

# Wine Aging and Monitoring Workshop

Wine/Enology-Grape Chemistry Group



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**[www.vtwines.info](http://www.vtwines.info)**  
*Enology Notes*  
**On-Line Publications**

# The Enology Analytical Services Laboratory

**Fee-Based Commercial  
Lab Service, no students**

- **Chemical**
- **Physical**
- **Microbiological**
- **Sensory**

**TTB Certified Lab**



## *Our Brettanomyces Studies*

**H.M. McMahon and B.W. Zoecklein.**

*J. Ind. Micro. Biotech.* 23:198-203.

**A.K. Mansfield and B.W. Zoecklein.**

*Am. J. Enol. Vitic.* 53:303-307.

**K. Fugelsang and B. Zoecklein**

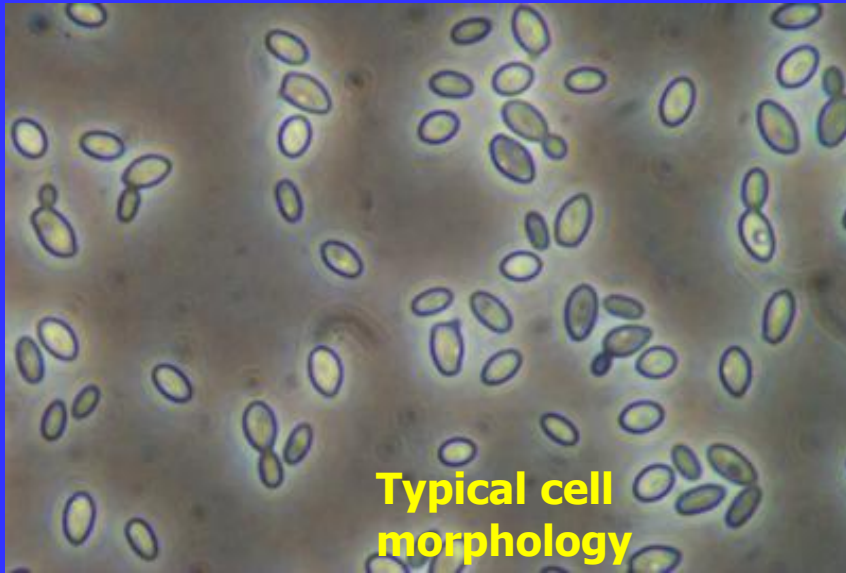
*Am. J. Enol. Vitic.* 54:294-300

*The faster the scientific advances, the greater the risk of widening the gap between what we know and what we do.*

**-Emile Peynand, 1984**



# The many faces of *Dekkera/Brettanomyces*...



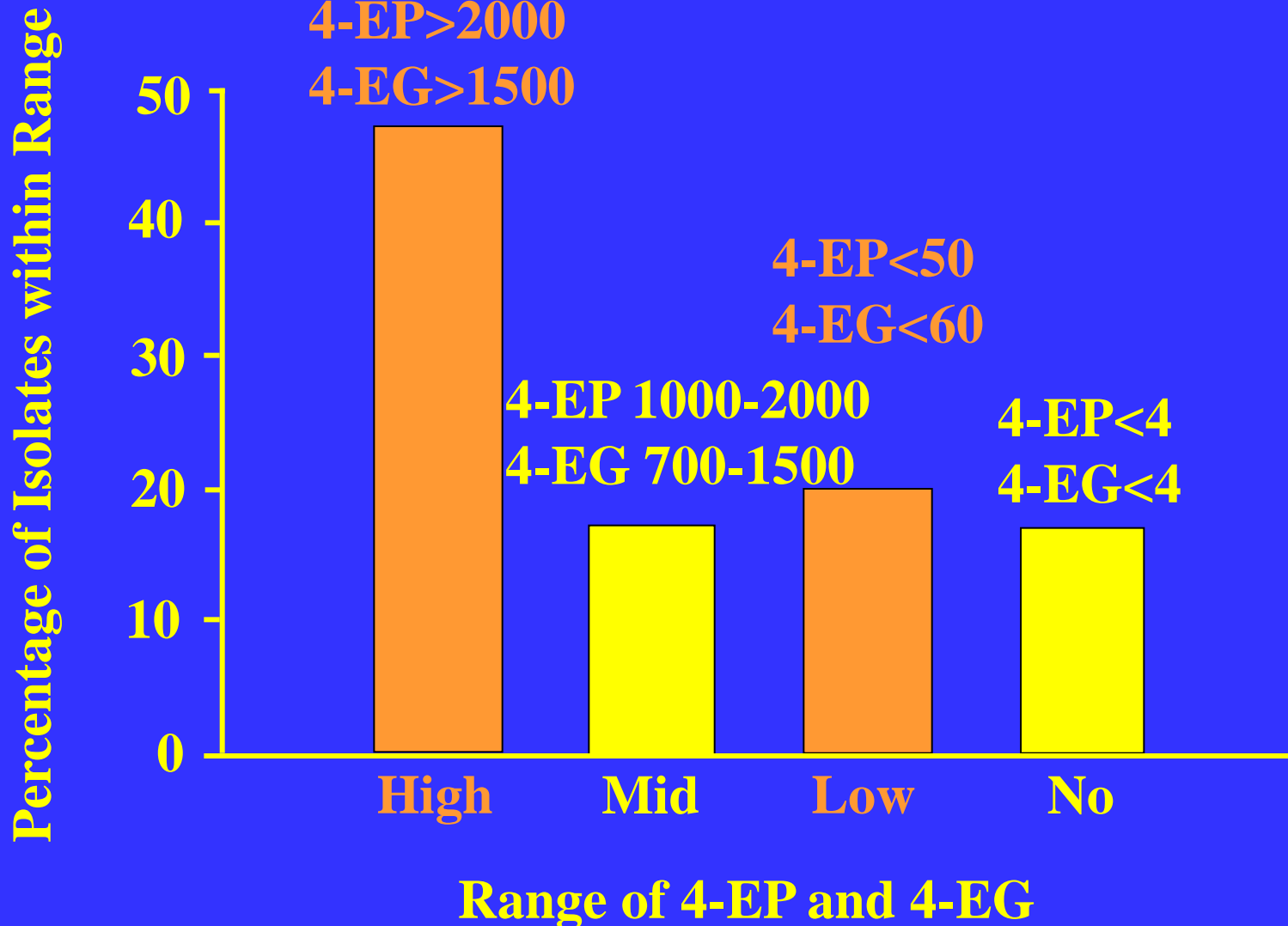
# *Brett* Descriptors

- **Positive**
  - **Complex**
  - **Mature**
  - **Spicy**
- **Negative (partial list)**
  - **Animals**
    - **Sweaty horse/saddle**
    - **Wet dog**
    - **Manure**
    - **Barnyard**
    - **Mousy aftertaste**
  - **Plastic**
    - **Band-aids**
    - **Burnt plastic**
  - **Other**
    - **Burnt beans**
    - **Rancid**
    - **Metallic**

# *Brett* Metabolites

- 4-Ethylphenol
- 4-Ethylguaiacol
- Furfural
- 3-methyl-2-buten-1-ol
- Guaiacol
- Isobutyl alcohol
- Isobutyric acid
- Isovaleric acid
- Propionic acid

# Significant Strain Variation in 4-EP and 4-EG Production





# Palate Balance Equation

**Volume / Body  $\longleftrightarrow$  Acid + Phenols**

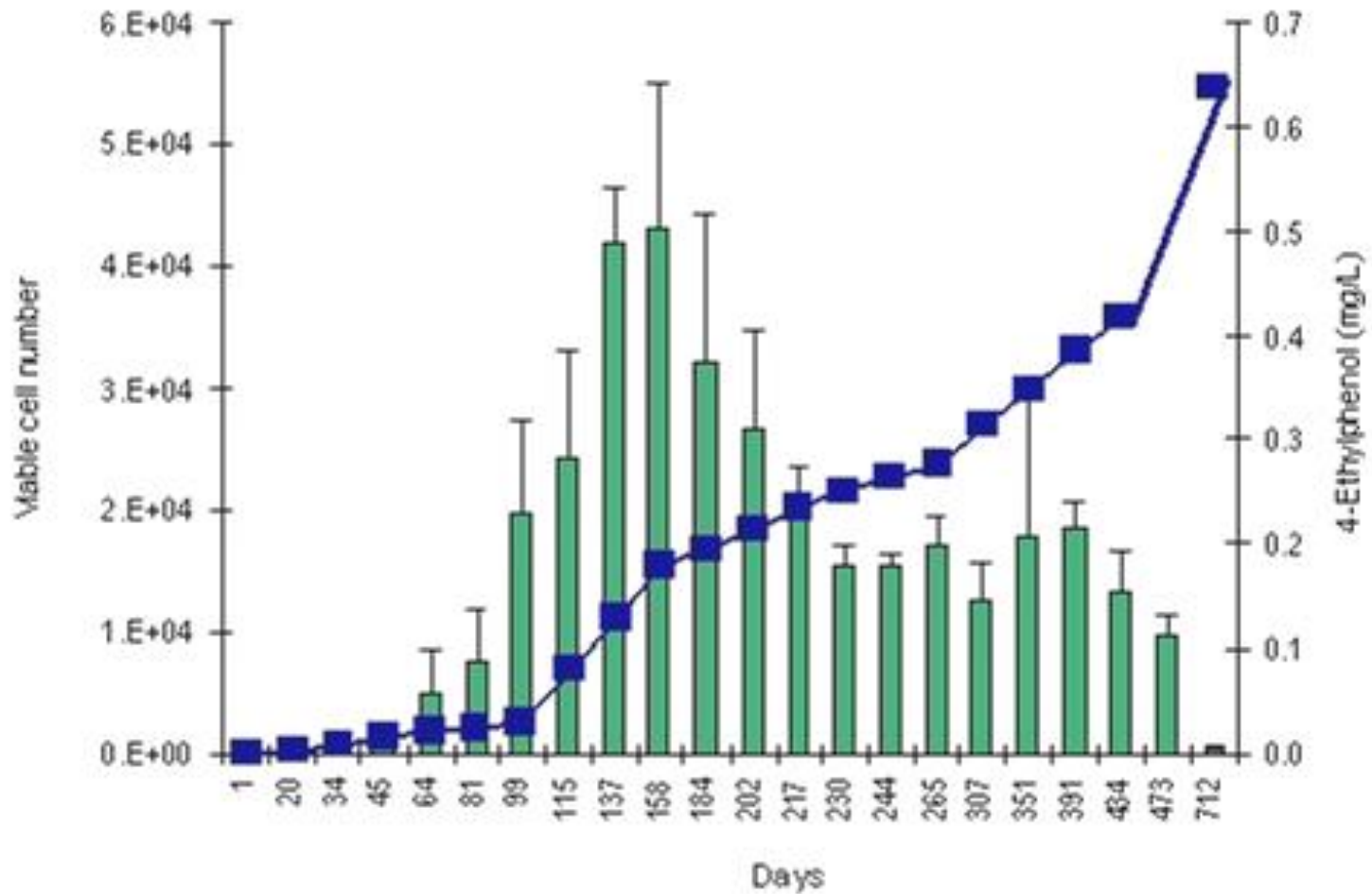


# *Brett Growth*

## Physical effects

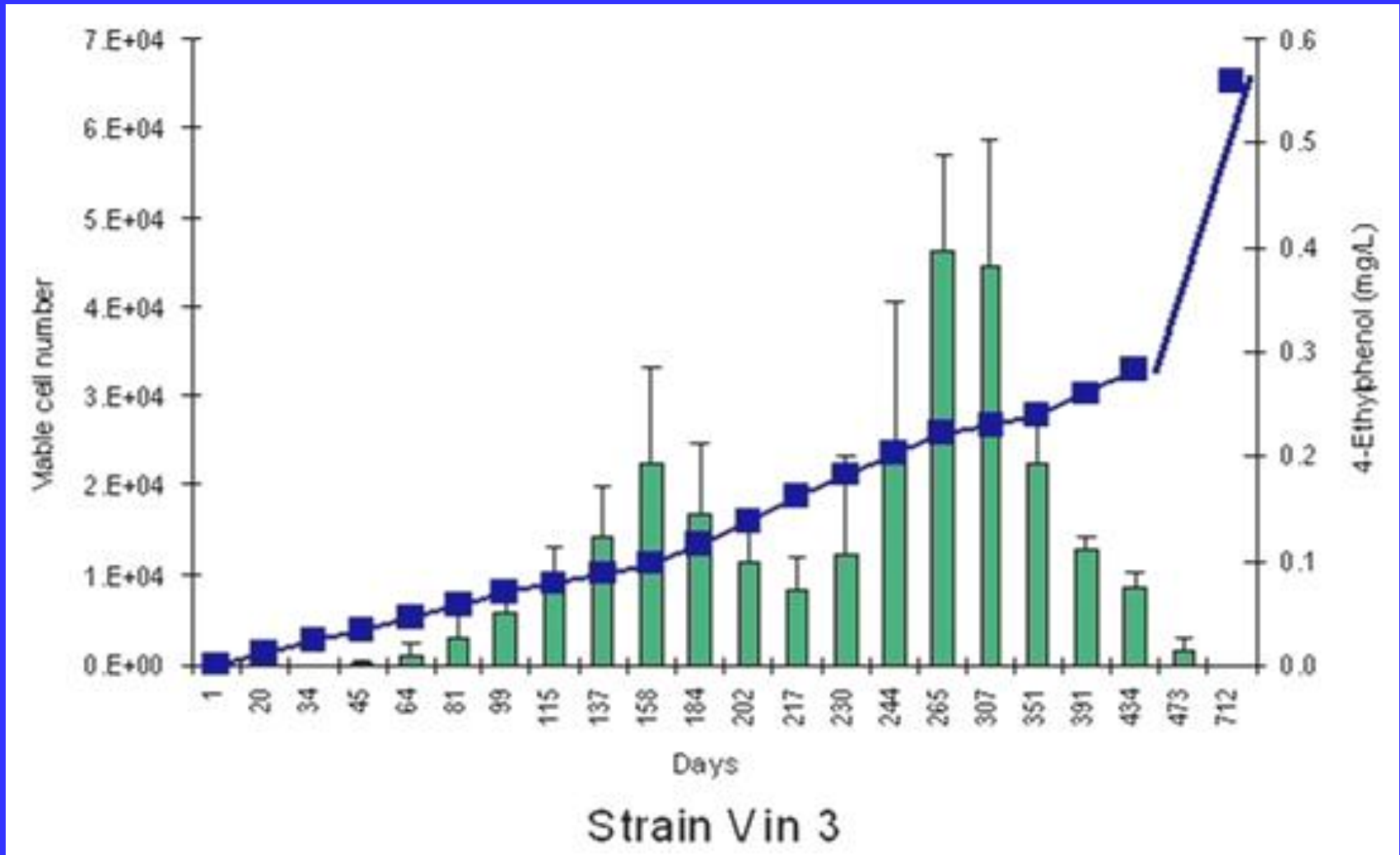
- Usually grows slowly, over many months
- Can grow within weeks if conditions are favorable
- Grows in the wine, almost never as a surface film
- Growth is stimulated by oxygen, but very little is required
- Slight CO<sub>2</sub> gas
- Sediment in bottle

# Results (cont.)

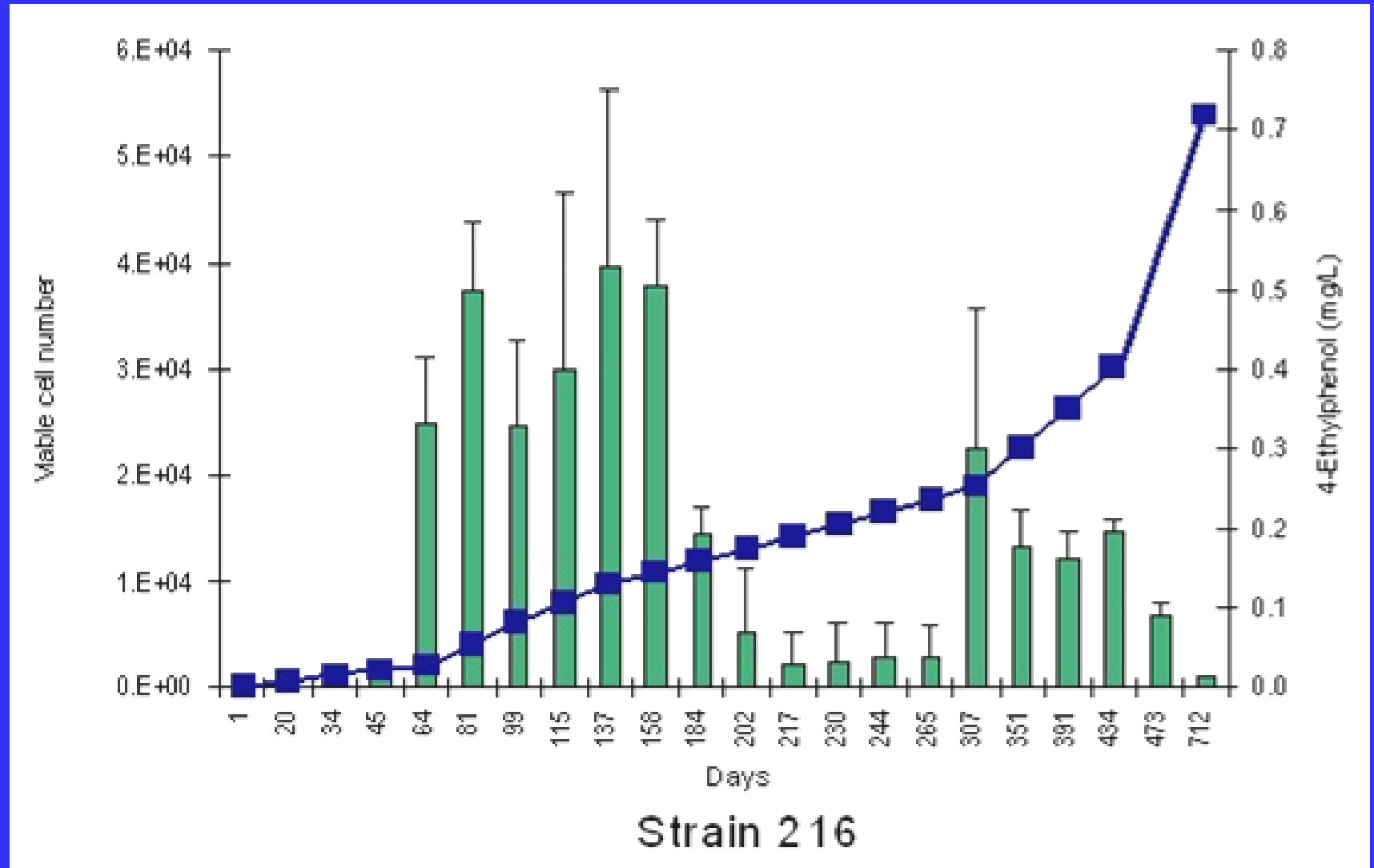


Strain 213

# Results (cont.)



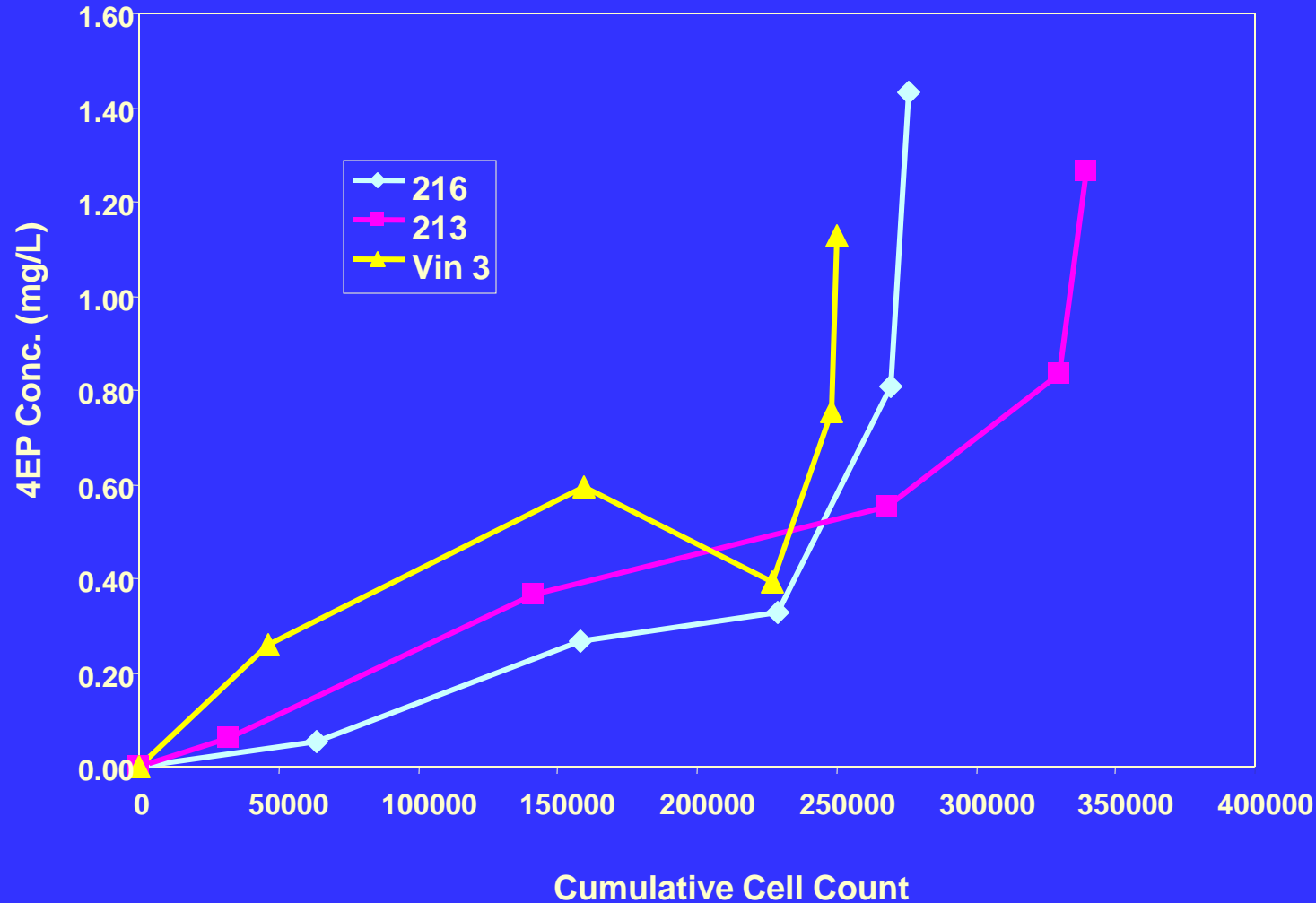
# Results (cont.)





# *Brettanomyces*

4-EP vs Cum. Cell Count, averaged





## Things to Consider

- Blooms may be explained by VNC.
- Large range of 4-ethylphenol (4-EP) and 4-ethylguaicol (4-EG).
- Most 4-EP and 4-EG produced after the population reached maximum cell density.
- Strong correlation between 4-EP and viable cell density is not as strong as the correlation with cumulative cell density.
- There were significant sensory differences among strains.



# Monitoring Brett

**The key to monitoring and management is to have a good HACCP plan in place (See [www.vtwines.info](http://www.vtwines.info))**

## **Methods & Problems:**

- **Metabolite analysis**
- **Culturing**
- **Sensory analysis**
- **Antibody methods**
- **Genetic markers: PCR, Scorpions**

# Practical Culturing Method

**Brett Sniff/ Sniff Brett**

- **Growth media for the detection of ethyl phenols**
- **Allows detection in as short as 2-3 days**
- **Simple-does not require any pipettes, filters or plating**
- **Evaluate ethyl phenol odor intensity vs. incubation time to estimate cell population**
  
- **Some Limitations**

**Available from several sources**

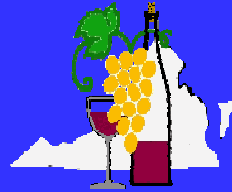
# Monitoring Brett

## Methods & Problems:

- **Metabolite analysis**
- **Culturing**
- **Sensory analysis**
- **Antibody methods**
- **Genetic markers: PCR, Scorpions**

**The key to monitoring and management is to have a good HACCP-like plan in place (See [www.vtwines.info](http://www.vtwines.info))**

# Sensory Evaluation Brett



Virginia Tech

- **Evaluate representative samples**
- **Evaluate under proper conditions (temperature, TNSS, environment)**
- **Use reference standards and contrast**
- **Minimize prejudice and bias**
- **Interpret results appropriately**

# Monitoring *Brett*: Sensory Detection

- Train to recognize danger signals using standards
  - When sensory effects are noticeable, it may be too late
- Matrix effect: cultivar, phenol composition Q and Q, metabolites:

Tempranillo 4-EP 125, Cabernet Sauvignon 420 ug/L

- Synergistic effect on detection level:
  - 4-EP + 4-EG=426 ug/L
  - 4-EP alone=620 ug/L
- ❖ High 4-EP can mean High Brett character
- ❖ Low 4-EP can mean High Bret Character

# Brett Aromas

Sensory threshold levels depend on the matrix

- **4 EP 120-1200 ng/L**  
**Band-aids, Plastic**
- **4 EG 70-150 ng/L**  
**Smokey, Spice, Burnt Beans, Medicinal**
- **Isovaleric Acid 1200 ng/L**  
**Rancid, vomit, barnyard**
- **Combination of these and other metabolites**  
**Provide the typical sweet horse, leather, horse blanket-type odors**



# Encourage/Discourage *Brett*

- To ENCOURAGE

## Winemaking operations

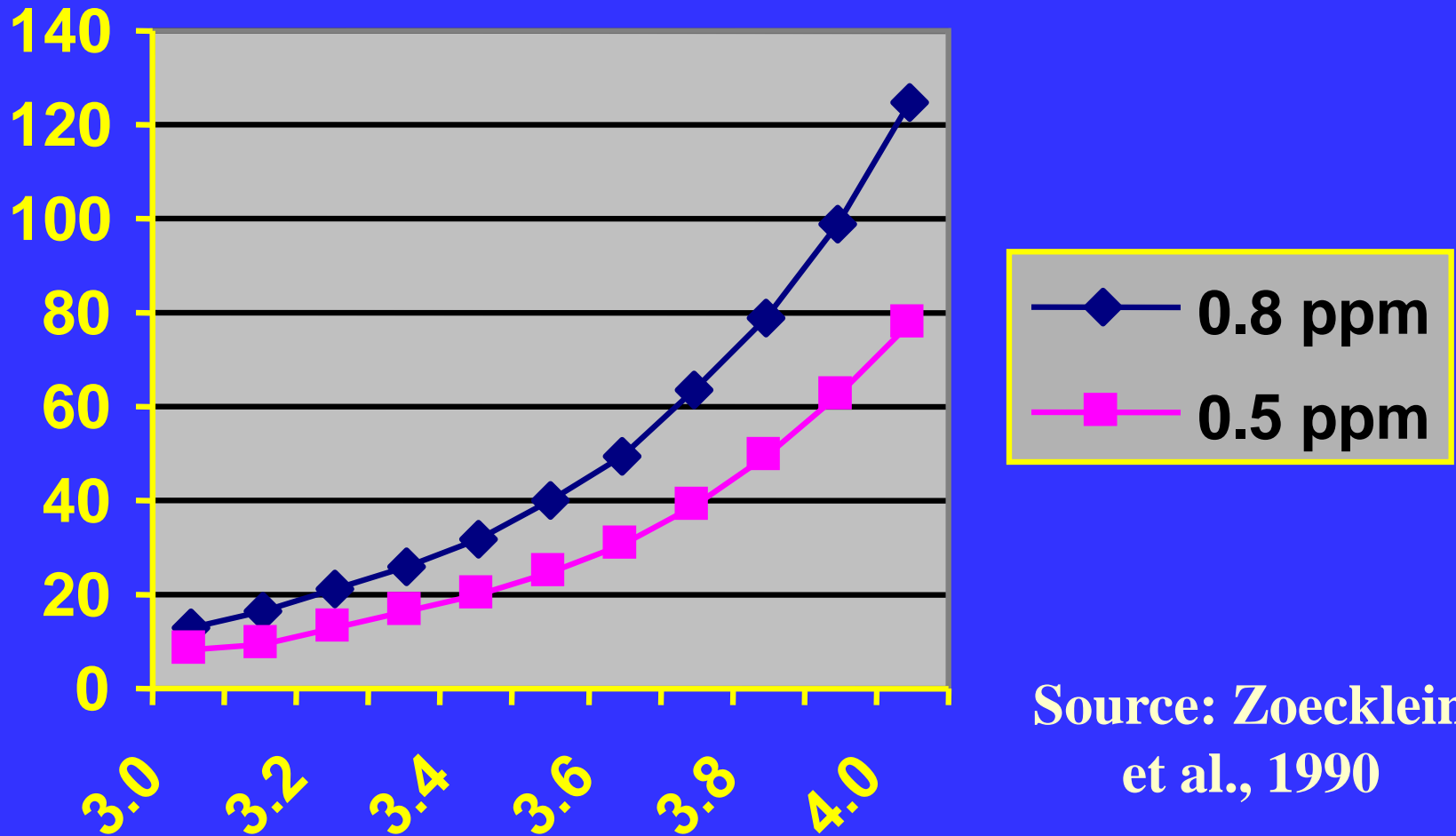
- High N
- Heavy lees
- Slow MLF
- Temperature 25-30 C
- Oxidative conditions
- New barrels
- Poor sanitation
- Cross-contamination
- Barrels washed in cold water
- No aggressive barrel sanitation

- To DISCOURAGE

## Winemaking operations

- Low N
- Light lees
- Rapid MLF completion
- Temperature < 16 C
- Low pH
- Keep containers topped / closed
- Older but uninfected barrels
- **Good hygiene**
- **Keep infected wine separate**
- **Optimum SO<sub>2</sub> management**
- High-pressure hot water wash
- Ozone/burn sulfur wick in barrel

# Free SO<sub>2</sub> Needed to Achieve 0.5 and 0.8 ppm Molecular SO<sub>2</sub>, at Different pHs



Source: Zoecklein  
et al., 1990

# Effect of Barrel Sanitation Procedures -

## Barrel sanitation experiments

- *Brett / Dekkera* was found 8 mm deep in staves.
- Barrels cannot be “sterilized” with SO<sub>2</sub>, rinsing, or ozone.

Isolate *Brett*+ barrels.

# Ozone Summary

- Strong oxidizing agent
- No chemical residue
- Half-life at ambient conditions 10-20 minutes
- Degrades microbial bio-films
- Degrades natural rubber
- Is a surface active agent-does not penetrate

# Ozone Treatment

- **High-pressure water wash barrel**
  - Thorough blast with sharp stream of hot water
  - Rinse for 2-3 minutes
  - Must remove all organics
  - Cool down completely
- **Treat with ozonated water**
  - Filter and deionize water before ozonating
  - At least 2-2.5 mg/L ozone in barrel, 0.1 mg/L out
  - Time x Concentration

# Wine Chemistry and Brett

- Alcohol synergistic but will not control
- Glucose and Fructose: 0.275 g/L = 1000 ug/L 4-EP
- VA concentration not correlated with 4-EP or 4-EG
- pH effects molecular FSO<sub>2</sub>
  
- Sulfur dioxide and pH synergistic
  - Fewer additions but larger concentration
- Old wood vs. new wood
  - cellobiose
  - 0.275 g/L can produce 1000 ug/L 4-EP
- Oxygen stimulates growth
- Biofilm formation

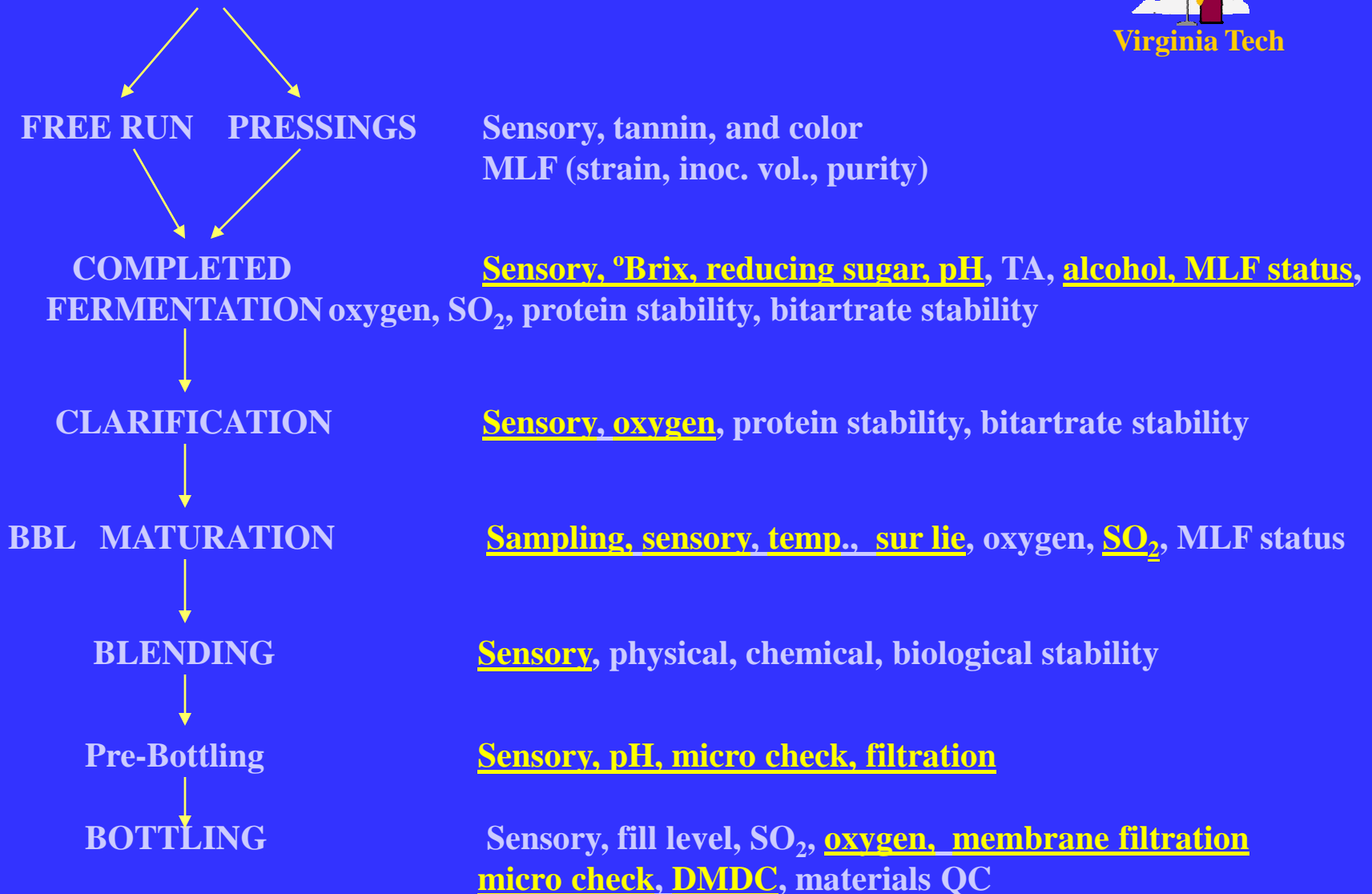


# HACCP Critical Control Points for Brett

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## Sanitation and Monitoring





# Monitoring *Brett*

- **Have a HACCP-like plan ([www.vtwine.info](http://www.vtwine.info))**
- **Isolate contaminated barrels**
- **Sample barrels with disposable plastic pipets**
- **Top with *Brett*-free wine (filtered, pasteurized and/or Velcorin-DMDC)**
- **Keep barrels topped-up or not opened**
- **Monitor carefully before bottling**

# Additional Info/Assistance

- Enology Notes
- On-Line Publications
- VT Enology Service Laboratory