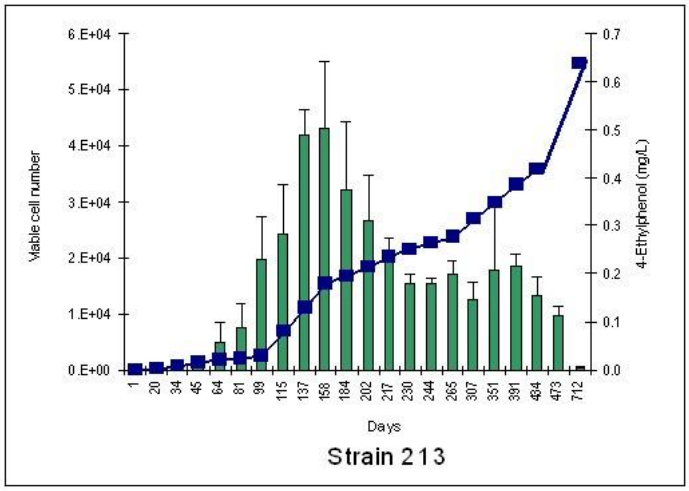
CONTROLLING MICROBIAL GROWTH IN WINE

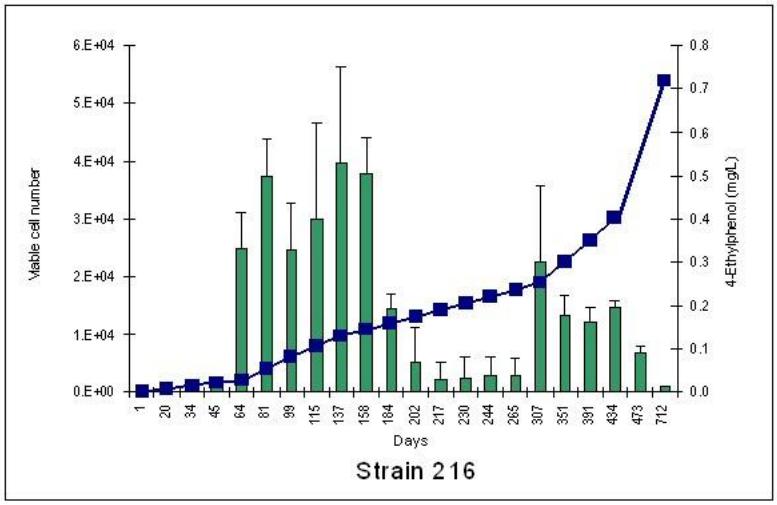
Section 6.

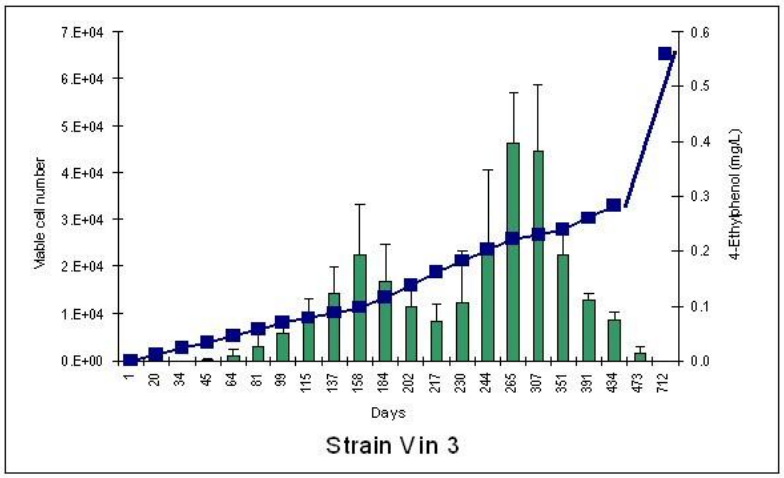
Brettanomyces

Brettanomyces continues to be a problem for premium wine producers. In a study funded by the Virginia Winegrowers Advisory Board (Fugelsang and Zoecklein, 2002), replicated sterile Pinot noir (*Vitis vinifera* L.) wines were individually inoculated with one of eight strains of *Brettanomyces bruxellensis* at initial viable cell numbers < 50 cfu (colony-forming units)/mL. In two separate studies, population changes were monitored for 23 months, or until cell densities peaked and subsequently declined to < 30 cfu/mL. Significant variation was noted in both growth rate and population densities among strains. Significant increases in the concentration of 4-ethylphenol occurred after accumulated cell populations reached 2.5×10^5 cfu/mL.

Figure 2. Growth (Green Bars) and Production of 4-Ethylphenol (Blue Squares) Over Time by Three Strains of *Brettanomyces*







This study demonstrated that strains of Brett could have very different growth patterns (Figure 2). As can be noted, several strains appeared to decline in cell population, and then bloom again. This may have resulted from a phenomenon now known as “active but not culturable.” This calls into serious question the validity of the traditional culture plate method used by industry to determine the concentration of viable *Brettanomyces.*

Brettanomyces has the ability to produce a number of organic compounds which can impact wines. However, the sensory attributes of Brett wines relate not simply to concentration of certain metabolites, but to the ratio of these components and their interactions with the wine matrix. For example, it is possible to have wines with high concentrations of metabolites, including the traditional troublemaker, 4-ethylphenol, and not have a Brett character.

Therefore, wine composition can greatly influence the sensory threshold of volatile compounds produced by Brett. Wine composition is a function of the fruit and processing. Wines like Cabernet Sauvignon have a high detection threshold, while Temprenillo and others have low detection thresholds.

Tests conducted at the Wine/Enology-Grape Chemistry Group lab, and elsewhere, have demonstrated that volatile phenols can bind with higher molecular weight phenols during thermal processing and microoxygenation. This lowers volatility and makes some Brett metabolites less perceptible. On the other hand, oxygenation in the presence of viable Brett (splash racking, microoxygenation) can increase the population and may increase the production of taint metabolites.

Regardless, the analysis of 4-ethylphenol does not always correlate to the intensity of Brett character, or to the concentration of viable *Brettanomyces* species present.

There seems to be a significant strain variation. A survey conducted in Australia demonstrated that the ratio of two important metabolites, 4-ethylphenol to 4-ethylguaiacol, was lower in cooler, than in warmer, climates. This may a function of malvidin-3-coumaroyl glucoside production. This grape skin phenol is believed to be a precursor to 4-ethylphenol, and is found in higher concentrations in shaded fruit. Thus, there may be a link between vineyard management and *Brettanomyces*.

Variation among Brett strains was highlighted by Joseph and Bisson (2004). 16% of the Brett strains surveyed produced no 4-ethylphenol or 4-ethylguaiacol.

Some Brett strains can metabolize ethanol. It had been assumed that Brett used sugars derived from barrels, micro-molar concentrations of glucose remaining in dry wines, or possibly the glucose derived by glycoside hydrolysis. Mansfield, Zoecklein, and Whiton (2002) demonstrated that Brett has the ability, in model solutions, to break down phenolic glycosides, like anthocyanins, liberating glucose. This breakdown can provide a carbon source and renders the anthocyanin molecule unstable, a reason why Brett wines frequently lack desirable color.

Joseph and Bisson (2004) reported that 30% of the Brett strains surveyed grew at 10°C, but some grew at elevated temperatures (37°C). 50% of the Brett strains reviewed grew in 30 mg/L free sulfur dioxide at pH 3.4. Brett strains demonstrate a large diversity in sulfur dioxide tolerance, from 14 - 56 mL molecular free SO_2. This would suggest that it is much better to attempt control with a few large doses of sulfur dioxide, rather than a number of smaller doses.

Brettanomyces may be able to utilize DAP (diammonium phosphate) and nutrients in some commercial nitrogen supplements. This would suggest that the addition of excess N is counter-indicative. Too much N increases the fermentation rate, and changes the ratio of esters to long-chain alcohols produced by *Saccharomyces*. Additionally, excess nitrogen may stimulate the growth of undesirable organisms such as *Brettanomyces*.

50% of the strains surveyed by Joseph and Bisson (2004) formed biofilms. An example of a biofilm is the plaque which forms on teeth. At this liquid-solid interface, microorganisms such as *Brettanomyces* can slough off into the environment. How well a sanitation procedure breaks down the biofilm determines, to a large degree, its effectiveness.

Winemaking Operations to Encourage Brett Growth

- temperature 25 - 30°C
- oxidative conditions
- new barrels
- poor sanitation
- cross-contamination
- barrels washed in cold water
- no aggressive barrel sanitation

Winemaking Operations to Discourage Brett Growth

- temperature < 16°C
- keep containers topped/closed
- older, but uninfected barrels
- good hygiene

- keep infected wine separate
- high-pressure hot water wash
- ozone/burn sulfur wick in barrel

Ozone Treatment

Ozone is one of several tools that winemakers use to help control biological growth, including *Brettanomyces* yeast. Ozone is frequently the method of choice in attempts to sanitize barrels and help prevent Brett growth. Ozone may be effective in helping to control Brett growth but only under the proper conditions:

- High-pressure water wash of barrel.
- Thorough blast with sharp stream of hot water.
- Rinse for 2-3 minutes.
- Remove all organics.
- Cool barrel down completely.
- Treat with ozonated water.
- Filter and deionize water before ozonating.
- Ozone effectiveness is a function of time and concentration. At least 2-2.5 mg/L ozone in barrel, 0.1 mg/L out.

In the case of any and all sanitation procedures, monitoring is the only way to know the true effectiveness.

Brett monitoring can be accomplished by the winemaker by using simple Brett Sniff or Sniff Brett commercial culture bottles. These small jars contain a growth medium conducive to Brett's formation of metabolites. A small sample of wine from a barrel or tank is added to the culture bottles. They are kept warm for a day or two. The presence of a Brett-type smell is presumptive indication of viable Brett.

Addendum: pH vs. Titratable Acidity

It is important that the winemaker understand the relationship between pH and titratable acidity. When an organic acid, or the salt of an acid, is dissolved in solution, it dissociates (separates) into charged components known as ions. Organic acids dissociate partly; salts dissociate completely:

CH_3COOH	→	CH_3COO^-	+	H^+
acetic acid		acetate ion		hydrogen ion

CH_3COONa	→	CH_3COO^-	+	Na^+
sodium acetate		acetate ion		sodium ion

pH is directly related to the hydrogen ion concentration; acidity is dependent upon the acid concentration and the extent of dissociation. If sodium acetate and acetic acid are added to a solution, the following would be present:

CH_3COOH	CH_3COONa	CH_3COO^-	H^+	Na^+
acetic acid	sodium acetate	acetate ions	hydrogen ion	sodium ions

If additional acetic acid is then added to this solution, some of the dissociated acetate ions will bind with the H^+ ions already in solution (opposite charges attract), and some of the H^+ ions from the added acid will bind with the acetate ions in solution, forming acetic acid. The result of this binding of hydrogen ions is to cancel, somewhat, the effect the addition of acetic acid has upon pH.

Thus, although an acid addition has been made, this addition did not change the hydrogen ion concentration (pH). This resistance to change is known as

buffering, and it is particularly important in grape juice and wine. This principle explains why it is sometimes difficult to affect changes in a must or wine pH by adding organic acids.

Practical Summary of Winemaking Issues

- The ability of sulfur dioxide to control microbiological growth is dependent upon the organism, its stage of growth, the composition of the wine, and the temperature.
- Microbial growth can be affected by sorbic acid, lysozyme, temperature, and levels of oxygen, carbon dioxide, alcohol, and phenolic and nitrogen compounds.
- Fermentation issues include vineyard environment, yeast preparation, strains, and population levels, nutrient addition and timing, oxygen, carbon dioxide, sulfur dioxide, sugar, and alcohol levels, pH, non-soluble solids, sedimentation, presence of native yeast and bacteria, fruit rot, poor sanitation, long settling, late inoculation, temperature, fructose, yeast hulls, and pesticides.
- Volatile acidity levels are determined by fermentation parameters, spoilage yeasts and bacteria, and cellar practices.
- “Acetic nose” character may be due to ethyl acetate, rather than acetic acid.
- Volatile acidity may contribute to wine complexity.
- Sugar and alcohol levels have an effect on the perception of high VA levels.
- Blending may be the best treatment for excessive VA levels.
- Brett character in wines is dependent on the ratio of wine components and *Brettanomyces* metabolites, not on absolute amounts of metabolites.

- Production of Brett metabolites may be dependent on both vineyard and winery operations.

Study Questions

1. What are the three principle groups of wine microorganisms? How do they differ in the following: oxygen requirements, sulfur dioxide tolerance, pH optima?

2. What are the major methods for controlling spoilage yeast?

3. What are the best means of controlling Brett growth?

4. Why is sulfur dioxide alone not always an effective means of controlling microbial growth in and on Virginia wines?

5. What are the advantages and disadvantages of having an MLF and yeast fermentation occurring at the same time? What are the factors which suggest that this may be a good idea?

6. Acetic acid bacteria are aerobic, yet it is possible to have a high VA produced prior to the completion of the alcoholic fermentation. How?

7. Under Virginia conditions, what pH increase would you expect post-MLF? What are the factors that influence this increase?

8. It has been noted in Virginia that free run and press run wines can have a very different rate of MLF. What is the likely cause?

9. What are the advantages and disadvantages of using sorbic acid?

10. Some winemakers in Virginia prefer not to use cultured yeasts. What are the advantages and disadvantages of such an approach? When would not using cultured yeast be a potential determent?

11. List features that differentiate *Brettanomyces* yeast from *Saccharomyces* spp.

12. Some products use a muté to have sweet juice to be able to blend back into a wine post-fermentation. One way of producing a muté is to pressurize the juice. What is the principle behind this practice? What are some of the cautions?

13. Oxygen is considered a yeast nutrient. Discuss the effect oxygen has on the yeast cell and the practical concerns or problems that little oxygen causes.

14. Why do some wines with a high spoilage metabolite concentration not seem to have any issues, whereas other wines with much lower concentrations of the same compound seem very spoiled?

15. One reason there has been such a diversity of opinion regarding Brett is that there are a number of different strains. List and discuss strain differences among this group.

16. In Virginia, we have high wine pH which can impact biological stability. For this and other reasons, many winemakers would add acid to the fermentor in hopes of lowering the pH. From one season to another, there can be a different degree of pH reduction per unit addition of acid. Why?

17. Copper sulfate, in the form of Bordeaux mix, is a common vineyard spray material used in Virginia. List and discuss the advantages and possible disadvantages with regard to wine quality.