekkera/Brettanomyces*. Annual Review of Food Science and Technology **4**, 57-78.

1509 **Table 1.** Reported trend of yeast diversity with regard to spray treatment applied in the
1510 vineyard.

Reference	Organic spray		Conventional spray	
	Diversity trend†	Diversity index‡	Diversity trend	Diversity index
Cordero-Bueso et al. (2011)	↑	✓¶, ††	↓	✓¶, ††
Setati et al. (2012)	↑	✓§	↓	✓§
Milanovic et al. (2013)	↓	×	↑	×
Cordero-Bueso et al. (2014)	↑	✓¶, ††	↓	✓¶, ††
Martins et al. (2014)	↑	✓§	↓	✓§
Grangeteau et al. (2017)	↓	✓§	↑	✓¶
Agarbati et al. (2019a, b)	↑	×	↓	×
Cachón et al. (2019)	↑	✓¶, ††	↓	✓¶, ††

1511 †↑ diversity increased, ↓diversity decreased; ‡ Diversity index reports whether diversity
1512 indexes were calculated (✓) or not (×);§Shannon diversity index;¶Simpsons diversity index,
1513 ††Shannon-Wiener index.
1514

Table 2. Fungal diversity as a function of grape cultivar and fungicide treatment. Relative abundances are reported, in decreasing order of abundance, when available.

Fungicide(s) (number of applications)	FRAC Code (Fungicide group)	Grape cultivar tested	Detection method	Diversity method	Most abundant fungal species reported (100–10%)	Low abundance species reported (<10%)	Reference
No treatment							
N/A	N/A	Verdicchio	Culture dependent	Relative abundance (count/total)	*H. uvarum*¶, *St. bacillaris, M. pulcherrima,*	*A. pullulans*, *C. californica, P. fermentans*	Agarbati et al. (2019a)
N/A	N/A	Montepulciano	Culture dependent	Relative abundance (count/total)	*H. uvarum*	*A. pullulans*, *I. terricola*, *St. bacillaris, Z. meyerae*	Agarbati et al. (2019a), Agarbati et al (2019b)
N/A	N/A	Montepulciano	Culture independent (NGS)	Relative abundance (count/total)	*A. pullulans*, *H. uvarum*	*St. bacillaris, Z. meyerae*, *Rh. nothofagi*, *M. pulcherrima* (filamentous fungi reported); *Bot. caroliniana*, *Alternaria* sp., *Cl. ramotenellum*, *Cl. Delicatulum*	Agarbati et al. (2019b)
N/A	N/A	Tempranillo	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*, *H. osmophila*, *L. thermotolerans*, *R. babjevae*, *R. nothofagi*, *S. cerevisiae* (Relative abundance not reported)	N/A	Escribano-Viana et al. (2018)
N/A	N/A	Tempranillo	PCR-DGGE	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*	N/A	Escribano-Viana et al. (2018)
N/A	N/A	Passerina, Malvasia, Montepulciano	Culture dependent	Cell count only	*H. uvarum*, *M. pulcherrima*, *Cry. macerans*	*A. pullulans, T. delbrueckii, C. krusei*	Comitini et al. (2008)

		and Sangiovese (individual variety differences not reported)					
N/A	N/A	Tempranillo	Culture dependent	Shannon-Wiener index a Simpson's diversity index	*R. mucilaginosa*, *L. thermotolerans*	*S. cerevisiae*, *M. pulcherrima*, *T. delbrueckii*	Cordero-Bueso et al. (2014)

Fungicide(s) (number of applications)	**FRAC Code (Fungicide Group)**	**Grape variety tested**	**Detection method**	**Diversity method**	**Most abundant fungal species reported (100-10%)**	**Low abundance species reported (<10%)**	**Reference**
Bio-fungicide†							
2 (21 days and 3 days before harvest)	P6, BM2	Tempranillo[1]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*, *H. osmophila*, *Hyp. pseudoburtonii*, *R. babjevae*, *R. glutini*, *R. nothofagi*, *S. cerevisiae*	N/A	Escribano-Viana et al. (2018)
2 (21 days and 3 days before harvest)	P6, BM2	Tempranillo[1]	PCR-DGGE	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*	N/A	Escribano-Viana et al. (2018)

Fungicide(s) (number of applications)	**FRAC Code (Fungicide Group)**	**Grape variety tested**	**Detection method**	**Diversity method**	**Most abundant fungal species reported (100-10%)**	**Low abundance species reported (<10%)**	**Reference**
Organic fungicides‡							
15	M (multi-site inhibitors)	Verdicchio[1,2]	Culture dependant	Relative abundance (count/total)	*A. pullulans*, *Cryptococcus* spp., *H. uvarum*, *St. bacillaris*,	*M. pulcherrima, D. hansenii, P. membranifaciens, I. terricola*	Agarbati et al. (2019a)

15	M (multi-site inhibitors)	Montepulciano[1,2]	Culture dependant	Relative abundance (count/total)	*A. pullulans*, *St. bacillaris*, *H. uvarum*, *I. terricola*	*Cryptococcus* spp., *C. californica, P. membranifaciens, Z. meyerae, Rhodotorula* spp.	Agarbati et al. (2019a), Agarbati et al (2019b)
15	M (multi-site inhibitors)	Montepulciano[1,2]	Culture independent (NGS)	Relative abundance (count/total)	*A. pullulans*, *H. uvarum*, *St. bacillaris*	*Z. meyerae*, *P. terricola* *filamentous fungi reported; *Bot. caroliniana*, *Alternaria* sp., *Cl. ramotenellum*, Cl. *delicatulum*	Agarbati et al. (2019b)
10	M (multi-site inhibitors)	Chardonnay[1,2,3] (2012 vintage)	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Botryotinia* sp., *Cladosporium* sp.	*Alternaria* sp., *Metschnikowia* sp., *Cryptococcus* sp., *Candida* sp., *Hanseniaspora* sp.,	Grangeteau et al. (2017b)
13	M (multi-site inhibitors)	Chardonnay[1,2,3] (2013 vintage)	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Hanseniaspora* sp., *Cladosporium* sp.	*Cryptococcus* sp., *Saccharomyces* sp., *Alternaria* sp., *Erysiphe* sp.	Grangeteau et al. (2017b)
10	M (multi-site inhibitors)	Chardonnay[1,2,3] (2014 vintage)	Culture independent (454[#])	Shannon biodiversity index	*Hanseniaspora* sp.,	*Saccharomyces* sp., *Meyerozyma* sp.	Grangeteau et al. (2017b)
9	M (multi-site inhibitors)	Verdicchio[1]	Culture dependent	ANOVA and Duncan test (diversity index not reported)	*C. zemplinina*, *H. uvarum*, *Cry. carnescens*	*Cry. wieringae*, *R. glutinis*, *R. nothofagi*, *Cry. magnus*, *Cry. flavescens*, *M. pulcherrima*, *P. fermentans*, *R. babjevae*	Milanović et al. (2013)
Not reported	M (multi-site inhibitors)	Treixadura[1,2,3] (location 1)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Cry. carnescens*	*Cry. victoriae*, *Sp. ruberrimus*	Cachón et al. (2019)
Not reported	M (multi-site inhibitors)	Mencía[1,2,3] (location 1)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* spp., *Cry. carnescens*	*Metschnikowia* sp., *Cry. stepposus*, *L. thermotolerans*, *R. graminis*, *S. cerevisiae*, *H. uvarum*	Cachón et al. (2019)
Not reported	M (multi-site inhibitors)	Brancellao[1,2,3] (location 2)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Cry. terrestris*, *Aureobasidium* sp., *Metschnikowia* sp.	None reported	Cachón et al. (2019)

Not reported	M (multi-site inhibitors)	Treixadura[1,2,3] (location 2)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Cry. victoriae*	*Cry. carnescens*, *Cry. af. victoriae*, *R. graminis*	Cachón et al. (2019)
Not reported	M (multi-site inhibitors)	Mencía[1,2,3] (location 3)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*H. uvarum*, *Metschnikowia* sp., *P. kluyveri*	*Aureobasidium* sp., *C. apicola*, *Cry. af. victoriae*, *Cyst. macerans*, *Zygos. Bisporus*	Cachón et al. (2019)
Not reported	M (multi-site inhibitors)	Albariño[1,2,3] (location 4)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *H. uvarum*, *Cry. terrestris*	*D. hansenii*, *Metschnikowia* sp., *R. nothofagi*	Cachón et al. (2019)
Not reported	M (multi-site inhibitors)	Treixadura[1,2,3] (location 4)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*D. hansenii*, *H. uvarum*	*Cry. stepposus*, *Metschnikowia* sp., Aureobasidium sp., *Cry. af. victoriae*, *Cry. carnescens*, *I. terricola*, *C. oleophila*, *P. kluyveri*	Cachón et al. (2019)
6	M (multi-site inhibitors)	Passerina, Malvasia, Montepulciano and Sangiovese[2,4] *individual variety differences were not reported	Culture dependent	Cell count only	*H. uvarum, M. pulcherrima, Cry. macerans*	*A. pullulans, Tri. pullulans*	Comitini et al. (2008)
4	M (multi-site inhibitors)	Temperanillo[2]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*R. mucilaginosa*, *S. cerevisiae*, *L. thermotolerans*	*W. anomalus, H. guilliermondii, M. pulcherrima, C. sorbose, T. delbrueckii*	Cordero-Bueso et al. (2014)
2	M (multi-site inhibitors)	Temperanillo[2]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*R. mucilaginosa*, *L. thermotolerans*, *S. cerevisiae*	*T. delbrueckii*, *M. pulcherrima*, *H. guilliermondii*, *W. anomalus*	Cordero-Bueso et al. (2014)
Not reported	M	Merlot[2,5,6]	Culture dependent	Shannon-diversity index	*Aureobasidium* sp.	*Phoma sp., Cryptococcus* sp.	Martins et al. (2014)

	(multi-site inhibitors)						
7	M (multi-site inhibitors)	Cabernet sauvignon[1,2,6,7]	Culture dependent	Relative abundance (count/total) & Shannon diversity index	*A. pullulans, Cryptococcus* spp.	*Rhodosporidium diobovatum*, *Kazachstania* sp., *Rhodotorula slooffiae*, *Sporobolomyces roseus*, *Sporisorium* sp., *Ustilago* sp., *Pichia caribbica*, *Candida parapsilosis*, *Meira geulakonigii*, *Exophiala* sp.	Setati et al. 2012
Not reported	M (multi-site inhibitors)	Shiraz[2]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*K. thermotolerans* (now *L. thermotolerans*), *S. cerevisiae, C. stellata, M. pulcherrima, H. guilliermondii*	None reported	Cordero-Bueso et al. (2011)
Not reported	M (multi-site inhibitors)	Grenache[2]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*H. guilliermondii*, *K. thermotolerans*, *P. anomala*, *S. cerevisiae*, *C. stellata*	None reported	Cordero-Bueso et al. (2011)
Not reported	M (multi-site inhibitors)	Barbera[2]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*S. cerevisiae*, *H. guilliermondii*, *K. thermotolerans*, *C. stellata*	*T. delbrueckii*	Cordero-Bueso et al. (2011)

Fungicide(s) (number of applications)	**FRAC Code (Fungicide Group)**	**Grape variety tested**	**Detection method**	**Diversity method**	**Most abundant fungal species reported (100-10%)**	**Low abundance species reported (<10%)**	**Reference**
Synthetic fungicides[§]							
12 (Also used organic fungicides[1,2])	M1, M2, 40, 46, P7, other	Verdicchio[1,2,3,4,5]	Culture dependant	Relative abundance (count/total)	*A. pullulans*, *H. uvarum*, *St. bacillaris*	*Cryptococcus* sp., *C. californica*	Agarbati et al. (2019a)

	(unknown FRAC)						
9 (Also used organic fungicides[1,2])	M1, M2, 3, 4, 5, 13, P7	Montepulciano[1,6,7,8,10]	Culture dependant	Relative abundance (count/total)	*A. pullulans*, *H. uvarum*	*L. thermotolerans, D. hansenii, C. californica, Rhodotorula* spp., *Cryptococcus* spp.	Agarbati et al. (2019a), Agarbati et al (2019b)
1 (21 days before harvest)	17	Tempranillo[9]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*, *H. osmophila*, *P. sporocuriosa*, *Tri, cantarellii*, *S. cerevisiae*	N/A	Escribano-Viana et al. (2018)
1 (21 days before harvest)	17	Tempranillo[9]	PCR-DGGE	Shannon-Wiener index and Simpson's diversity index	*A. pullulans*, *Bot. cinerea*	N/A	Escribano-Viana et al. (2018)
9 (Also used organic fungicides[1,2])	M1, M2, 3, 4, 5, 13, P7	Montepulciano[1,6,7,8,10]	Culture independent (NGS)	Relative abundance (count/total)	*A. pullulans*, *H. uvarum*,	*L. thermotolerans*, *St. bacillaris*, *P. terricola*, *filamentous fungi reported; *Bot. caroliniana*, *Alternaria* sp., *Cl. ramotenellum*, *Cl. delicatulum*	Agarbati et al. (2019b)
9	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2012 vintage)[21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Botryotinia* sp., *Cladosporium* sp., *Aureobasidium* sp., *Erysiphe* sp. Unclassified genus	*Alternaria* sp., *Phoma* sp., *Metschnikowia* sp., *Cryptococcus* sp., *Hanseniaspora* sp., *Candida* sp., *Sporidiobolus* sp., *Saccharomyces* sp.	Grangeteau et al. (2017b)
9	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2013 vintage) [21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Hanseniaspora* sp., *Saccharomyces* sp., *Alternaria* sp.	*Mucor* sp., *Monilinia* sp., *Cryptococcus* sp., *Metschnikowia* sp., *Sporidiobolus* sp., *Erysiphe* sp.	Grangeteau et al. (2017b)
8	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2014 vintage) [21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Cryptococcus* sp., *Hanseniaspora* sp., *Sporidiobolus* sp.,	*Metschnikowia* sp., *Saccharomyces* sp.,	Grangeteau et al. (2017b)

7 (managed with a dose reduction and/or with a reduced number of treatments compared to conventional treatment - above)	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2012 vintage)[21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Botryotinia* sp., *Cladosporium* sp.,	*Erysiphe* sp., *Alternaria* sp., *Phoma* sp., *Metschnikowia* sp., *Cryptococcus* sp., *Candida* sp., *Hanseniaspora* sp., *Saccharomyces* sp., *Sporidiobolus* sp.	Grangeteau et al. (2017b)
8 (managed with a dose reduction and/or with a reduced number of treatments compared to conventional treatment - above)	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2013 vintage)[21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Aureobasidium* sp., *Cryptococcus* sp., *Saccharomyces* sp., Monilinia sp.,	*Hanseniaspora* sp., *Alternaria* sp., *Metschnikowia* sp., *Erysiphe* sp., *Sporidiobolus* sp.	Grangeteau et al. (2017b)
5 (managed with a dose reduction and/or with a reduced number of treatments compared to conventional treatment - above)	M1, M2, M3, M4, M9, 3, 5, 7, 11, 21, 27, 40, 43, 45, 50, P3	Chardonnay (2014 vintage)[21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36]	Culture independent (454)	Shannon biodiversity index	*Hanseniaspora* sp.,	*Cryptococcus* sp., *Saccharomyces* sp., *Aureobasidium* sp., *Meyerozyma* sp., *Itersonilia* sp.	Grangeteau et al. (2017b)
1-8 (Also used organic fungicides[1,2])	M1, M2, 3, 4, 5, 11, 13, 17, 40	Verdicchio[1,6,7,9,11, 12,13,14,15]	Culture dependant	ANOVA and Duncan test (diversity index not reported)	*H. uvarum*, *C. zemplinina*, *M. pulcherrima*	*R. nothofagi*, *R. glutinis*, *A. pullulans*, *R. babjevae*, *P. fermentans*, *Cryptococcus* sp., *Candida* sp.	Milanović et al. (2013)
Not reported	Not reported	Treixadura [spray names not reported] (location 1)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Cyst. macerans*, *Cry. terrestris*	None reported	Cachón et al. (2019)

Not reported	Not reported	Mencía spray names not reported (location 1)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Metschnikowia* sp.	*Cry. stepposus*, *S. cerevisiae*	Cachón et al. (2019)
Not reported	Not reported	Brancellao spray names not reported (location 2)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *D. hansenii*	None reported	Cachón et al. (2019)
Not reported	Not reported	Treixadura spray names not reported (location 2)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Cry. laurentii*	*Metschnikowia* sp.	Cachón et al. (2019)
Not reported	Not reported	Mencía spray names not reported (location 3)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Metschnikowia* sp., *Cry. terrestris*, *H. uvarum*,	*Z. hellenicus/meyerae*	Cachón et al. (2019)
Not reported	Not reported	Albariño spray names not reported (location 4)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*Aureobasidium* sp., *Cry. stepposus*, *Metschnikowia* sp.	*R. graminis*	Cachón et al. (2019)
Not reported	Not reported	Treixadura spray names not reported (location 4)	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*H. uvarum*, *Aureobasidium* sp.	*Metschnikowia* sp., *R, graminis*, *S. ruberrimus*	Cachón et al. (2019)
2 (Also used organic fungicides 2x applications[1,2])	9 and 12	Verdicchio[16]	Culture dependent	Cell count only	*A. pullulans, Cry. albidus, Cry. humicolus*	*Tri. pullulans, H. uvarum, R. aurantiaca*	Comitini et al. (2008)
4	3	Temperanillo[12]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*R. mucilaginosa*, *L. thermotolerans*	*M. pulcherrima*, *T. delbrueckii*, *W. anomalus*, *S. cerevisiae*	Cordero-Bueso et al. (2014)
2	3	Temperanillo[12]	Culture dependent	Shannon-Wiener index and Simpson's diversity index	*R. mucilaginosa*, *L. thermotolerans*, *S. cerevisiae*	*T. delbrueckii*, *W. anomalus*, *M. pulcherrima*	Cordero-Bueso et al. (2014)

Not reported (Also used organic fungicides[6])	M3, M4, 4, 22, 27, P7	Merlot[17, 18, 19, 20]	Culture dependent	Shannon-diversity index	*Sporidiobolus* sp., *Rhodotorula* sp., *Cladosporium* sp., *Aureobasidium* sp.	*Cryptococcus* sp., *Epicoccum* sp.	Martins et al. (2104)
8 (Also used organic fungicides[2, 5])	M2, M3, M4, 3, 13, 40, P7	Cabernet sauvignon [32, 35, 37, 38, 39, 40]	Culture dependent	Relative abundance (count/total) & Shannon diversity index	*A. pullulans, Cryptococcus* spp.	*Sporobolomyces roseus*, *Rhodotorula slooffiae*, *Bullera dendrophila*, *Candida sp.*, *Issatchenkia terricola*, *Rhodotorula nothofagi*, *Blastobotrys nivea*[*] ([*]only 87% identity)	Setati et al. 2012
8 (Integrated management system, also used organic fungicides[2])	M2, M3, 11, 13, 27, 40, P7	Cabernet sauvignon [19, 32, 37, 38, 39, 41]	Culture dependent	Relative abundance (count/total) & Shannon diversity index	*A. pullulans, Cryptococcus* spp.	*Rhodotorula glutinis*, *Issatchenkia terricola*, *Sporobolomyces roseus*	Setati et al. 2012
Not reported	3	Shiraz[12]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*S. cerevisiae*, *K. thermotolerans* (now *L. thermotolerans*) *P. anomala*	*P. toletana*, *C. sorbose*, *T. delbrueckii*	Cordero-Bueso et al. (2011)
Not reported	3	Grenache[12]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*K. thermotolerans*, *H. guilliermondii*	None reported	Cordero-Bueso et al. (2011)
Not reported	3	Barbera[12]	Culture dependent RFLP and PCR-RAPD analysis	Shannon-Wiener index and Simpson's diversity index	*C. stellata*, *T. delbrueckii*, *K. thermotolerans*	None reported	Cordero-Bueso et al. (2011)

1517

[†]Bio-fungicides include [1]*Bacillus subtilis* QST 713 (SerenadeÒ); [‡]Organic fungicides include: [1]Bordeaux mixture (copper (II) sulfate and calcium hydroxide), [2]sulfur, [3]pyrethrins, [4]copper sulphate, [5]copper hydroxide, [6]cuprous oxide, [7]chitosan (striker); [§]Synthetic fungicides include; [1]copper-oxychloride, [2]cyclohexanol + 1,2- propanediol + abamectin + 2,6-diterbutylp-cresol, [3]iprovalicarb + copper oxychloride, [4]sulfur (selenium free) + terpene alcohols + sodium salt of an aromatic polymer, [5]phosphorus pentoxide + potassium oxide, [6]spiroxamina, [7]metalaxyl-M14+ copper-oxychloride, [8]quinoxyfen+myclobutanil+coformulants, [9]fenhexamid based (TeldorÒ), [10]fosetyl-A1+ copper sulfate, [11]mandipropamid, [12]penconazole, [13]iprovalicarb + copper hydroxide, [14]quinoxyfen, [15]cymoxanyl + famoxadone, [16]ciprodynil and fludioxonil, [17]metalaxyl-M mancozeb, [18]zoxamide + mancozebe, [19]cymoxanil, folpet, fosetyl, [20]folpet, fosetyl, [21]benzamides, [22]pyridinyl-ethyl-benzamides, [23]pyridine-carboxamides, [24]oximino-acetates, [25]cyano-imidazole, [26]triazolo-pyrimidylamine, [27]triazoles, [28]spiroketal-amines, [29]cinnamic acid amides, [30]mandelic acid amides, [31]cyanoacetamide-oxime, [32]phosphonates, [33]benzophenone, [34]dithiocarbamates, [35]phthalimides, [36]quinones, [37]dimethomorph, [38]proquinazid, [39]mancozeb, [40]propiconazole, [41]kresoxim-methyl; ¶Yeast abbreviations: *A.* (*Aureobasidium*), *C.* (*Candida*), *Cry.* (*Cryptococcus*), *Cyst.* (*Cystofilobasidium*) *D.* (*Debaryomyces*), *H.* (*Hanseniaspora*), *Hyp.* (*Hypopichia*), *I.* (*Issatchenkia*), *K.* (*Kluyveromyces*), *L.* (*Lachancea*), *M.* (*Metschnikowia*), *P.* (*Pichia*), *R.* (*Rhodotorula*), *S.* (*Saccharomyces*), *Sp.* (*Sporobolomyces*), *St.* (*Starmerella*), *Tri.* (*Trichosporon*) *T.* (*Torulaspora*), *W.* (*Wickerhamomyces*), *Z.* (*Zygoascus*), *Zygos*. (*Zygosaccharomyces*). Other fungi abbreviations: *Bot*. (*Botrytis*), *Cl.* (*Cladosporium).* 454, pyrosequencing; L/A, low abundance; NGS, next generation sequencing (Illumina paired end); PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis); PCR-RAPD (Polymerase Chain Reaction-Random Amplified Polymorphic DNA); RFLP (Restriction Fragment Length Polymorphism).

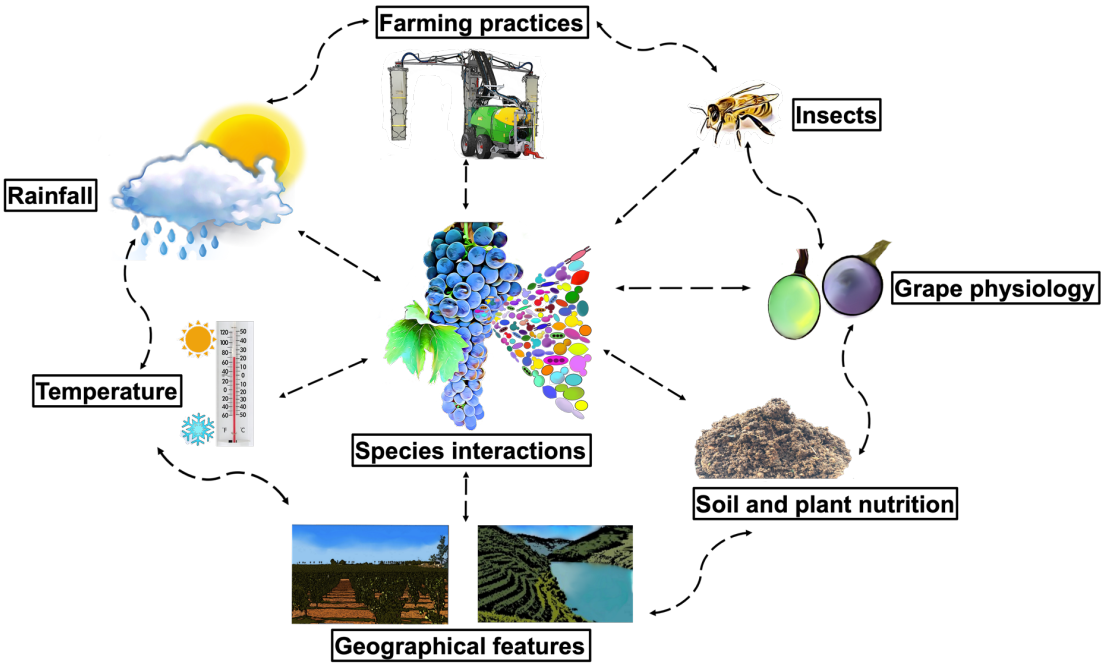

Figure 1. Factors to consider when investigating yeast biodiversity on grapes. These factors are not mutually exclusive and interactions between all these factors will determine both fungal diversity and the species present.

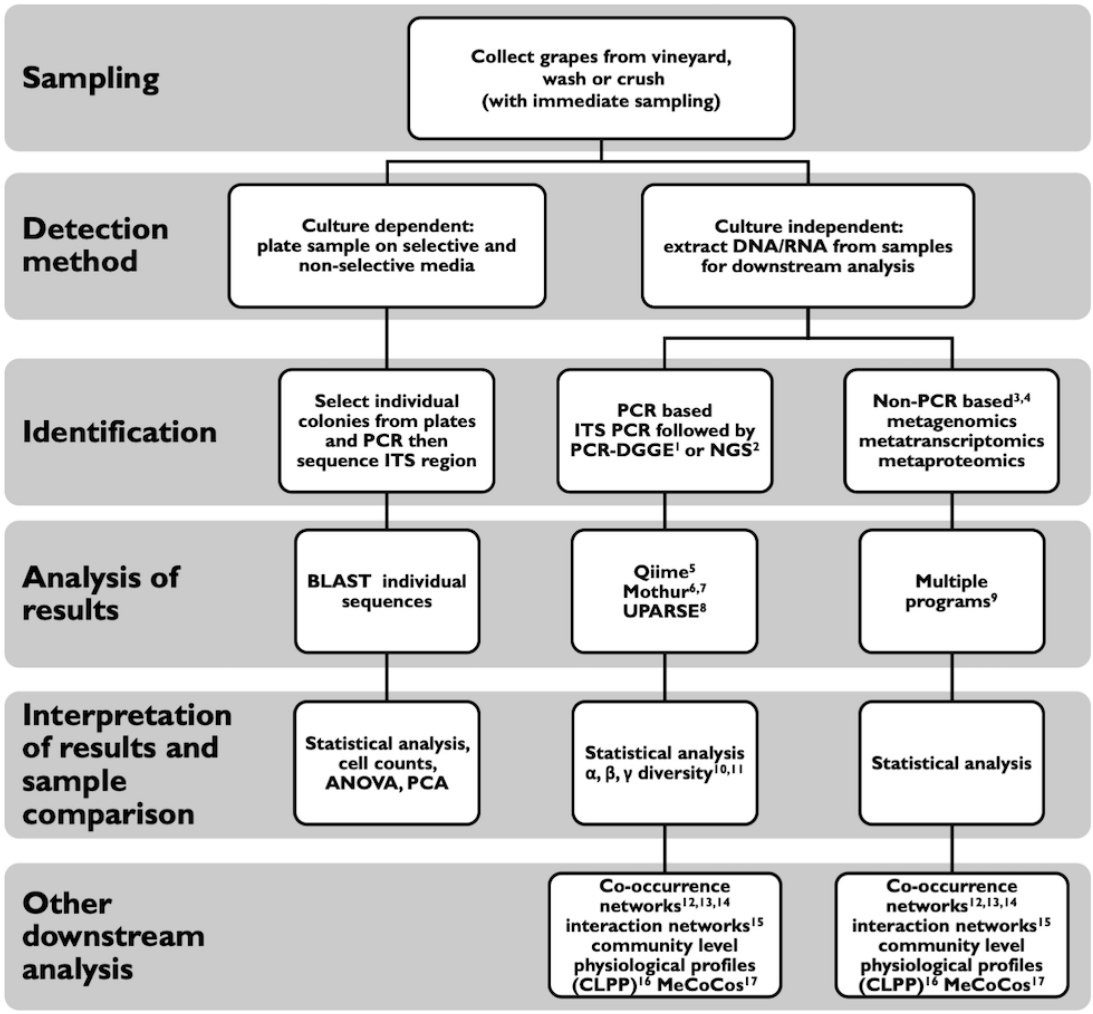

Figure 2. Multiple pipelines for fungal biodiversity analysis. [1]Escribano-Viana et al. (2018), [2]Agarbati et al. (2019b), [3]Sirén et al. (2019), [4]Vorholt et al. (2017), [5]Caporaso et al. (2010), [6]Schloss et al. (2009), [7]López-García et al. (2018), [8]Edgar (2013), [9]Lucaciu et al. (2019), [10]Whittaker (1972), [11]Morris et al. (2014), [12]Libis et al. (2019), [13]Pauvert et al. (2020), [14]Zhang (2019), [15]Abdelfattah et al. (2019), [16]Nerva et al. (2019), [17]Pascual-García (2020).

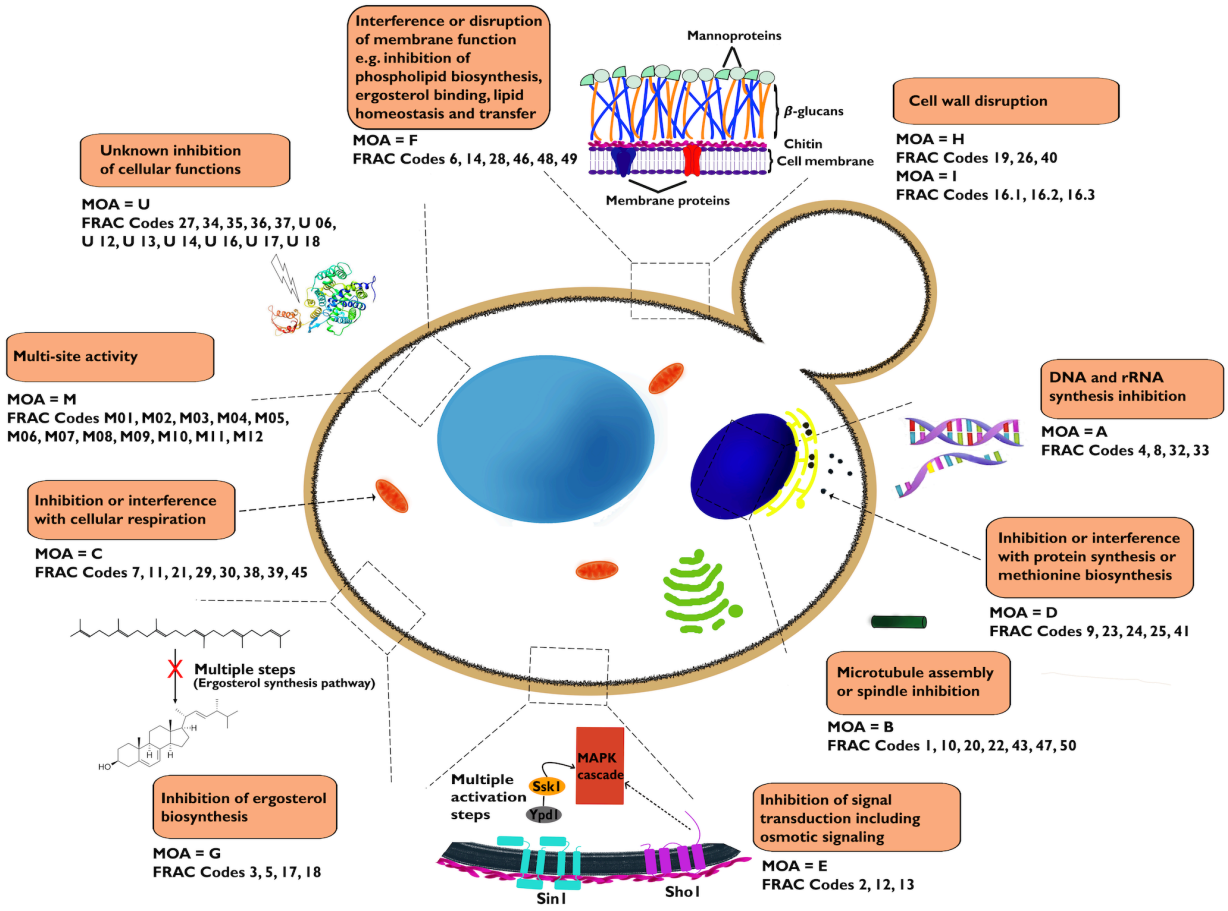

Figure 3: Mode of action (MOA) of antifungals. *Saccharomyces cerevisiae* is used as an example, but different yeast species will have different cell wall structure and may be more or less resistant to inhibition from fungicides because of this. Groups based on MOA: A, nucleic acid metabolism; B, cytoskeleton and motor protein; C, respiration; D, amino acids and protein synthesis; E, signal transduction; F, lipid synthesis or transport, membrane integrity or function; G, membrane sterol biosynthesis; H, cell wall biosynthesis; I, cell wall melanin synthesis; U, Unknown mode of action (Fungicide Resistance Action Committee 2020). In addition, FRAC codes are used to distinguish the fungicide groups according to their cross-resistance behaviour and define the GROUP Number on product labels (Fungicide Resistance Action Committee 2020).