Avoiding microbiological wine spoilage

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Abstract
Successful winemaking uses selected wine microorganisms to convert fruit flavours into wine flavours. These grape varietal and regional wine flavours can be partially or even totally obscured by wine spoilage yeast and bacteria. Potentially most damaging are apiculate yeasts like Kloeckera/Hanseniaspora spp. and Brettanomyces/Dekkera bruxellensis. Avoiding microbial wine spoilage starts with selecting fruit that is unbroken and free of mould infection and it continues by managing temperature, pH, and winery sanitation throughout the winemaking process. This paper focuses on the introduction of spoilage microorganisms with the grapes, in particular apiculate yeasts with grapes that are affected by diffuse powdery mildew infection and on the control of Brettanomyces yeast from grape maceration to barrel ageing.

It is well known that grapes with visible fungal infections (Botrytis, powdery mildew, and others) carry a much higher microbial load into the start of fermentation than grapes with are unbroken by insects, birds, or fungal hyphae. Such damaged fruit can carry more than several million viable yeast and bacteria per mL compared to sound fruit, which carries less than several thousand yeast and bacteria. Also, the types of microorganisms carried by damaged fruit are very different from those on undamaged fruit. Most of the microorganisms carried on sound fruit do not survive the juice and early fermentation stage while the oxidative yeast and bacteria in damaged fruit can survive throughout the wine fermentation and even be carried into the wine ageing stage. A study of the easily overlooked diffuse infection of powdery mildew showed a very large population of apiculate yeasts in the grape must of such infected grapes that appear undamaged. These potent spoilage yeast must be suppressed by heavy juice fining and possibly juice pasteurisation. Pre-fermentation time must be kept very short and the temperatures between 5 and 15°C must be avoided. The addition of yeast and bacteria starter cultures also helps to suppress these spoilage yeasts.

The feared wine spoilage yeast Brettanomyces bruxellensis can also get established in a vineyard and in early stages of vinification such as cold maceration and sluggish onset of alcoholic fermentation. The next critical stage for proliferation of Brettanomyces is during delayed malolactic fermentation. When, following completion of alcoholic fermentation, the wine is not protected by active malolactic bacteria or by pH control and addition of SO2, it is critically exposed to spoilage by Brettanomyces. During wine ageing, the wine must be protected against Brettanomyces contamination from the barrel surface and winery environment. Strategies to suppress growth of Brettanomyces will be presented.

Introduction
Critical to avoiding microbial spoilage of wine is the knowledge of where potential spoilage microorganisms can enter the winemaking process and how their growth and damaging metabolic activity can be limited or excluded. The winemaking process and resulting wine quality start with the microorganisms present on the grapes, on the harvest equipment and the winery surfaces, and all along the process of the grape must preparation, the fermentation, and the ageing of wines. Microorganisms are introduced into the wine and new environments are being created that can foster the development of spoilage microorganisms. The winemaker’s challenge is to select the desired microorganisms for the conversion of grape juice into wine and limit the presence of potential wine spoilage microorganisms. This paper reviews how the potentially spoilage microorganisms can be managed with sanitation, temperature control, SO2 additions, and use of yeast and bacteria starter cultures.

Microorganisms on grapes
Undamaged grapes harbour very small numbers of yeast and bacteria on the berry surface, the leaves and the stems. Most of these microorganisms are aerobic (require oxygen for growth) and they grow on surfaces with near neutral pH values. When these microorganisms enter the grape must they find themselves in a very acidic environment (pH below 4) and very soon in an anaerobic environment – as the fermentation starts, the oxygen dissolved in the grape must is very quickly consumed and with the choice of fermentation vessel and fermentation management, the winemaker can limit entry of oxygen from the air. Only yeast and bacteria tolerant of acidic environments and able to survive in an anaerobic environment can survive and grow in this new environment, the fermenting grape must. These are primarily Kloeckera/Hanseniaspora and Saccharomyces yeasts and lactic acid bacteria. Hanseniaspora yeasts can cause spoilage in grape must and wine because they can grow in anaerobic environments and produce large amounts of acetic acid and acetate esters (Moreira et al. 2005). In fact, these yeasts can grow to high cell density in grape must and persist all through the alcoholic fermentation. The sources of entry of Hanseniaspora yeast are broken berries (and exposed juice in grape picking bins) and unsanitary equipment surfaces in the winery (Renouf et al. 2006a, 2006b). Hanseniaspora can grow rapidly in picking bins that contain free juice and in winery juice storage and fermentation tanks, inside transfer hoses and in collected juice in the crusher/destemmer. They grow well at temperatures around 10°C, at temperatures near 20°C and higher and especially in the presence of some SO2. Hanseniaspora are outcompeted by Saccharomyces yeasts. In order to limit the entry of Hanseniaspora, winemakers must exclude damaged fruit and maintain sanitary conditions in the grape processing and fermentation areas. The growth of Hanseniaspora can be restricted by the use of SO2 and temperature control. Already an addition of 50 mg/L SO2 will reduce the population of Hanseniaspora in harvested fruit and in the grape must. Larger additions and even pasteurisation are required when severely damaged fruit is to be processed, or damaged fruit that has been held for several days prior to start of fermentation. In this case must pasteurisation is an excellent tool. During grape processing – harvest, destemming, crushing, pressing – the fruit should be kept at a low temperature, below 10°C. Growth of all grape and wine microorganisms is almost completely inhibited at temperatures around 5°C.
soaks are being used, it is important that the crushed grapes are kept at such a low temperature. If the temperature is allowed to reach 10 to 15°C then growth of *Hanseniaspors* is strongly favored. If low temperatures during grape processing cannot be maintained, then the microflora must be managed by additions of SO2 and warming the must quickly to 20°C before inoculation with a yeast starter culture. The SO2 addition and the warmer temperature encourage early and rapid growth of *Saccharomyces* yeasts which then can suppress the unwanted *Hanseniaspors*. In any case, the time which grape must remains at temperatures between 8 and 18°C should be minimised in order to limit the opportunity for growth of *Hanseniaspors*. The addition of a yeast starter culture (*Saccharomyces cerevisiae*) further helps to quickly close the ‘window of opportunity’ for growth of *Hanseniaspors*. The *Saccharomyces* starter culture should, of course, be added only when the grape must has been warmed to 18 to 20°C; otherwise it cannot take hold due to the temperature shock.

Occasionally, two other groups of potent spoilage microorganisms can be found in damaged grapes. It is well known that acetic acid bacteria multiply in damaged grapes and can enter the wine fermentation in large numbers. Again, sorting out of damaged fruit, SO2 addition, and a quick initiation of alcoholic fermentation (production of anaerobic environment) can lower the cell number of these damaging bacteria. Once an anaerobic environment have been established and is maintained by careful topping of tanks and barrels, the acetic acid bacteria have no opportunity to grow and damage the wine. Less known is that *Brettanomyces/Dekkera* yeasts can be found in vineyards on grapes and in soils. *Brettanomyces bruxellensis*, the only *Brettanomyces* species that can grow in wine can be brought into the winery with fruit damaged by mould, insects, birds, or split by rapid uptake of water following rains. Cold soaks which are not really cold – around 10°C to even 15°C instead of 5°C – also can serve to encourage the growth of *B. bruxellensis*.

**Fungal infections on grapes**

Infection of grape berries by the powdery mildew pathogen (*Uncinula necator*) degrades the quality of fruit, and juice and wine prepared from this fruit. We have discovered a form of diffuse powdery mildew infection that is both virtually invisible under field conditions on berries, and has unforeseen effects on juice and wine quality (Gadourey et al. 2007). Diffuse powdery mildew predisposes Chardonnay, Riesling, Pinot Noir, and Gewürztraminer berries to bunch rot by *Botrytis cinerea*, and to contamination by several microorganisms with proven deleterious effects on juice and wine. We also found elevated levels of insect infestation in Pinot Noir clusters with diffuse powdery mildew. Sap beetles, ants, and wasps were approximately 30 times more prevalent in clusters with diffuse infection. This was true despite the fact that the clusters had not yet begun to decay noticeably in the vineyard. Gas chromatography and mass spectroscopy analyses revealed that grapes with diffuse powdery mildew give off larger amounts of volatile ethyl acetate, ethanol, and acetic acid; all of which are used by the above insects to locate food. Our results indicate that the insects are responding to incipient decay, and at least one (a common yellowjacket) is actively involved in wounding the clusters and thereby accelerating decay.

Our overall goal has been to define when and to what degree that grapevines must be protected from powdery mildew for optimal disease control and for the production of high-quality juice and wines. We have now demonstrated that even inconspicuous disease gives rise to diffuse powdery mildew, and all of its attendant secondary effects, can be avoided if fruit are protected until ontogenic resistance to powdery mildew is fully expressed at four weeks after bloom is completed. Where suppression of disease has not been fully successful, preliminary studies indicate that treatments of juice with SO2 ultraviolet light, or pasteurisation may eradicate some spoilage microorganisms in the juice from berries with diffuse powdery mildew.

**Microflora of fruit with diffuse powdery mildew vs healthy fruit**

Diseased fruit has the potential to bring large numbers of potentially harmful bacteria and yeast into the grape must, and thereby into wine fermentation. The microflora of healthy Pinot Noir fruit vs. those with diffuse powdery mildew was analysed to estimate the differences in yeast and bacterial populations that are potentially detrimental to wine quality. We quantified acetic and lactic acid bacteria, as well as major yeast genera (*Kloeckera, Candida, Metschnikowia, Pichia, and Saccharomyces*) using a combination of techniques.

The summary of the data shows that there is a large increase in the number of total microorganisms (various yeasts and bacteria) in the berries with diffuse powdery mildew vs. the healthy berries. These data clearly show that a much larger load of yeast and bacteria enter the winemaking process. Many of these yeast and bacteria die during the early stages of fermentation, but yeast in the cycloheximide resistant group (*Brettanomyces/Dekkera-Smith 1981*) and in the lysine agar group (*Kloeckera/Hanseniaspors*) have a particularly high spoilage potential. Some *Brettanomyces* can produce the very potent “Bandaid™”, wet dog, horse sweat, and cow manure type odours which can spoil wine (Chatonnet et al. 1995, 1997; Heresty 1986b; Hesford et al. 2004). These odours have been attributed to the presence of ethyl phenols produced by *Brettanomyces* together with other less characterized compounds (Heresty 1986a; Liker et al. 1998; Brock et al. 2006). Some of the *Kloeckera* yeasts can produce very large amounts of acetic acid and acetate esters, which at high concentrations also spoil wine (Moreira et al. 2005).

The difference in the number of microorganisms between the clean fruit and the fruit with diffuse powdery mildew was striking. The total microbial population was much increased with diffuse infection with powdery mildew. Such large differences in total population should also result in different populations in the fermenting wines. Fruit with diffuse infection had a total microbial population greater than 5 million cells per mL. Such a large population in the must at the beginning of fermentation can be expected to have an effect on the sensory quality of the resulting wine. Against such a large population the addition of SO2 and the addition of a yeast starter culture may be only partially successful. This large indigenous population will only be partially suppressed. There did not appear to be a qualitative difference in the microbial populations between mildew-free and diffuse-infected fruit in 2001.

Powdery mildew, even at trace levels that cannot be seen in the vineyard, can predispose fruit to bunch rot, and result in contamination of grapes by various other spoilage microorganisms.

**Impact of powdery mildew on juice and wine quality**

Since 1999, we have prepared Pinot Noir wines from grapes with and without diffuse powdery mildew. In 1999, wines from fruit with diffuse powdery mildew tended to exhibit green, unripe flavours. The tasters noted green (vegetative) flavours, shorter in mouth-feel, some bitterness. The wine from the clean fruit had good mouth-feel and cherry, berry flavours. In 2000 wines from the fruit with diffuse powdery mildew again tended to be less ripe with green, herbaceous, tea, and dusty flavours. They also tended to have a thinner mouth-feel. The wines from the clean fruit were judged to have more ripe fruit flavours. In 2001, bitterness was noted in each of three replicated wines from the diffuse powdery mildew fruit, but not in the wines from the clean fruit. Also bretty (*Brettanomyces*)
off-flavours were noted in all three of the wines from the diffuse infected fruit. One of the wines from the clean fruit also possibly had a reduced sulfur off-odour, which might be confused with ‘Brett’.

Considering all of the sensory evaluations performed to date, in general the number of defects and their perceived severity is higher among the Pinot Noir wines prepared from grapes with diffuse infection, and among Chardonnay wines prepared with grapes bearing more than 5–10% mildewed grapes.

It should be noted that inconsistent and even rare defects should still be of great concern in the production and marketing of wines. Consumers will remember a single bottle contaminated by *Brettanomyces* (it smells like manure) long after they have forgotten several excellent vintages produced by the same winery. If this is their first experience with a particular winery, it is also likely to be their last.

**Alcoholic fermentation**

**Winery sanitation**

Maintaining sanitary environment is the first rule in managing fermentations for premium wines. We believe that the extra degree of definition of pure varietal and regional flavours, flavour intensity and fine texture can only be achieved in a clean winery environment. A dirty winery environment that allows entry and proliferation of various spoilage microorganisms at some or several stages of winemaking will only produce wines with dulled flavours, wines which are not clearly distinguished from other good or average wine, wines which do not truly carry regional distinctive flavours or expressive varietal flavour characteristics. A medium degree of contamination with spoilage microorganisms, be it acetic acid producers or *Brettanomyces*, does wipe out the distinction of varietal and regional flavours. Thus, a sanitation program is essential for a winery in which premium wines of consistent high quality are to be produced. Critical points for sanitation in the winery are of course during harvest time crushers and destemmers, juice settling tanks, transfer lines. During fermentation and ageing it is important to keep tanks clean and topped up, transfer hoses (lines) cleaned, barrels cleaned, and floors and drains clean. Sanitation of stainless steel and tile or epoxy floor coverings is relatively easy with cleaners, good rinsing, and steam. Barrel sanitation is more difficult because of the porosity of wood. For barrel sanitation it is most important to control the microorganisms that enter the barrel with the wine by assuring that the wine entering is of low pH, and has adequate free SO₂, or is still actively undergoing fermentation. Next, it is critical that the tannate, pigment and protein deposits on the inside are removed by careful washing (high pressure, warm water, even mild alkali treatments), and, the outside of the barrel is kept clean to prevent growth of mould and acetic acid bacteria and *Brettanomyces*. In winery sanitation surveys in New York State (Henick-Kling et al. 2005) we found that area around the bung hole of a barrel can harbour sizable numbers of *Brettanomyces* and acetic acid bacteria. Thus, this area is a dangerous source of contamination with spoilage microbes in wine management. We found that 70% ethanol and SO₂ treatments were the most effective in decontaminating the bung hole area on the barrels. Treatment with peracetic acid had limited efficacy at the concentrations tested which were the supplier’s recommended concentrations. The cleaning treatment with detergent alone confirmed the lack of effectiveness and was comparable to water alone. A common misconception by the food and beverage industry is that detergents are capable of killing microorganisms. The role of detergents is to remove dirt and organic debris to allow the sanitiser that is subsequently applied, to work more effectively.

Other critical areas of winery sanitation in are drains, hoses, and valves (Henick-Kling et al. 2005). Drains do need to be scrubbed and sanitised on a regular schedule, hoses need to be cleaned immediately after use and hung to allow complete draining after use, tank valves need to be of a sanitary design that does not include any unreachable spaces for brushes and hot water rinses. It is also important to properly set the sanitation plan to include the use of a detergent followed by rinsing prior to application of a sanitiser. A rotation in the use of sanitiser formulated with different active compound it is also recommended, in order to avoid development of microbial resistance.

**Fermentation management**

Fermentations need to be managed to ensure dominance of *S. cerevisiae*. Competitors to *S. cerevisiae* are mainly *Hanseniaspora* yeasts, lactic and acetic acid bacteria. These microorganisms compete for nutrients and they can produce antimicrobial compounds that selectively inhibit growth of certain microorganisms. Recent research in interaction of wine and juice microorganisms has shown that the competition for nutrients – energy sources and especially essential nutrients – is a key factor in the succession and dominance of wine microorganisms. In this interaction antimicrobial compounds such as organic acids and bacteriocins also play an important role. To assure dominance of the desired wine microorganisms it is critical that the establishment of *S. cerevisiae* and *O. oeni* is assured very early. In the case of *S. cerevisiae*, it is important to keep the number of competing microorganisms in the must low by keeping out damaged, infected fruit, next the must pH needs to below 3.5 and some SO₂ (20 to 50 mg/L) should be added. The grape must should be warmed to 20°C to encourage growth of native *S. cerevisiae* or to assure implantation of an added *S. cerevisiae* starter culture. Nutrients need to be managed to assure adequate supply of essential nutrients (year available nitrogen above 200 mg/L). During fermentation, the temperature needs to be controlled to avoid rapid heat rises and drops and excessive temperatures (over 35°C). Remember, the comfort zone for *S. cerevisiae* is 20 to 30°C, below 20°C it is not very competitive with other yeasts and above 30°C it is stressed by increasing alcohol content (of course higher temperatures help improve enzyme activity to increase extraction of flavours and color from grape tissue). Following completion of alcoholic fermentation malolactic fermentation (if desired) should be induced quickly by maintaining a favorable temperature (20°C) and/or adding a starter culture. An excellent way of encouraging malolactic fermentation right after alcoholic fermentation is to co-inoculate a yeast and malolactic starter culture.

It is critical that sluggish fermentation during alcoholic and malolactic is avoided. If fermentation becomes sluggish then the dominant yeast or bacteria culture have come under stress and are prone to be out-competed by other microorganisms.

To avoid growth of *Brettanomyces* yeast (Dias et al. 2003, Uscanga et al 2000, Suarez et al 2007), encourage malolactic fermentation immediately after alcoholic fermentation. Make sure the pH is below 3.5, there is no free SO₂, and the temperature is 18 to 20°C. Addition of a starter culture will help assure dominance of desired lactic acid bacteria and avoiding off-flavours and possible production of biogenic amines.

Within one to two weeks after completion of malolactic fermentation, establish free SO₂ (20 to 40 mg/L), and maintain a pH as low as possible. Maintain full containers, blanket the wine surface with inert gas. Bottle with sterilization – filtration or heat to assure it maintains is flavour qualities without destruction by *Brettanomyces* or other spoilage yeast.
And, of course, bottle the wines under screw caps to avoid uncontrolled oxidation and addition of cork off-flavours.

References


