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Review

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Grapevine Bunch Rots: Impacts on Wine Composition, Quality, and Potential Procedures for the Removal of Wine Faults

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ABSTRACT: Bunch rot of grape berries causes economic loss to grape and wine production worldwide. The organisms responsible are largely filamentous fungi, the most common of these being *Botrytis cinerea* (gray mold); however, there are a range of other fungi responsible for the rotting of grapes such as *Aspergillus* spp., *Penicillium* spp., and fungi found in subtropical climates (e.g., *Colletotrichum* spp. (ripe rot) and *Greeneria uvicola* (bitter rot)). A further group more commonly associated with diseases of the vegetative tissues of the vine can also infect grape berries (e.g., Botryosphaeriaceae, *Phomopsis viticola*). The impact these fungi have on wine quality is poorly understood as are remedial practices in the winery to minimize wine faults. Compounds found in bunch rot affected grapes and wine are typically described as having mushroom, earthy odors and include geosmin, 2-methylisoborneol, 1-octen-3-ol, 2-octen-1-ol, fenchol, and fenchone. This review examines the current state of knowledge about bunch rot of grapes and how this plant disease complex affects wine chemistry. Current wine industry practices to minimize wine faults and gaps in our understanding of how grape bunch rot diseases affect wine production and quality are also identified.

KEYWORDS: *off-flavor, wine quality, Vitis vinifera, mycotoxin, fining agents, fungal rot*

INTRODUCTION

Commercial wine production relies almost exclusively on the European grapevine *Vitis vinifera*, a hardy perennial plant, capable of withstanding environmental stress grown in temperate regions of the world.[1] Grapevines are susceptible to a number of plant diseases, those that affect the reproductive structures, and, more specifically, the fruit are the most destructive in terms of grape and wine quality and composition. Collectively referred to as bunch rots, the organisms are primarily, although not exclusively, fungal organisms. Estimates of economic losses are difficult to gauge and are region and variety specific. As an example, if one assumes an overall 1% loss of yield, then this equates to approximately AUD$8 million per annum for the Australian wine industry alone.

Viticultural management practices are frequently ineffective for bunch rot control in seasons when rainfall occurs close to harvest. Furthermore, pesticide usage in agriculture on crop products is becoming increasingly restrictive. For instance, the number of available pesticides registered in the European Union fell from 900 to approximately 200 products between 1995 and 2008, and further restrictions are anticipated.[2] This is likely to lead to the disappearance of some of the major fungicides from the market, such as those belonging to the triazole structure group, with serious consequences for the management of many plant diseases including those that affect grapes. Increasing restrictions on pesticide usage will translate to greater disease incidence in vineyards. In the absence of suitable vineyard disease control measures, wineries will need to explore options to minimize loss of wine quality. Climate change also represents an additional challenge, with various models predicting heightened rainfall events in dry-inland areas of Australia,[3] whereas other important grape-producing regions may experience diminished rainfall.[4] Such challenges to the growing conditions of grapevines are likely to lead to increased outbreaks of bunch rot disease in many of the major grape-growing regions of the world.

Much has been published on microbial spoilage of wine, that is, contamination of ferments by unwanted microorganisms and resulting wine faults.[5] Less has been published on the impact of microbial infection by phytopathogens at the grape production as opposed to the wine production stage of winemaking. The purpose of this review is to examine the impacts that those fungi associated with diseases of grape berries (i.e., bunch rots) have on wine composition and quality. An overview of the organisms associated with grape berry diseases is presented followed by a review of specific effects on grape and wine chemistry together with current knowledge and potential winery practices to minimize quality losses.

ORGANISMS ASSOCIATED WITH BUNCH ROTS OF GRAPES

The grape berry surface supports microflora of filamentous fungi, yeast, and bacteria,[6] and many of these organisms have little if any impact on grape and wine quality. Indigenous organisms do have a role in fermentation, but defining a specific influence is difficult, because microbial populations vary between vineyards and seasons. Numerous studies have reported grape berry microflora and their contribution to natural fermentation; however, most of these studies have sampled grapes from a specific region.[7−10] Microorganisms that survive beyond grape harvesting generally do not survive the

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Table 1. Organisms Associated with Grape Bunch Rots, Trivial Names, and a Summary of Documented Impacts on Wine Quality and Selected References

organism	trivial name and appearance	reported impacts on wine quality	selected refs
Alternaria spp.	black mold	unknown	104, 209
Aspergillus spp.	black sooty mold	mycotoxin production (e.g., ochratoxin A (5) in some strains of *A. niger* and other *Apergillus* species)	150, 151, 210
Botrytis cinerea	gray mold/noble rot	loss of red wine color, earthy mushroom aromas	47, 115, 118, 134
Cladosporium spp.	dark green velvety mold	unknown	62
Colletotrichum spp.	orange sporulation − ripe rot	Hessian sack and musty off-flavors, higher VA, glycerol and gluconic acid	116, 211
Elsinoë ampelina	black spot	unknown (disease primarily affects table grape varieties)	68
	black spot or lesion on immature berry that becomes a black hardened scar on ripening		
Greeneria uvicola	bitter rot, black rings of sporulation around circumference of berry	bitter off-flavors	49, 78
Guignardia bidwellii	black rot	unknown	63, 64, 212
Penicillium spp.	blue-green mold	earthy, mushroom aromas. mycotoxin production (patulin) by some strains	118,163−165, 213−215
Rhizopus	black mold	unknown	118, 209
indigenous yeasts, bacteria, and filamentous fungi	sour rot	largely formation of ethyl acetate and acetic acid, but variable depending on the particular complex of organisms involved	50, 114, 170
	watery berries, smell of vinegar		

Table 2. Organisms Capable of Infecting Grape Berries That Are Normally Regarded as Pathogens of the Vegetative Tissues and Selected References

organism	trivial name and appearance on berries	vegetative tissue more commonly affected	reported impacts on wine quality	selected refs
Botryosphaeriaceae	macrophoma rot	trunk and other woody tissues	unknown	112
	bunch rot of mature berries			
Erysiphe necator	powdery mildew of preveraison berries	leaves	wine haze	135
			reduction in levels of 3-mercaptohexanol associated with Sauvignon varietals	175
Phomopsis viticlola	bunch rot of mature berries	leaves, green shoots, and lignified canes	unknown	109, 111
Plasmopara viticola	downy mildew on preveraison berries	leaves	unknown	106

alcoholic and anaerobic conditions of the winemaking process. Wild yeasts such as *Candida stellata*, *Kloeckera apiculata*, and *Metschnikowia pulcherrima*[11,12] are easily isolated from grape berries but unable to tolerate alcohol concentrations above about 3% v/v and rapidly decline during fermentation. Similarly, bacterial species associated with the surface of the berry also do not survive the fermentation process.[13] Acetic acid bacteria are largely strict aerobes, and although associated with spoilage, their numbers similarly decline under anaerobic conditions. Lactic acid bacteria, which are tolerant of low-oxygen environments, are also weak competitors with *Saccharomyces cerevisiae* during alcoholic fermentation. Control of these spoilage organisms is achieved by a combination of good winery hygiene, sanitation, minimization of air contact, and judicious use of sulfur dioxide (SO_2).[14] Filamentous fungi found on the surface of the grapes are also largely aerobic organisms and are unable to tolerate high alcohol concentrations. Detrimental effects of filamentous fungi therefore largely arise from metabolic activities during growth as plant pathogens, and it is these organisms (Tables 1 and 2) that will be the subject of this review.

***Botrytis cinerea*, Gray Mold, Botrytis Bunch Rot.** The most frequently encountered bunch rot pathogen of mature grape berries is the filamentous fungus *B. cinerea* (teleomorph *Botryotinia fuckeliana*). Responsible for gray mold or *Botrytis* bunch rot, this pathogen occurs worldwide, particularly in vineyards exposed to cool and wet conditions during the ripening period. There is a wealth of literature on this organism,[15,16] and several reviews dealing with specific aspects of *B. cinerea* have been produced, especially disease management issues such as chemical control,[17] potential biocontrol agents,[18] the role of the fungus in eliciting host-defense mechanisms in grapevines,[19] and pathogen virulence factors.[20] Some of the earliest documented cases of fungicide resistance in agriculture involved *B. cinerea*.[21] Reports of fungicide resistance in this fungus continue to be published around the globe on a regular basis.[22−28] Aside from the indiscriminate use of fungicides, the rapid development of fungicide resistance in this organism can be explained by the variable nature of the fungus due to the complex asexual and sexual reproductive cycles of the fungus resulting in multinucleate conidia and hyphae.[29]

B. cinerea is non-host-specific and can infect a wide range of horticultural and ornamental host plants.[16] It is a necrotophic plant pathogen[30] that is able to survive in the soil and grow on plant material on the vineyard floor. The adaptability of the fungus means that aside from the development of fungicide resistance, grapevine host-defense mechanisms are readily overcome.[20,30−33] The life cycle on grapevines involves an overwintering or sclerotial stage on the wood[34] with conidial

release in the spring. Released conidia infect both the young foliage and reproductive structures. Grapevine flowers are susceptible to infection,[35,36] and studies on the epidemiology of the fungus suggest that floral infection in the spring may correlate with the degree of gray mold at berry maturity in Australia and New Zealand[37] but not necessarily so in France.[38] When infection of flowers occurs, this can result in flower abortion or latent infections after fruit set. Flower infection represents one of many pathways that can account for gray mold at harvest.[39] Excessive irrigation and rainfall leads to skin splitting, which further increases the chances of gray mold development. Conversely, vines with less dense canopies and reduced foliage have a lower incidence of gray mold.[40,41] Climatic conditions and microclimatic conditions (e.g., canopy density) are the primary risk factors for *Botrytis* development.[41] Despite occasional reports of midseason gray mold,[42] the immature or preveraison berry is largely resistant to *Botrytis*. This natural resistance has been attributed to the presence of resveratrol[43,44] and other phenolic substances that have antifungal activity. Concurrently *B. cinerea* produces enzymes such as stilbene oxidases to combat these host-defense mechanisms.[45] The antioxidant properties of resveratrol and these other secondary metabolites produced by vine tissues are believed to account for the cardiovascular protective properties associated with moderate wine consumption.[46]

Under climatic conditions when cold damp nights are alternated with relatively warm dry days, the fungus slowly dehydrates the berry, concentrating the sugar and forming glycerol, causing a syndrome known as noble rot. This type of rot is desirable if grapes are grown for the purpose of late-harvested desert wines, for example, Sauternes from France[47] or Tokaj from Hungary.[10] Different stages of *Botrytis* rot can be identified, that is, pourri plein (white-skinned varieties develop a brown coloration) and pourri rôti (berry dehydrates and sugars concentrate), and it is this latter stage that is used in sweet desert wine production.[48]

Aside from *B. cinerea*, a number of other filamentous fungi are associated with grape bunch rot. Collectively referred to as non-*Botrytis* bunch rots or bunch rots other than *Botrytis*, these fungi can be divided into those that are true pathogens, invading undamaged healthy berries, and those that are opportunistic pathogens, invading the berry when the natural resistance of the berry is compromised by injury (e.g., berry splitting after rainfall and other weather events or insect damage, etc.). The most important non-*Botrytis* bunch-rotting organisms are reviewed below.

Bunch Rots Other than *Botrytis*. Most of the non-*Botrytis* fruit-rotting pathogens are hard to differentiate by visual inspection, particularly on the surface of dark-skinned berries, as many of these fungi produce black molds (Table 1). Frequently, bunch rot of grapes is caused by a complex of organisms,[49] making differentiation of the specific influence of individual organisms on wine quality difficult to elucidate.

Sour rot is caused by a complex of filamentous fungi, saprophytic yeasts, and bacteria and occurs after periods of warm and wet climatic conditions.[50] Acetic acid bacteria (AAB) are always associated with sour rots; *Aspergillus* spp. may or may not be present,[51] whereas the profile of saprophytic yeasts, filamentous fungi such as *Rhizopus*, and other bacteria is variable. Insect damage and adverse weather events increase the probability of sour rot development, and it is likely that the organisms associated with sour rot are transmitted by insect vectors and in particular *Drosophila* spp.[52,53] Grape berries with sour rot have a distinctive smell due to the formation of ethyl acetate and acetic acid, compounds with low odor thresholds. Some of the yeasts associated with sour rot (e.g., *Zygosaccharomyces bailii*) are capable of surviving the fermentation process and can contribute to wine spoilage.[54] *B. cinerea* can occasionally be linked with sour rot; however, sour rot is more commonly associated with other filamentous fungi, in particular, *Aspergillus* spp.

Alternaria spp., *Aspergillus* spp. and *Rhizopus niger* are largely opportunistic pathogens or secondary bunch rot invaders infecting grape berries after a prior wounding event.[55] *Alternaria* spp. can be isolated as both epiphytic and endophytic fungi from a range of plant species and readily isolated from grape berries, particularly if other fruit-rotting fungi are present.[49,56,57] *Aspergillus niger* and *Aspergillus carbonarius* tend to be associated with hot dry Mediterranean climates and are frequently the most commonly encountered filamentous fungi associated with sour rot.[51] Secondary invading organisms such as *Penicillium* spp.m which produce blue−white molds and *Cladosporium* spp., producing dark green velvety molds, are readily identifiable and occur when grapes have been damaged. *Alternaria*, *Cladosporium*, and *Penicillium* have been identified by several authors as fungal endophytes, secondary invaders, or producing quiescent infection in grapevines.[54,58−61] Generally, these fungi are regarded as weak opportunistic pathogens of *V. vinifera*; however, there are reports of severe disease bunch rot problems with two species of *Cladosporium*, *C. herbarum* and *C. cladosporioides*.[62]

Guignardia bidwellii (black rot), a member of the Botryosphaeriaceae fungi, attacks both the vegetative tissues and reproductive structures. The fungus originates from the United States of America[63] and was introduced to Europe in the late 19th century.[64,65] This disease is described as extremely destructive and can cause up to 100% fruit loss in some vineyards and some seasons, particular in those regions that experience high summer rainfall.[66] To date, black rot has not been recorded in Australia. *Elsinoë ampelina* causes black spot or anthracnose of grapes, affecting the immature berry and the vegetative tissues.[67,68] This disease is largely confined to table grape varieties such as Sultana and Waltham Cross, whereas wine grape varieties such as Shiraz and Cabernet Sauvignon are highly resistant.[68,69]

Greeneria uvicola (syn. *Melanconium fuligineum*)[70,71] causes bitter rot of grapes and is associated with subtropical viticulture. Bitter rot has been recorded in diverse grape-growing countries including Australia,[72] Brazil,[73] India,[74−76] Taiwan,[77] and the United States[78,79] and in the past has been confused with black rot caused by *G. bidwellii*.[80] Warm wet conditions close to harvest such as those experienced in coastal regions of Australia and southern U.S. states predispose grapevines to outbreaks, and it is from these regions where cases of bitter rot are most frequently reported.[78,81] European (*V. vinifera*)[78] and American grapevines (*Muscadinia* spp.)[82] are susceptible to infection, and whereas infection of other horticultural crops is reported,[79] the economic importance of the disease is most significant for grapevines. Symptoms of bitter rot include olive-brown lesions that develop into a soft rot[78] and occasionally a series of concentric rings of black sporulation around the circumference of the berry.[49] In addition to attacking the reproductive structures, *G. uvicola* is associated with diseases of grapevine wood.[82−86]

Ripe rot caused by *Colletotrichum* spp. is similarly associated with subtropical vineyards and often occurs concurrently with *G. uvicola*. Two species, *C. acutatum* and *C. gloeosporioides*,[87,88] cause ripe rot, which is more distinctive than the other bunch-rotting fungi in that it is easily recognized by a discharge of pink to orange spore from the berry surface. *C. acutatum* and *C. gloeosporioides* are generally non-host-specific[89] and are pathogens on a wide range of fruit crops such as apple,[90] blueberry,[91] capsicum,[92] citrus,[93] olives,[94] strawberry,[95] tropical fruits, for example, mango,[96,97] and various nut crops, for example, almond and pistachio.[98−100] Although distributed worldwide, *Colletotrichum* species are a problem on grapes only in subtropical vineyards in countries such as Japan,[100] Southeast Asia,[77,87] southeastern U.S. states,[102] and subtropical regions of Australia.[81] It is not unusual to find ripe rot (*Colletotrichum* spp.) and bitter rot (*G. uvicola*) concurrently within the same bunch.[49] These bunch rots are largely a problem postveraison, although as with *B. cinerea*, evidence is gathering to indicate that flowering is a crucial time for disease management in the vineyard.[103]

Most if not all of the fungi described above can lead to postharvest rots in table grapes.[104,105] When they occur on wine grapes, they can lead to wine faults, that, to date, have been poorly characterized.

ORGANISMS MORE COMMONLY ASSOCIATED WITH THE VEGETATIVE TISSUES OF THE VINE THAT CAN ATTACK THE GRAPE BUNCH

Aside from the fruiting structures, the vegetative tissues of the vine are also susceptible to a range of plant diseases. These pathogens can also attack grape berries depending on the climatic and other environmental conditions of the vineyard (Table 2).

Erysiphe necator (syn. *Uncinula necator*) and *Plasmopara viticola*, respectively, cause powdery and downy mildew of grapevine leaves.[69] Powdery mildew develops in the spring after bud burst and affects the young leaves and appears as a white growth of mildew on the upper surface of the leaf. Downy mildew can be distinguished from powdery mildew in that the initial symptoms include yellow discoloration referred to as oil spots on the upper leaf surface followed by a white mildew on the underside of the leaf. Once established in a vineyard, both mildews can then progress to infect immature or preveraison berries.[69] Berry development in *E. necator*-affected fruit is arrested; the berries become purple, shrivel, and dry out. *Pl. viticola* infections of the immature berry result in a white powdery surface growth of mildew. Although no growth occurs after the onset of ripening (veraison),[106,107] the berry develops brown scar tissue with a web appearance over the surface. Berries attacked by powdery mildew early in the growing season are reported to have a greater incidence of *Botrytis*, other spoilage organisms, and wine faults. [108]

Phomopsis viticola and members of the Botryosphaeriaceae are also normally pathogens of grapevine vegetative tissues; *Ph. viticola* causes a cane and leaf blight, whereas the Botryosphaeriaceae cause cankers on the trunk and arms of the vine. Both groups of fungi can cause bunch rots in seasons with high disease pressures (e.g., unusually high rainfall) and possibly when existing wood infections are present.[109−111] Among these *Botryosphaeria dothidea* causes Macrophoma rot[69,82] and is reported from the southern United States. Other members of the Botryosphaeriaceae including *Diplodia seriata*, *Neofusiccoccum luteum*, and *Neofusiccoccum parvum* have similarly been isolated from grape bunches as pathogens in Australia.[112,113]

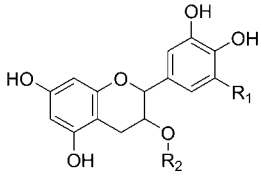
WINERY TOLERANCE LEVELS FOR BUNCH ROT CONTAMINATION

How much bunch rot can be tolerated in a vintage depends on the type of rot and also the winemaking processes. Unfortunately, many studies on the impact of bunch-rotting organisms on wine composition have used field-grown material in which numerous organisms are likely to contribute to the pathology.[114] Few studies have employed disease-free berries and inoculated the material with characterized strains, so specific effects are hard to determine. Low levels of infection can potentially have a detrimental effect on wine quality. Red wine quality has been reported to have been impaired when made from grapes with infection rates as low as 5% for *B. cinerea*[115] and 1.5% for *C. acutatum*.[116] However, these figures depend upon the wine type and style. The effect of bunch rots on wine made from red grapes can be considerable as the red winemaking process involves extended skin maceration. The oxidative enzymes (laccases, discussed below) produced by these fungi break down anthocyanins and proanthocyanidins. Wine quality is diminished as these phenolic compounds help provide the important palate structure features of bitterness and astringency as well as red wine color The influence of bunch rots on white wines can be manifested in several ways (see below), but desirable aromatic characters may be oxidized[117] and earthy aromas produced[118] with varying infection rates.

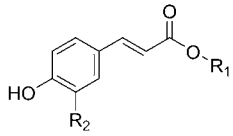
Determining the relative amounts of a particular bunch rot organism in harvested grapes is often difficult. Visual assessments based on incidence (i.e., % of bunches affected) or severity (degree of infection in a given bunch) have been used.[49] Measurements of laccase activity[119,120] give an indication of the total rot and likely impacts on wine quality, but this test is not organism-specific. Molecular-based PCR methods have been developed for analysis of bunch rot pathogens on grapes,[121−123] but these tend not to be suitable for field situations. Commercially available kits based on ELISA technology for the detection of the *Botrytis* antigens in wine and grapes are available[124−126] and employ the monoclonal antibody BC-12.CA4. These kits give a more precise estimation of gray mold than laccase[119] and are suitable for field use. Spectroscopic techniques such as NIR have been used for the analysis of grape and wine composition,[127] and this technology may prove to be useful for specific fungal bunch rot detection in the future. So far, the technique has been explored for the detection of powdery mildew contamination of grapes;[128] however, the technique is still very much under investigation at the time of this writing.

Fungal infection of grape berries may lead to the de novo synthesis of compounds not present in healthy grapes or wine or the modification of existing grape substrates. *B. cinerea* infection of grapes leads to a degradation of proanthocyanidins, catechin, and epicatechin with consequential changes in wine color.[115] The production of mycotoxins by *Aspergillus* and *Penicillium* strains is well documented[129] along with the modification of grape metabolites by fungal laccase. Although there is information in the literature on how fungal contaminants modify standard wine quality analytes (e.g., titratable acidity (TA), volatile acidity (VA), total soluble solids (TSS), ethanol content), knowledge of specific effects on flavor and aroma compounds is lacking. There is a significant knowledge gap of how fungal pathogens influence berry

Flavonoids



Non-Flavonoids



Compound	R1	R2	Compound	R1	R2
(epi)-catechin	H	H	p-coumaric acid	H	H
(epi)-gallocatechin	OH	H	caffeic acid	H	OH
galloyl-(epi)-catechin	H	gallic acid	ferulic acid	H	OCH_3
			coutaric acid	tartrate	H
			caftaric acid	tartrate	OH
			fertaric acid		

Figure 1. Grape-derived flavonoid and nonflavonoid phenolic compounds that are readily oxidized to corresponding quinone forms.

metabolism and subsequent wine composition. Some of the known effects are discussed below.

IMPACT ON WINE QUALITY

For many of the fungi involved in bunch rot disease of grapes little is published on their impact on wine quality. Wine made from bunch rot affected grapes typically has moldy or fungal aromas and flavors (Tables 1 and 2). Surprisingly, there is some evidence that a low level of bunch rot infection[114] may be desirable for the sensory characteristics of certain wine styles associated with *B. cinerea*. An understanding of the chemistry behind these off-flavors will facilitate a greater understanding of remedial actions that can be undertaken in the winery.

The majority of studies on the impact of bunch rot infection on wine quality have largely related to *B. cinerea*,[47,115,130−134] and less has been published on the impact of non-*Botrytis* rots.[108,116,133,135] Determining if undesirable compounds arise as a direct result of fungal metabolism or from chemical transformations occurring in juice and wine constituents is difficult to elucidate. Aside from the formation of off-flavors and aromas, grapevine pathogens affect wine quality through the degradation of grape phenolics and by the production of extracellular polysaccharides.

Altered Carbohydrate Metabolism. The fungi responsible for bunch rots often produce high molecular weight polysaccharides that cause processing difficulties. These include β1−3 and β1−6 glucans, which serve to protect the pathogen against host-defense mechanisms.[136] Glucans are also the products of plant-cell wall degrading enzymes produced by the fungus during infection. During wine production the glucans aggregate, and exogenous glucanase enzymes are necessary to prevent filtration blockage.[137] *B. cinerea* and other bunch-rotting fungi metabolize glucose to form glycerol and gluconic acid, which can then be metabolized by other micro-organisms to form acetic acid and dihydroxyacetone. The ratio of glycerol to gluconic acid has been proposed as an indicator to determine the level of gray mold versus noble rot.[48]

Laccase and Color Reduction. Laccases are a large group of oxidative enzymes produced by many fungi and some higher plants and exhibit a great diversity of activity and catalytic action. The range of substrates that laccases may oxidize is wide but are generally characterized by a diphenolic structure, although it is recognized that some fungal laccase enzymes are capable of attacking monophenolic compounds.[138] In grapes, a range of suitable substrates exist for laccase action with the principal nonflavonoid compounds being caffeic and *p*-coumaric acids, their tartaric acid esters, and, to a lesser extent, ferulic acid and flavonoid compounds such as (+)-catechin and (−)-epicatechin[48] (Figure 1). The presence of grape polyphenol oxidase also enables conversion of monophenolic substrates to diphenols, which are subsequently rapidly oxidized by laccase.

Laccase production by phytopathogenic fungi facilitates the infection process, and the amount of laccase activity in must has been taken as indicative of the degree of *Botrytis* rot.[115] *B. cinerea* laccases detoxify stilbene phytoalexins[32] and appear to inactivate pathogenesis-related proteins.[139] Furthermore, *B. cinerea* is known to produce several laccase enzymes.[140] Other genera of fungi associated with bunch rots have also been documented as laccase-producing, including *Aspergillus* spp. and *Colletotrichum gloeosporioides* from host plants other than grapes.[141] To date, laccase production in isolates of these fungal species originating from grape berries has not been reported, although it is likely. Detrimental effects on must and wine include a loss of red wine color as well as other problems associated with wine oxidation. Diphenolic compounds are rapidly transformed to their corresponding quinones, which are unstable and rapidly react with a range of phenolic and nonphenolic compounds, such as thiols, that ultimately lead to the formation of brown end products[142,143] In the presence of ascorbic acid, the quinones thus formed can be regenerated and browning reactions moderated until consumption of the ascorbic acid occurs.[144,145] These reactions are illustrated in Figures 2 and 3. Laccase in wine grapes presents a number of processing challenges due to its tolerance to high concentrations of sulfur dioxide, wine and grape pH, alcohol, and fining agents.[146−148] Wine made from grapes infected with *C. acutatum* has less color, suggesting that this species also produces laccase on wine grapes[116] or other polyphenol oxidases such as tyrosinase or catecholase.

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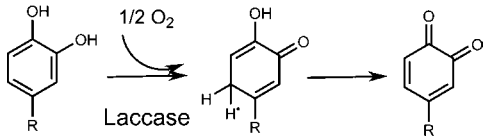


Figure 2. Oxidation of diphenolic compounds to the quinone form in grapes can be initiated by grape polyphenol oxidase or laccase.

Mycotoxins. Several fungal species associated with the rotting of grape berries have the potential to form mycotoxins, harmful to human health (Table 3). Mycotoxin production is well reported in species of *Aspergillus* and *Penicillium* that infect grape berries, but considerable strain and species variation occurs. However, there are a number of fungi other than *Aspergillus* and *Penicillium* that are capable of producing mycotoxins.

Ochratoxin A (**5**) produced by *Aspergillus* spp. is a potent nephrotoxic carcinogen that has been associated with tumors in the upper urinary tract and with fatal human kidney disease, referred to as Balkan endemic nephropathy. The European Union regulatory limit in wine made from 2005 is 2 μg/L,[149] and although ochratoxin A (**5**) survives the winemaking process, it is partially removed with marc and lees.[150] The amount of ochratoxin A (**5**) found in wine varies considerably from region to region and depends on the vineyard environment and the winemaking practices.[129,151,152] Ochratoxin A (**5**) is common in *Aspergillus carbonarius* [153] but occurs less frequently in *A. niger*. Isolates of *A. niger* that are ochratoxin A (**5**) producers produce lower levels than isolates of *A. carbonarius*.[154,155] Ochratoxin A (**5**) has also been reported from other species of *Aspergillus*, such as *A. tubingensis*,[56] although there is some doubt in the literature concerning the production of ochratoxin A (**5**) by *A. tubingensis*.[156] *Aspergillus* spp. tend to be associated with bunch rots in warmer and drier climates, so not surprisingly wines made in southern Europe have a higher propensity for ochratoxin A (**5**) than those from northern Europe.[129,159] The microbial profile of the grape berry surface also influences the amount of ochratoxin A (**5**) found on grapes, because there is evidence that different fungal species are able to degrade ochratoxin A (**5**).[158]

Some strains of *A. niger* also produce fumonisins B_2 and B_4 (**54**).[157,159,160] The fumonsins are a group of mycotoxins produced by *Fusarium* species not normally associated with grapes. A survey of 77 wines from 13 different countries found that 23% contained fumonsin B_2 in the 1−25 μg/L range.[161]

Patulin (**6**) and citrinin (**3**) (Table 3) are mycotoxins produced by *Penicillium* spp. on grapes and most notably *P. expansum*. [162] *P. brevicompactum* isolates are also reported to produce patulin (**6**).[163] Both mycotoxins could be readily detected in grape and apple products derived from mold-infected fruit; however, neither compound could be detected following alcoholic fermentation.[164,165] Strains of *Alternaria alternata* are reported to produce the mycotoxins alternariol (**1**) and alternariol methyl ether (**2**) (Table 3),[166] on grapes and other fruits, although to what extent this is a problem on wine grapes is unknown. *Alternaria* species largely occur on grape berries as endophytes,[58,59] although as opportunistic pathogens they have the potential to cause crop losses under high disease pressure situations. Mycotoxin production in other fungal genera associated with bunch rot of grapes has, to date, not been reported. Clearly the production of mycotoxins in grapes and their fate during fermentation are areas deserving of further research despite the many papers published in this area.

Off-Flavors and Aromas. A number of compounds potentially responsible for off-flavors and aromas in wine have been identified in the literature from grapes affected by bunch rot pathogens (Table 4). These include fenchol (**8**), fenchone (**14**), (−)-geosmin (**18**), 2-heptanol (**7**), 2-methylisoborneol (**19**), 1-nonen-3-one (**15**), 1-octen-3-one (**16**), 1-octen-3-ol (**11**), and 2-octen-1-ol (**12**), found in grapes infected, or wine made from grapes infected, with *B. cinerea*, *Penicillium* species, *Aspergillus* spp., and *Rhizopus nigricans* or, in some cases, combinations of these organisms.[118,167,168] Collectively these compounds are described as having varying degrees of mushroom, or earthy odors. Odor perception thresholds depend upon the medium used, for example, water versus model wine solution versus wine type, but of these

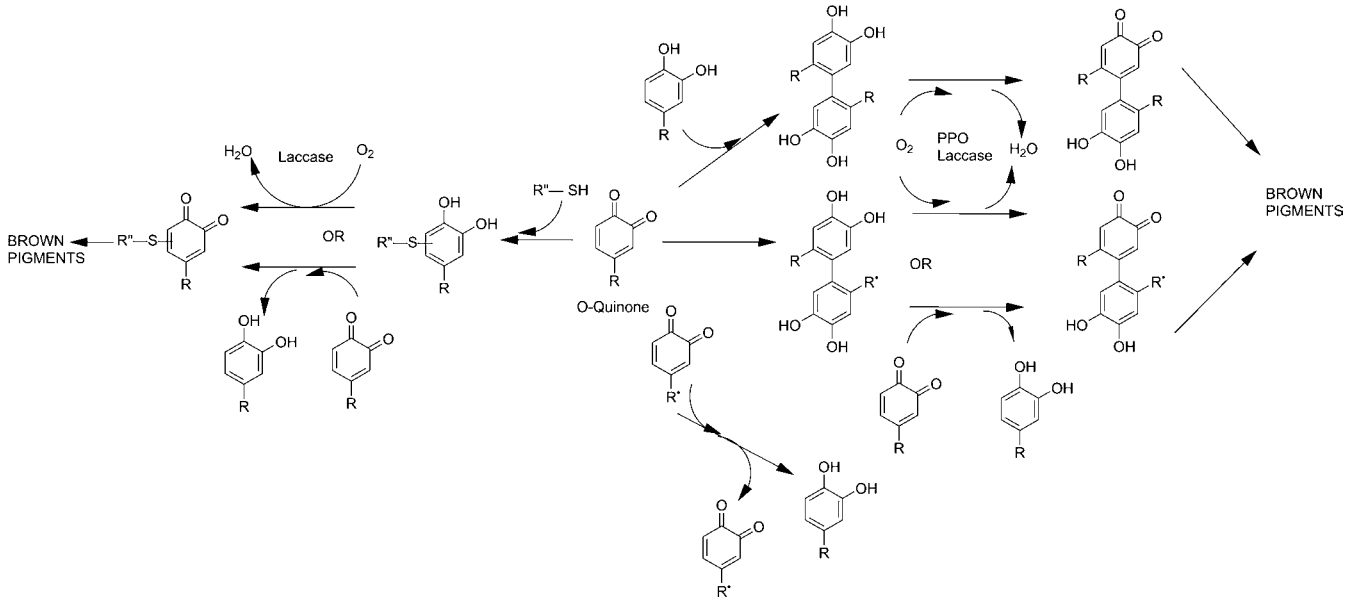
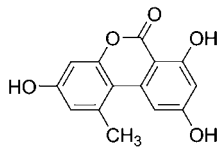
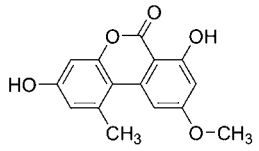
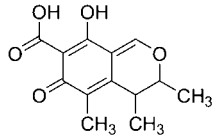
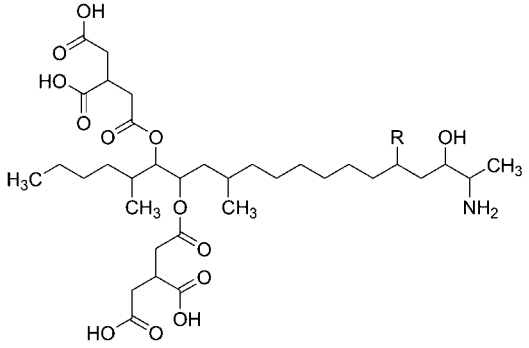
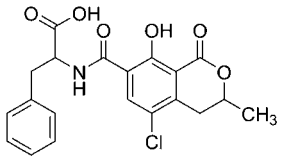
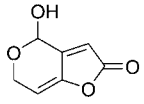


Figure 3. Formation of brown polymeric phenolic compounds by rapid dimer and trimer condensation reactions. Modified with permission.[142]

Table 3. Chemical Structures of Mycotoxins Produced by Fungi Associated with Bunch Rot Diseases of Grapes

Mycotoxin	Chemical structure	Associated Fungal Species	Selected References
Alternariol	 **1**	*Alternaria alternata*	166
Alternariol Methyl Ether	 **2**	*Alternaria alternata*	166
Citrinin	 **3**	*Penicillium expansum*	162, 213, 215
Fumonisin B_2 & B_4 B_2: R=OH B_4: R=H	 **4**	*Aspergillus niger*	159-161
Ochratoxin A	 **5**	*Aspergillus carbonarius* *Aspergillus niger* *Aspergillus tubigensis*	151, 155, 156, 216
Patulin	 **6**	*Penicillium expansum*	162-165

compounds geosmin (**18**), 2-methylisoborneol (**19**), and 1-octen-3-one (**16**) appear to be the most odorous (Table 4). Little information is available on the removal of these compounds from wine, although there is some evidence that at least some of these compounds are partially degraded during fermentation.[168] The rate of degradation may be enhanced by the nitrogen status of the must, because inclusion of either glycine or glutathione to wines made with bunch rot complexes leads to wines having lower concentrations of 1-octen-3-one (**16**) and a diminished mushroom odor.[168] Studies on the formation of these compounds by bunch rot fungi in planta are complicated because of strain and species variation and because of the complex of different organisms that can be isolated from one bunch. Furthermore, the production of these secondary metabolites is influenced by environmental conditions. In an in vitro study using two different media, one based on white grapes and the other on red grapes, production of these compounds was found to differ.[167] Similarly, geosmin (**18**) production by *Penicillium expansum* is influenced by the metabolic activities of *B. cinerea* present on the same bunch. This effect depends on the strain that is present and is maybe related to the nitrogen status of the grape berry as alluded to above.[133] *B. cinerea* colonization of grape berries leads to amino acid degradation within the berry, which then influences geosmin (**18**) production by *Penicillium* spp. Geosmin (**18**) production in *P. expansum* is also further influenced by other *Botrytis* metabolic factors that are yet to be fully described. Thus, there is a complex relationship between the metabolic activities of these two organisms.

Wine made from ripe rot (*Colletotrichum acutatum*) affected grapes has been described as having a musty, Hessian sack aroma, coupled with elevated levels of glycerol, gluconic acid, and volatile acidity.[116] These off-flavors and odors appear to be distinct from those described above for some of the other bunch rot fungi. As far as the authors are aware, no detailed analysis of wine made from grapes infected with some of the

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Table 4. Chemical Structure and Odor Properties of Compounds in Wine Associated with Bunch Rot Infection

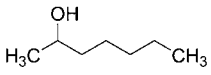
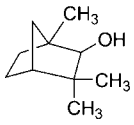
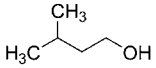
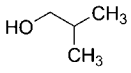
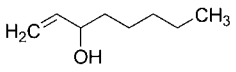
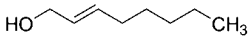
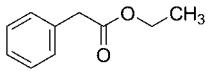
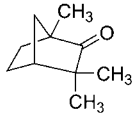
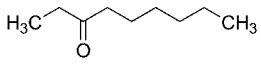
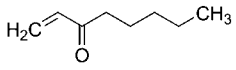
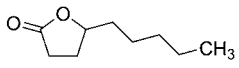
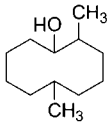
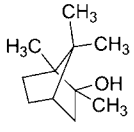
Compound	CAS	Chemical Structure	Odour Properties	Olfactory Detection threshold µg/L
Alcohol Compounds				
2-Heptanol	543-49-7	 **7**	mushroom [118]	100 §§ [118]
Fenchol	2217-02-9	 **8**	earthy-camphor [118]	50 §§ [118]
3-Methyl-1-butanol	123-51-3	 **9**	cheese, pungent [218]	300 §§ [217] 30000 † [219] 60000-180000 ‡ [220]
2-Methyl-1-propanol (isobutyl alcohol)	78-83-1	 **10**	fusel [170]	40 000 †† [219] 75 000 ††† [221]
1-Octen-3-ol	3391-86-4	 **11**	mushroom [218] earthy [222]	1.0 §§ [217] 2.0 §§ [118] 20.0 § [118]
2-Octen-1-ol	18409-17-1	 **12**	mushroom [118]	50 §§ [118]
Carbonyl Compounds				
Ethyl phenyl acetate	101-97-3	 **13**	sweet, honey, waxy [170]	20 §§ [223] 650 §§ [224]
Fenchone	1195-79-5	 **14**	earthy- camphor [118] eucalyptus like, musty (1R, 4S) [225]	110 §§ (1R,4S)+ [225] 240 §§ (1R,4S)+ [226] 440 §§ (1S, 4R)- [226]
1-Nonen-3-one	24415-26-7	 **15**	mushroom [168]	0.008 § [168]
1-Octen-3-one	4312-99-6	 **16**	mushroom [118]	0.016 §§ [225] 0.03 § [118]
Lactone Compounds				
(R/S)-γ-Nonalactone	104-61-0	 **17**	musty [222] coconut [225]	9.7 §§ [225] 30 †† [227]
Miscellaneous Compounds				
Geosmin (-*trans*-1,10-Dimethyl-*trans*-9-decalol)	19700-21-1	 **18**	earthy [118]	0.01 §§ [118] 0.04 § [118]
2-Methylisoborneol	2371-42-8	 **19**	earthy-camphor [118]	0.02 §§ [118] 0.04 § [118]

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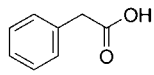
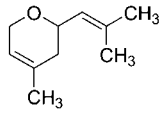
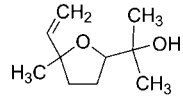
Table 4. continued

Compound	CAS	Chemical Structure	Odour Properties	Olfactory Detection threshold μg/L
Phenyl acetic acid	103-82-2	 **20**	urine [222] beeswax [225]	1000 [§§ 228] 2000 [§§ 223] 6100 [§§ 225]
Terpenoid Compounds				
Nerol oxide	1786-08-9	 **21**	geranium [229]	Not available
trans-Furan linalool oxide	34995-77-2	 **22**	burnt [218] green [222] earthy (3S, 6R) [230]	60 [§§ 223] 6000 [§§ 231]

[†]Determined in water/ethanol (90 + 10, w/w). [††]Determined in 10% ethanol. [†††]Determined in 9.45% w/w ethanol. [ψ]Determined in 10% ethanol/water mixture containing 5 g/L tartaric acid, pH 3.2. [‡]Range reported for wine and beer. [§]Determined in a model solution of ethanol 12%, tartaric acid 5 g/L, pH 3.5. [§§]Determined in water. [§§§]Determined in a model solution of ethanol 11% v/v, glycerol 7 g/L, tartaric acid 5 g/L, pH 3.4.

other fungi involved in bunch rots such as bitter rot, black rot, and macrophoma rot has been conducted. Consequently, the chemical identities of compound(s) responsible for these off-flavors in grapes and wine are unknown.

The specific effects of sour rot on wine quality are difficult to define, because of the variability in the organisms involved in any given sour rot complex. In Riesling wine sour rot reduces the levels of linalool, nerol, and geraniol and increased the α-terpineol, *trans*-furan linalool oxide (**22**), nerol oxide (**21**), benzyl alcohol, 2-phenylethanol, 2-methyl-1-propanol (**10**), 3-methyl-1-butanol (**9**), and 1-octen-3-ol (**11**),[50] resulting in loss of varietal flavor. Higher levels of ethyl phenyl acetate (**15**), phenyl acetic acid (**20**), and γ-nonalactone (**17**) (Table 4) have been reported in Trincateria and Cabernet Sauvignon wines made from sour rot affected grapes,[170] although surprisingly there was no difference in the sensory properties of wine with infections rates of up to 30%.[171]

Although not regarded as a bunch rot in the classical sense, wine made from powdery mildew affected grapes typically has elevated acidity, phenolics, flavonoids, and levels of hydoxycinnamates than wine made from unaffected grapes, whereas total suspended solids and spectral color are reduced.[172,173] Furthermore, pathogenesis-related (PR) proteins produced by the host tissue in response to powdery mildew infection can result in protein hazes in white wine.[135] Sensory analysis of wine made from powdery mildew affected Chardonnay grapes has fungal, earthy, and cooked tomato attributes,[175] although the chemical identities of compounds contributing to these flavor profiles have not been fully characterized. The level of the varietal aroma 3-mercaptohexanol is reduced in response to powdery mildew infection in Sauvignon wines,[175] and as discussed above, other common odors associated with mushroom and earthy taints such as 1-octen-3-one (**16**) are elevated.

A number of authors have investigated the impact of powdery mildew on wine quality,[135,174−176] but nothing has been published on the impact of downy mildew on wine as far as the authors are aware. It is likely that downy mildew affected berries, being hard and dehydrated, would not be collected by mechanical harvesters or would be excluded with stalks.

WINEMAKING PRACTICES THAT AMELIORATE FUNGAL PATHOGEN IMPACT

Harvest and Sorting. In the absence of adequate vineyard disease control measures, minimizing the use of diseased grapes for winemaking is a critical step for maintaining wine quality. Selective hand harvesting of grapes with concurrent disposal of diseased bunches can be accomplished when the pickers are sufficiently skilled to identify fruit of suboptimal quality. Alternatively, the diseased fruit can be dropped in the vineyard prior to subsequent hand or machine harvesting. Sorting and the removal of grapes at the winery using manual or automated grape sorting tables is also undertaken in small, premium wineries. These practices are time-consuming and expensive and would seldom be an economically viable solution, particularly in high-volume production.

Laccase is not particularly susceptible to sulfur dioxide and requires several days contact for appreciable loss of activity. Addition of sulfur dioxide at high rates (100−200 mg/L)[48] to harvest bins with diseased fruit or to must is common to mitigate laccase activity, although oxidation will only be marginally impeded. The sulfur dioxide does inhibit other microorganisms such as *Gluconobacter* and *Acetobacter*, which will inevitably be present in higher numbers.[177] To further minimize the effects of laccase prior to processing, harvesting should be completed in the coolest conditions possible,[178] fruit transported to the winery as quickly and gently as practical, and processing at the winery initiated expeditiously.

Thermovinification of Grapes and Must. *Botrytis* laccase activity decreases at temperatures exceeding 60 °C, although the observed effects are substrate dependent.[179] Pasteurization of juice and must at temperatures exceeding 60 °C is therefore required for inactivation of laccase,[146] and 80 °C with a holding time of 5 s has been recommended[178] to treat heavily infected

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juice prior to winemaking. Whole grapes may be heated with steam or boiling water,[146] although more commonly performed via a tubular heat exchanger. This closed system has the advantages of vastly reduced oxygen contact and greater temperature control.

Minimizing Oxygen Exposure. Laccase activity has an absolute requirement for oxygen to elicit substrate transformation; thus, limiting exposure of infected grapes to normal atmospheric conditions is an important management strategy to control oxidative and browning reactions. Whereas an anaerobic environment is difficult to achieve throughout the entire winemaking procedure, the use of inert gas covers during pressing, transfers, and in-tank ullage space will displace atmospheric oxygen and thereby lessen the impact of laccase.[178] During white wine production whole bunch pressing will lessen oxygen exposure compared to the macerating steps of crushing and destemming. Pressing cycles with minimal tumbling and relatively low pressures are also beneficial to lessen oxygen exposure of infected fruit and decrease release of the laccase from infected grape skins. Constant monitoring of press fractions will minimize the inclusion of moldy taints and overtly oxidized juice, and heavy pressings should be kept separate or discarded if not of sufficient quality. Small additions of enological tannin during crushing provide an alternative substrate for the oxidative effects of laccase and may be useful in red and white juice ferments.[178] The implications of tannin additions to the final mouthfeel properties of the wine must be carefully considered if additions are contemplated. Rapid initiation of all fermentations using commercially prepared yeast inoculum will ensure a vigorous fermentation in which evolved carbon dioxide from ethanol production will assist in oxygen exclusion. Red ferments are best pressed off skins prior to completion of alcoholic fermentation to ensure adequate carbon dioxide presence during the press cycle and transfer to tank.

Clarification and Settling. Removal of *Botrytis* material and laccase in white grape juice prior to fermentation may require settling at colder than normal temperatures to reduce enzyme activity.[180] Larger sediment volumes arise in grapes with bunch rots due to their susceptibility to mechanical stress,[181] and a more compact lees may be produced by the use of commercial pectolytic enzymes added at the higher range of manufacturer's recommendations.[182] Wineries also utilize centrifugation for juice clarification, and increased centrifugation speeds may be beneficial. Bentonite, an aluminum−silicate clay routinely used for protein stabilization during wine production, adsorbs to proteinaceous compounds and is subsequently removed by centrifugation or following a settling period. Whereas laccase is not completely removed by bentonite,[64] low addition rates of 0.2−1.0 g/L[183] may facilitate lees compaction and laccase removal in affected fruit. Enzyme additions should be made after Pasteurization to avoid their inactivation during the heating process. If both pectolytic enzymes and bentonite are used, it is important to consider the timing of additions as bentonite will also adsorb the added pectolytic enzymes. To help ameliorate this problem, enzymes should be allowed at least 6 h to break down the grape pectins prior to bentonite addition. Alternatively, the bentonite may be added following an initial clarification procedure.

Fermentation Management. Fungal growth on grape bunches will diminish nutrient availability for yeast to complete alcoholic fermentation, along with the secretion of inhibitory substances that retard yeast growth.[184] Thus, management of the nutritional status of the ferment is an important aspect for wine production when using rot-infected grapes. *B. cinerea* uses ammonium nitrogen during growth and depletes levels of thiamine and pyridoxine.[183] *Botrytis*-infected juice has been reported to display 2−7-fold reductions in the total amino acid concentrations when compared to uninfected berries.[185] Therefore, *Botrytis*-infected grapes often require nitrogen supplements to avoid formation of hydrogen sulphide produced by *S. cerevisiae* and vitamins to help avoid stuck alcoholic fermentations.[183] It is advantageous to measure yeast assimilable nitrogen of harvested grapes prior to yeast inoculation so that nitrogen deficiencies can be corrected prior to fermentation. Addition of complex yeast nutrients containing trace elements and vitamins should also be considered for overall ferment management. Manual punch-downs and particularly delestage, the oxidative procedure that involves the fermenting juice being separated to a separate vessel before being pumped back on top of the ferment cap, should be avoided. The gentler pump-over procedure should instead be used for color and tannin extraction in red ferments. Fewer pump-overs and addition of pectinolytic enzyme to degrade the grape skin wall will hasten color and tannin extraction. Prior to the completion of fermentation, the wine should be racked from the yeast lees as quickly as possible as *Botrytis* material and laccase will also settle to the bottom of the tank during fermentation. If malolactic fermentation is desired, inoculation should be performed as soon as practical to help minimize oxygen contact with the wine, and, when complete, sulfur dioxide added at higher than normal levels.

Postfermentation Winemaking. The transformation of the highly odor potent compounds 1-octen-3-one (**16**) and (*Z*)-1,5-octadien-3-one to less potent 3-octanone and (*Z*)-5-octen-3-one, respectively, by *S. cereivsiae* is reported and provides some evidence of the fate of these earthy aromas during fermentation.[169] Of interest is the conjugation of 1-octen-3-one (**16**) with glycine and glutathione in model solutions that demonstrates potentially alternative methods for remediation of wine tainted with carbonyl compounds.[168] However, even after careful grape selection and vigilant winemaking, wine taints, undesirable aromas, and oxidized phenolic compounds produced by various bunch rots have been reported in the final wine and thus require strategies for their removal. Wine fining is the addition of an adsorptive compound followed by the removal of partially soluble or precipitated compounds through settling or centrifugation.

Mycotoxin removal by addition of yeast hulls is reported by several researchers, although addition rates and efficacy of several preparations in various wines do not reveal any specific trends.[186,187] As an alternative, must can be passed over ochratoxin A (**5**)-free grape pomace, which absorbs some of the ochratoxin without affecting either color or other phenolics associated with wine quality.[188] Similarly, some yeast species (viable and heat-treated) such as *Candida famata* are able to bind ochratoxin A (**5**) and may have a role in its removal from wine.[187] Sodium bentonite has been evaluated for the removal of ochratoxin A (**5**) from white wine and spiked phosphate-buffered saline solutions and found not to be particularly effective, whereas much greater reductions were found using activated carbon.[189] Wheat gluten was found to significantly decrease the concentrations of 1-hexanol, 3-methyl-1-butanol acetate, 2-methyl-1-butanol, and benzyl alcohol[190] and so may have some potential to reduce some of the earthy aromas found in bunch rot affected wine. However, there are issues with the

addition of gluten to wines because this is not acceptable for consumers who suffer from celiac disease. Despite this, these promising approaches to wine fining are deserving of greater attention to elucidate important mechanisms and chemical basis for taint removal in wines.

Whereas fining includes additions such as the adsorption of wine proteins by bentonite or copper sulfate to remove hydrogen sulfide, the remainder of this review will concentrate on fining agents that are routinely used for the removal of phenolic compounds in wine as this chemical class comprises the principal substrates for fungal enzyme activity. Furthermore, the formation of weak hydrogen bonds with carbonyl compounds may provide opportunities for the use of these fining agents for selective removal of undesirable taint compounds.

FINING AGENTS

Protein Fining Agents. Protein fining agents depend primarily on hydrogen bonding between the carbonyl oxygen of the protein and the phenolic hydroxyl group to form insoluble protein−phenol complexes.[183,191,192] Hydrophobic interactions play a minor role,[192] but for the purpose of the following discussion hydrogen bonding will be considered the primary complexing mechanism. In wine, protein fining agents are positively charged species and form hydrogen bonds with negatively charges species. Hydrogen bonds are relatively weak, and the efficacy of protein fining agents is therefore reliant on the formation of many such bonds with phenolic compounds, and minimizing competing bonds between amide groups within the native proteins.[183,191,192] Proline and 4-hydroxyproline may also comprise up to 18% of gelatin, and these amino acids lack a hydrogen atom on the amide group as their structural configuration involves a ring formation. Consequently, protein fining agents with high proportions of proline and hydroxyproline have a reduced capacity for internal hydrogen bonds to form[192] and have a more open tertiary structure that increases binding capacity for phenolic compounds.[48] Fining agents with high concentrations of proline and hydroxyproline are therefore favored as these preparations have a higher efficacy for removal of undesirable phenolic compounds. Gelatin and isinglass have a regularly repeating sequence of amino acid residues in which glycine occurs at every third residue. Increased glycine content of protein fining agents also improves phenol binding efficacy. A high proportion of glycine increases the number of hydrogen bonding sites per weight of protein and enables the protein to more efficiently fold around the target phenolic compounds as lower side-chain formation reduces steric hindrance.[192]

It is important to note that fining agents are not specific for undesirable phenolic compounds and that a deleterious effect on wine quality can result from wine fining due to a possible loss in wine flavors and aromas. Trials are therefore necessary to judge the most appropriate dosage rate before the fining agent is added to the wine.

Gelatin. Gelatin is derived by the hydrolysis of collagen to produce a protein with increased solubility and molecular weight range from 15 to 150 kDa. Smaller molecular weight gelatins are reported to selectively bind to polymerized tannins[192,193] as the smaller peptides are more able to adapt their conformational structure to the tannin molecules.[48] However, larger gelatins may be more efficient at removing phenolic compounds as less phenolic substances are required to form insoluble complexes, and entrapment of smaller phenolic compounds occurs once precipitation is initiated.[192] Increased polymerization and galloylation of tannins increases the number of hydroxyl groups, which can interact with the protein and lead to precipitation of the complex.[183,191,194]

White wine does not usually contain high concentrations of large polyphenolic molecules, and therefore gelatin is seldom used for fining these wines. Low rates (20−50 mg/L) are sometimes used to reduce bitterness in low-quality white wine. Higher dosages (100−200 mg/L) are used to remove astringency and bitterness of red wine.[195] The preferential removal of high molecular weight galloylated proanthocyanidins with gelatin has also been reported.[196] As the phenolic products of fungal infections appear to be relatively small in size, most gelatin forms seem to have limited use for fungal taint removal, although the larger gelatin forms potentially have the greatest efficacy. The relatively poor efficacy reported for gelatin removal of ochratoxin A (**5**)[197] and removal of pesticides[198] are consistent with the action of gelatin. Similarly, it has been reported that gelatin showed poor specificity for smoke taint affected wines, which contained high levels of compounds such as guaiacol and 4-methylguaiacol.[199]

Isinglass. Isinglass is a protein derived from the sturgeon swim bladder and purchased in a dehydrated, fibrous form. Isinglass has an average molecular weight of 140 kDa and an isoelectric point of 5.5[191] and primarily binds to small phenolic compounds, including monomeric phenols, but not condensed tannins.[183] It is therefore used extensively in fining white wine to remove undesirable bitterness. Isinglass is also reported to enhance fruit characteristics and optical brilliance and improve clarification[183] at low addition rates.[195]

Little scientific literature is available that describes the efficacy of isinglass and removal of specific phenolic species; however, dosage rates of 100 and 200 mg/L decreased the total phenolic content of a Neuburger wine by 2.5 and 3.3%, respectively, without any loss of catechin or epicatechin.[200] It has been reported that isinglass size is an important factor in the selective removal of certain phenolic compounds,[201] a possible explanation for the low effectiveness of isinglass to remove smoke taint[202] and ochratoxin A (**5**)[186] in wine.

Casein. Casein is the principal protein found in milk and, when purified, is practically insoluble in acidic conditions due to the combined properties of high molecular weight (375 kDa) and isoelectric point of 4.7.[183] Therefore, casein is commonly purchased in the far more soluble form of powdered potassium caseinate. In wine, casein flocculates quickly, and thorough mixing is required to avoid loss of fining action.[48] Casein is typically used in white wine and sherry to reduce bitterness, overdeveloped characters, and brown color from oxidized phenolic material.[191] In the Champagne region, casein is purportedly used successfully on oxidized must when grapes have been infected by *B. cinerea*.[203]

The nature of the casein preparation is reported to influence the removal of various phenolic compounds. Significant reductions of both monomeric and polymeric flavonols were described when casein fining was employed, whereas potassium caseinate reduced only the polymeric species.[201] A later study by the same author[204] indicated that potassium caseinate significantly decreased polyphenolic flavonoids and some nonflavonoids. Other reports on the influence of casein reducing polyphenolic compounds appear somewhat contradictory. It has been reported that casein reduced the polymeric phenol fraction of wine and improved color.[200,205] However, an earlier study found that potassium caseinate produced 20 and 34% reductions of the monomeric catechin using dosages of

0.25 and 0.50 g/L.[206] The varying forms of either casein or potassium caseinate possibly explain the study that indicated casein had a low impact on smoke taint affected wines, which contained high levels of compounds such as guaiacol and 4-methylguaiacol.[199] More encouragingly for possible removal of taint compounds, it has been reported that casein was able to decrease a large range of pesticides,[198] whereas potassium caseinate has been shown to remove 82% of ochratoxin A (**5**) when used at a the higher than usual dosage rate of 1.5 g/L.[197]

Polyvinylpolypyrrolidone (PVPP). PVPP is a synthetic, high molecular weight polymer composed of cross-linked monomers. The high concentration of carbonyl groups on PVPP form hydrogen bonds with phenolic compounds,[183] and unlike the soluble protein fining agents that are effective in removing larger polyphenols, insoluble PVPP contacts relatively few of the hydrogen binding sites of the phenolic compound. The insolubility of PVPP implies a rigid structure, which is unable to conform to that of larger polyphenolic compounds. Therefore, PVPP reacts more specifically with smaller phenolic species such as monomers and dimers[191,206] as well as oxidizable cinnamic acids and the quinones formed when they oxidize.[48] PVPP fining is reported to decrease flavonol monomers and dimers[204,207,208] and lead to improved wine color.[205] It is also routinely used to reduce bitterness and improve wine aroma[191] and may be successfully used in both juice and wine for removal of oxidized and brown phenolic compounds.[183]

Surprisingly, PVPP has been unsuccessful when trialled for removal of ochratoxin A (**5**),[186,197] pesticides,[198] and smoke taints.[202] These studies utilized PVPP at typical winemaking concentrations to investigate the removal of the array of phenolic compounds. It is hypothesized that higher dosages of PVPP may remove these compounds, in addition to wine taints caused by bunch rots, due to the known specificity of PVPP for relatively small phenolic molecules.

IMPLICATIONS FOR FUTURE RESEARCH DIRECTIONS

Grape berries are susceptible to infection by a range of microorganisms, and aside from a loss of yield, these infections affect grape and wine quality. Management of these diseases in the vineyard remains limited. In many cases the precise nature of the off-flavors and taints found in wine as a result of processing disease-affected grapes is not known. This is especially so as naturally occurring bunch rot infections in vineyards are caused by a complex mixture of organisms and strains. In many cases a common group of compounds are responsible for similar off-flavors and aromas, although individual fungal species may have specific effects. There is the potential to remove or rectify some faults such as those derived from oxidized phenolic compounds during wine production, and the use of fining agents is a potential tool that can be useful. Although there is considerable knowledge on the use of fining agents for the removal of phenolic compounds implicated in microbial spoilage of wine, winemakers still do not have the technology to remove wine taints produced as a result of microbial contamination of the grape berries in the vineyard. Furthermore, the chemical nature of many of the compounds that elicit undesirable aromas and flavors are not well characterized. This knowledge gap represents an avenue for future research.

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Notes

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