Brettanomyces Research Findings and Management

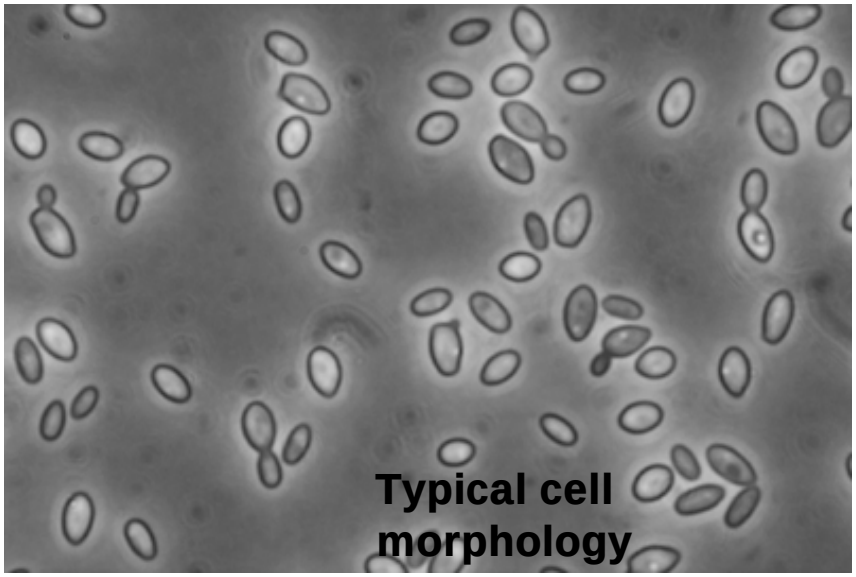
Wine/Enology-Grape Chemistry Group

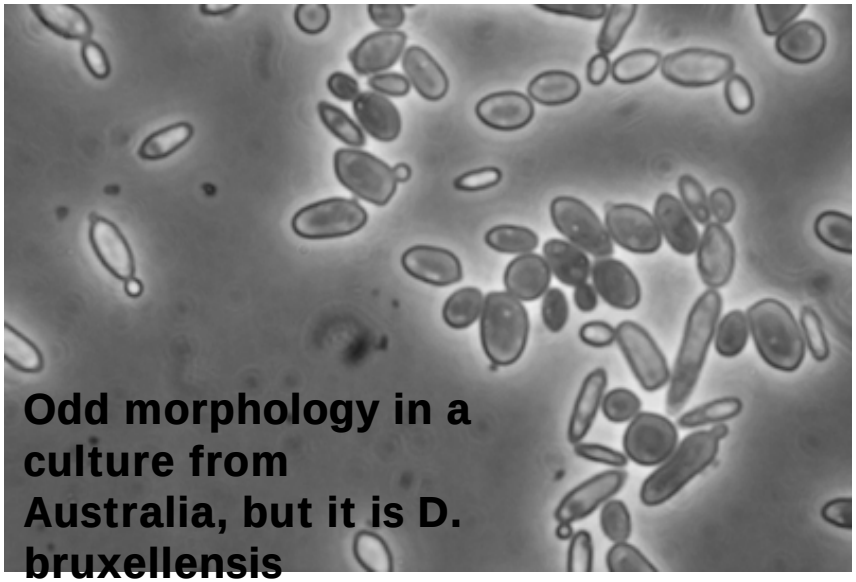
Virginia Tech

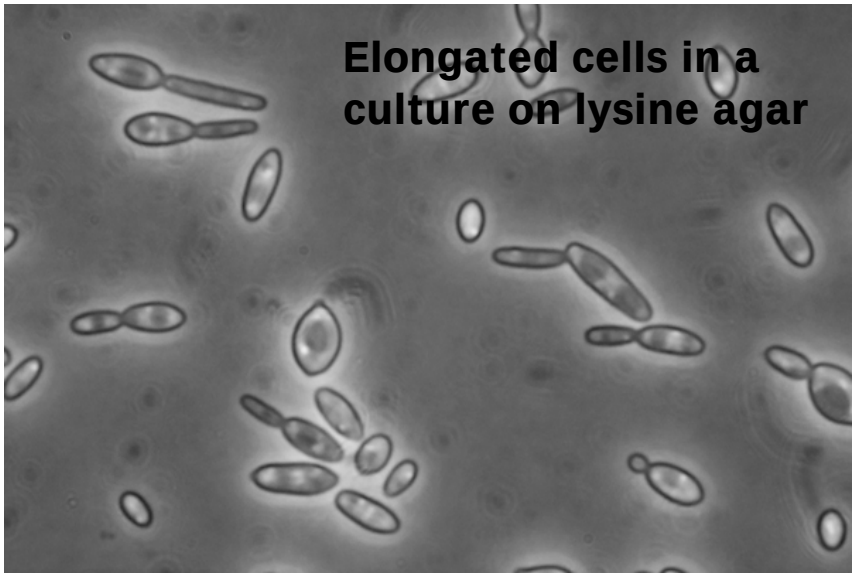
Bruce Zoecklein
Head, Enology—Grape Chemistry Group
Virginia Tech
Blacksburg, Virginia

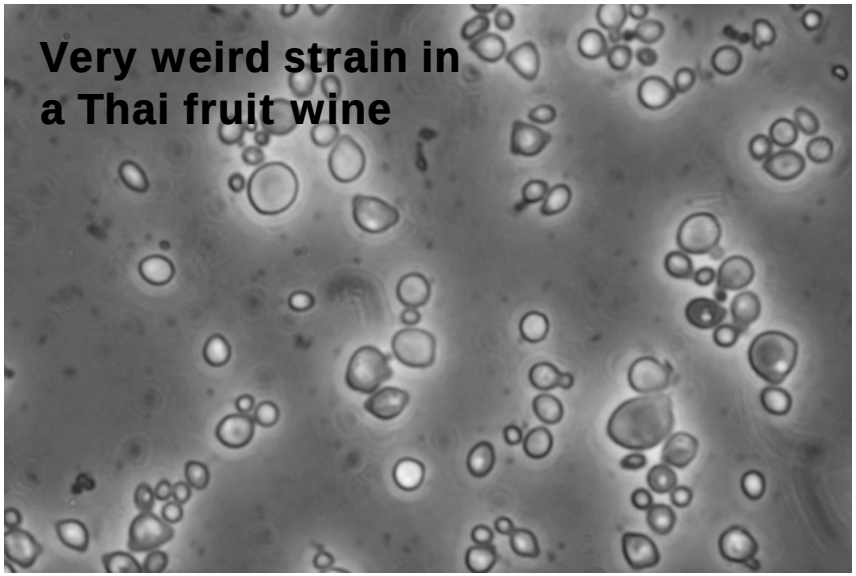
www.vtwines.info
***Enology Notes* #92**

The many faces of *Dekkera*/*Brettanomyces*…









Source: Lisa Van de Water

Brett Descriptors

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

Population dynamics and effects of *Brettanomyces bruxellensis* strains on Pinot noir wines

Bruce Zoecklein
Enology-Grape Chemistry Group
Virginia Tech, Blacksburg

Ken Fugelsang
Department of Vitculture and Enology
California State University, Fresno

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

Brettanomyces bruxellensis: Comparison of Growth Profiles and Metabolites among Ten Strains in Pinot Noir Wine

- ***Question: Can differences in winemaker's experiences with* Brettanomyces *growth in wine be attributed to strain differences?***

Experimental Design:

Ten genetically-characterized strains of *B. bruxellensis*

- **Pinot noir: 30 mg/L sulfur dioxide at crush. Ferment to dryness, press, clarify at 5°C (6 weeks).**
- **Rack to sterile containers, DMDC @ 700 mg/L.**
- **Bottle.**
- **Initial inoculum: 50 CFU/mL (10 strains x 4 replications) + controls.**

Sampling

Weekly samples were plated for growth and chemical analysis for up to 712 days.

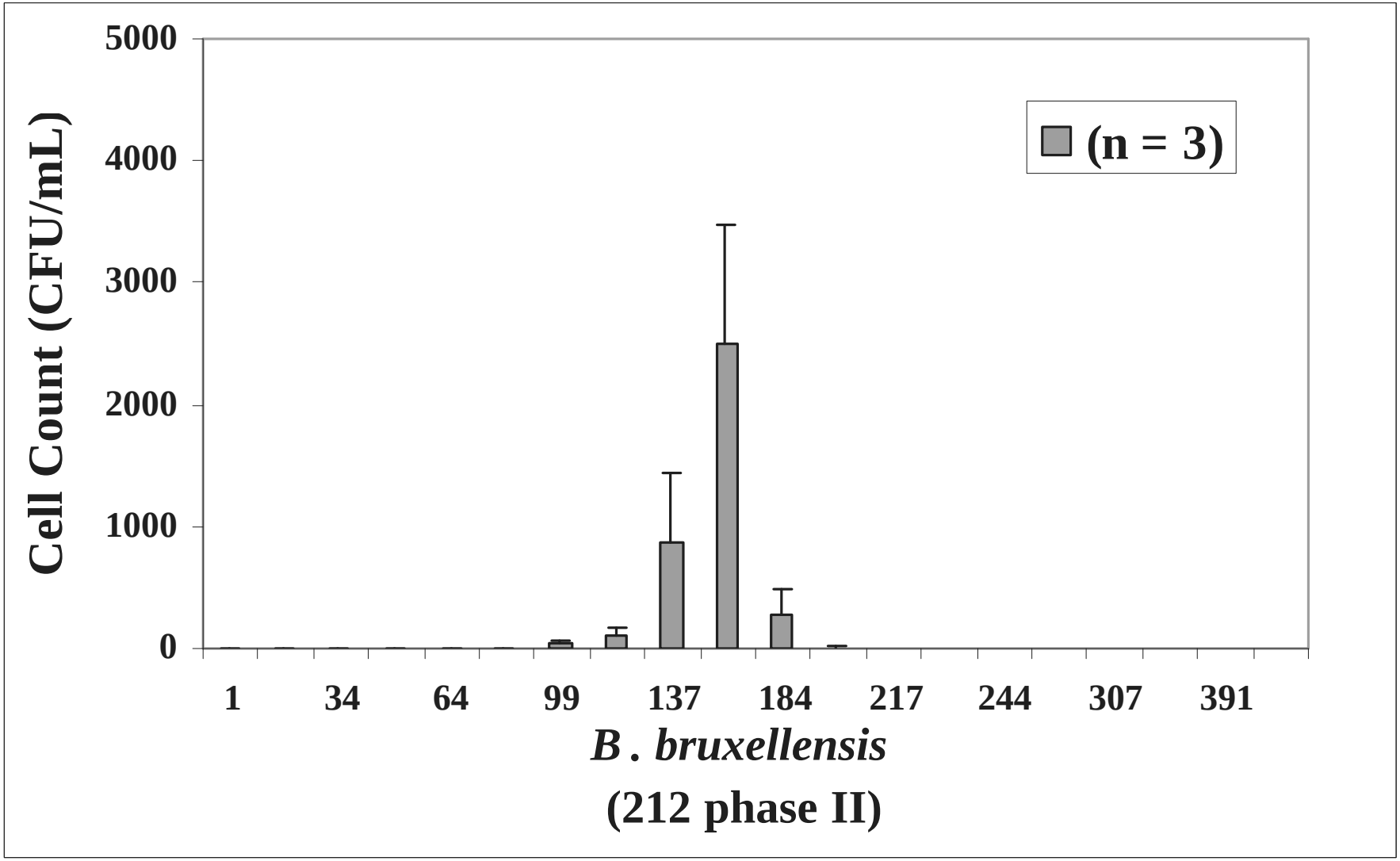
Analyte quantification by HE-SPME, GC/MS:

4-Ethylphenol (4-EP)

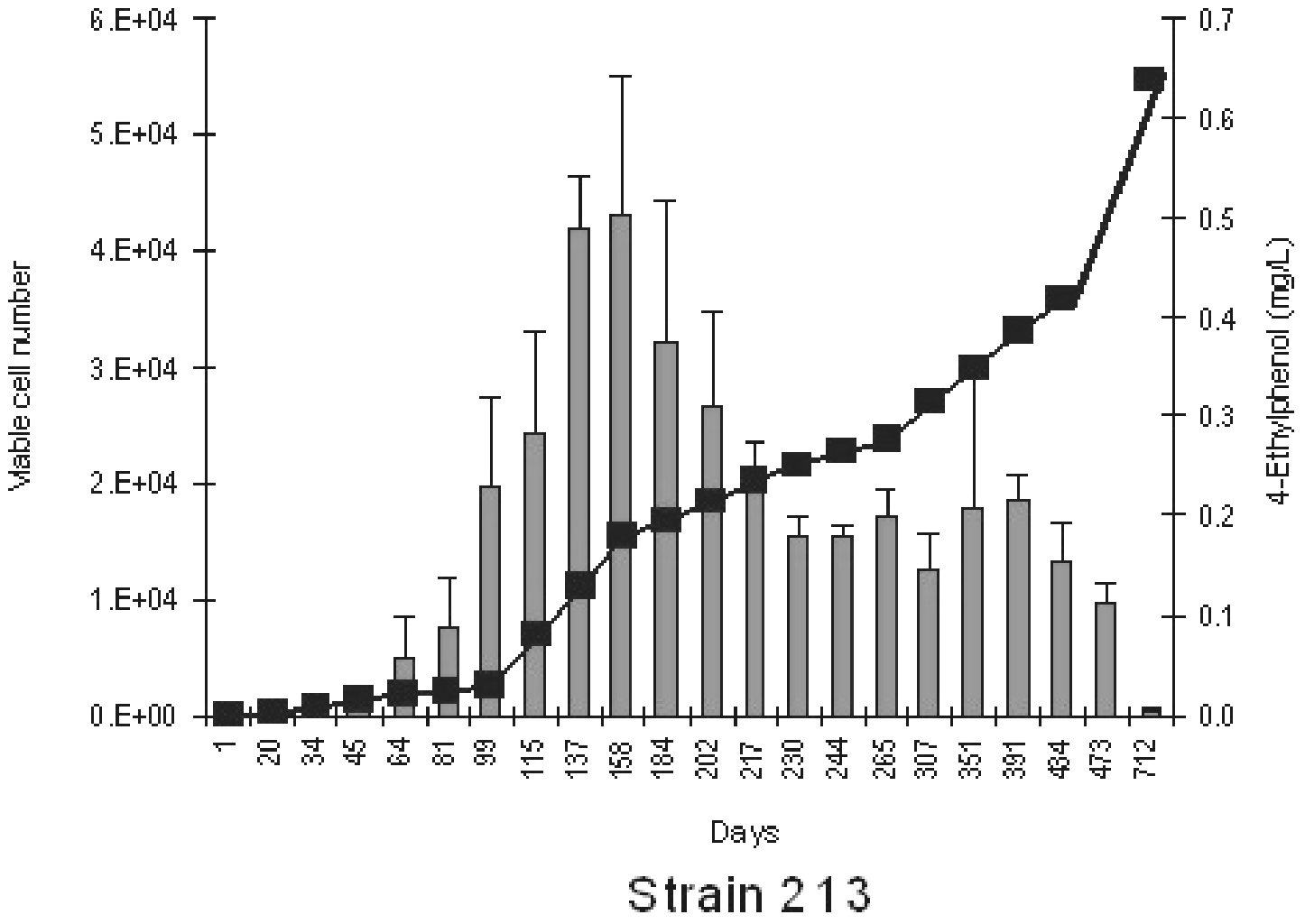
4-Ethylguaiacol (4-EG)

2-phenylethanol
Guaiacol
Isovaleric acid
Ethyldecanoate
trans-2-Nonenal
Isoamyl alcohol
Ethyl-2-methylbutyrate

Results

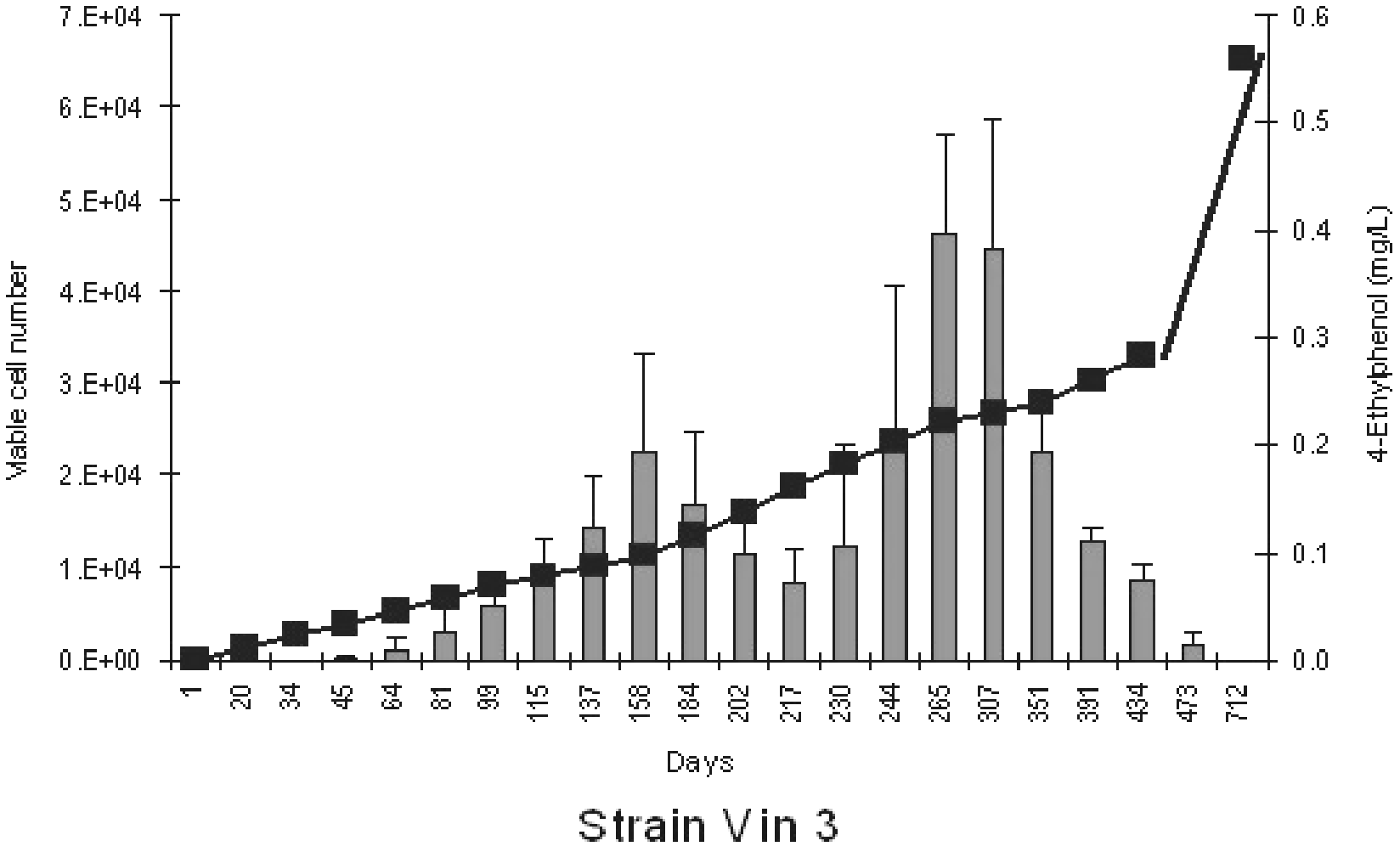

5000
4000
3000
2000
1000
0
Cell Count (CFU/mL)
(n = 3)
1
34
64
99
137
184
217
244
307
391
B . bruxellensis
(212 phase II)

Results (cont.)

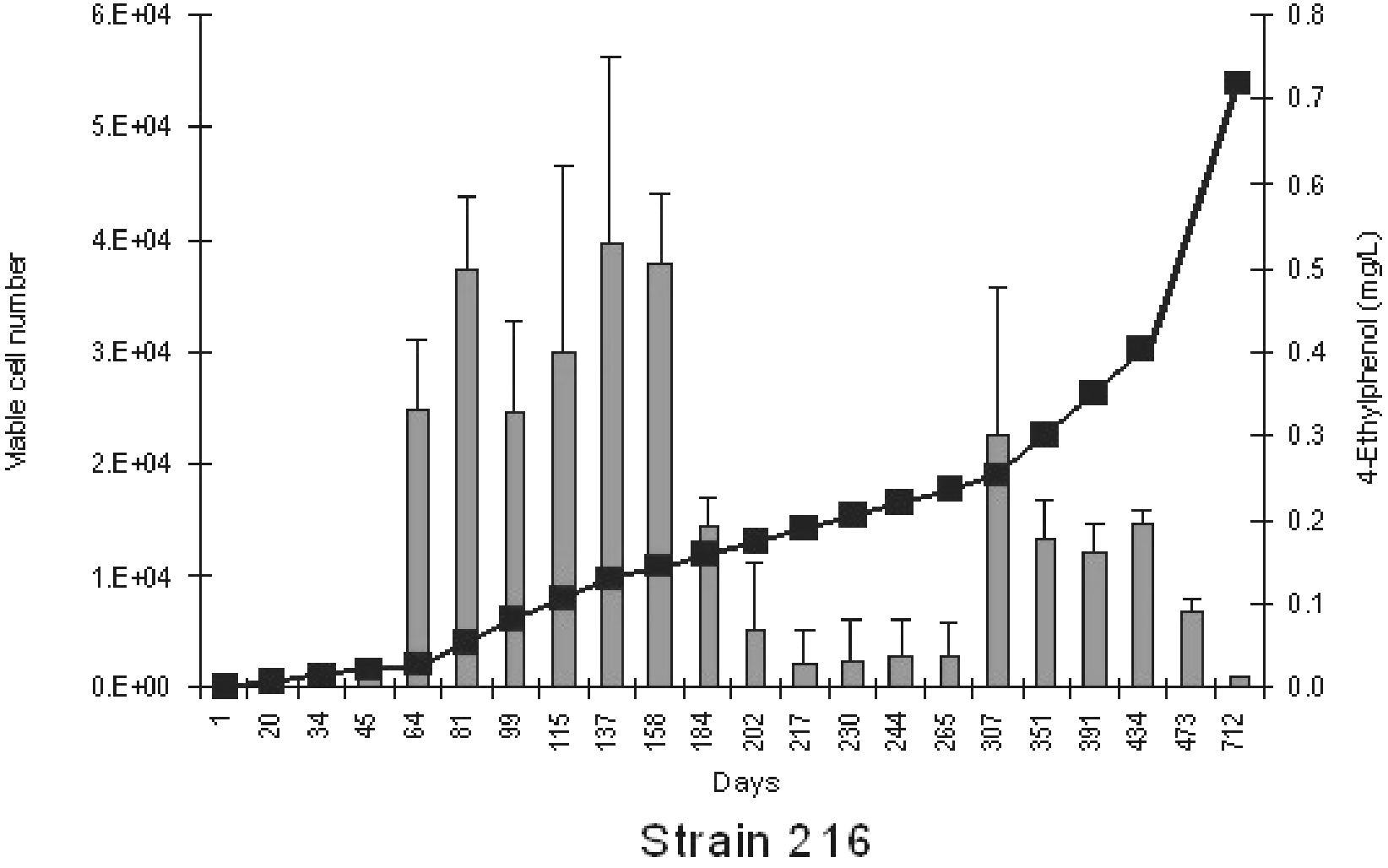


Strain 213

Results (cont.)



Results (cont.)



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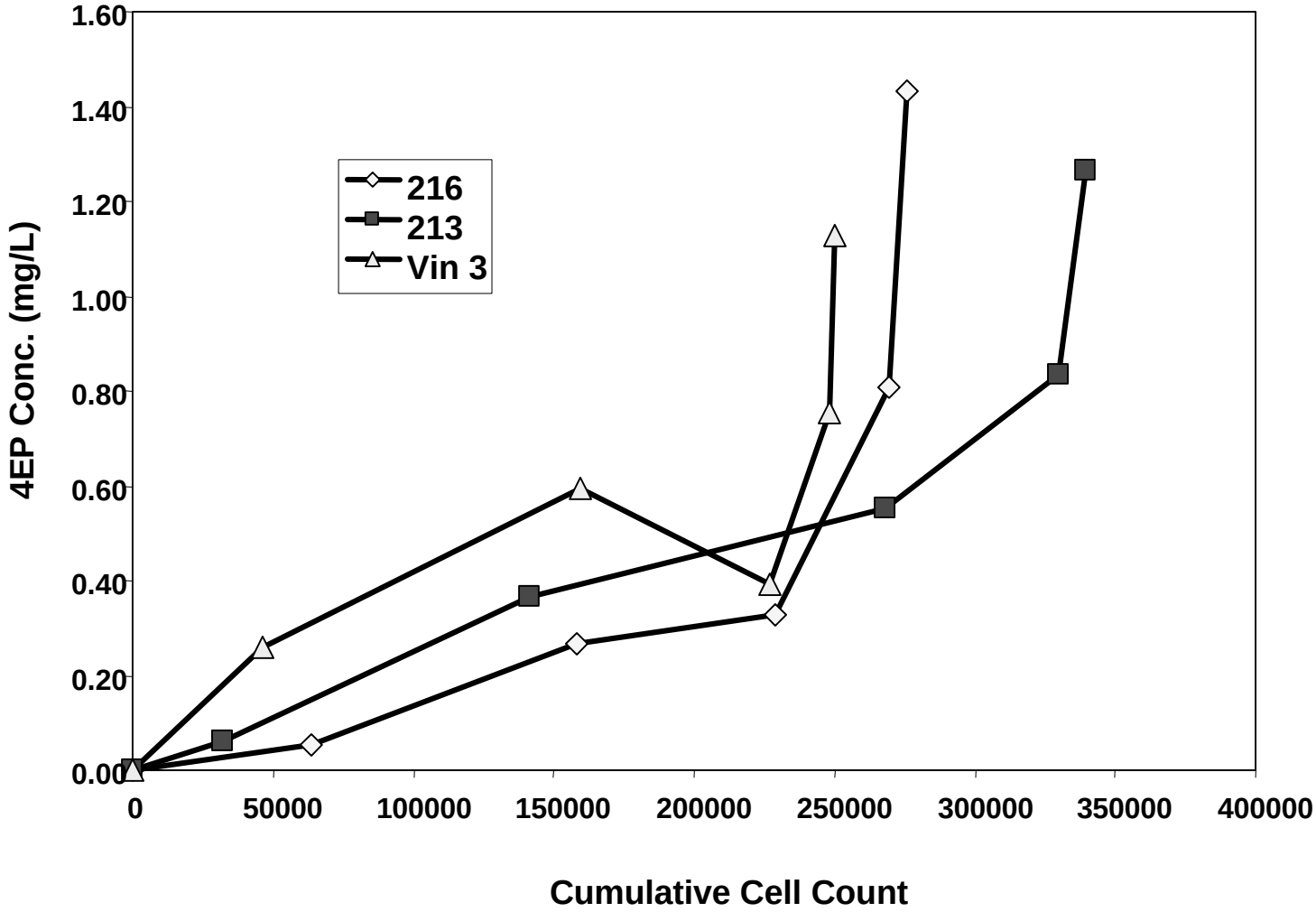
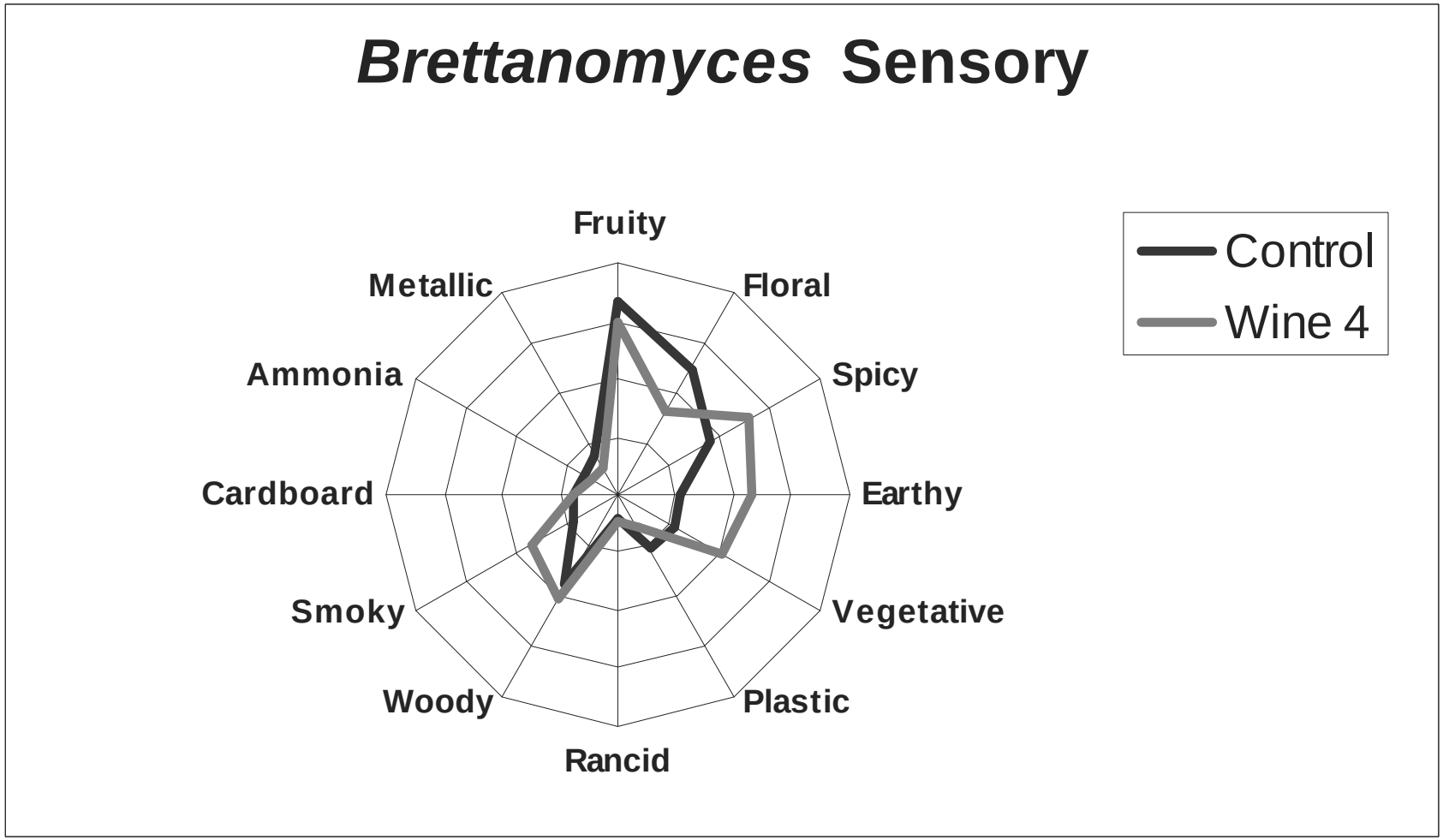
Brettanomyces

4-EP vs Cum. Cell Count, averaged



Sensory Evaluation



Conclusions

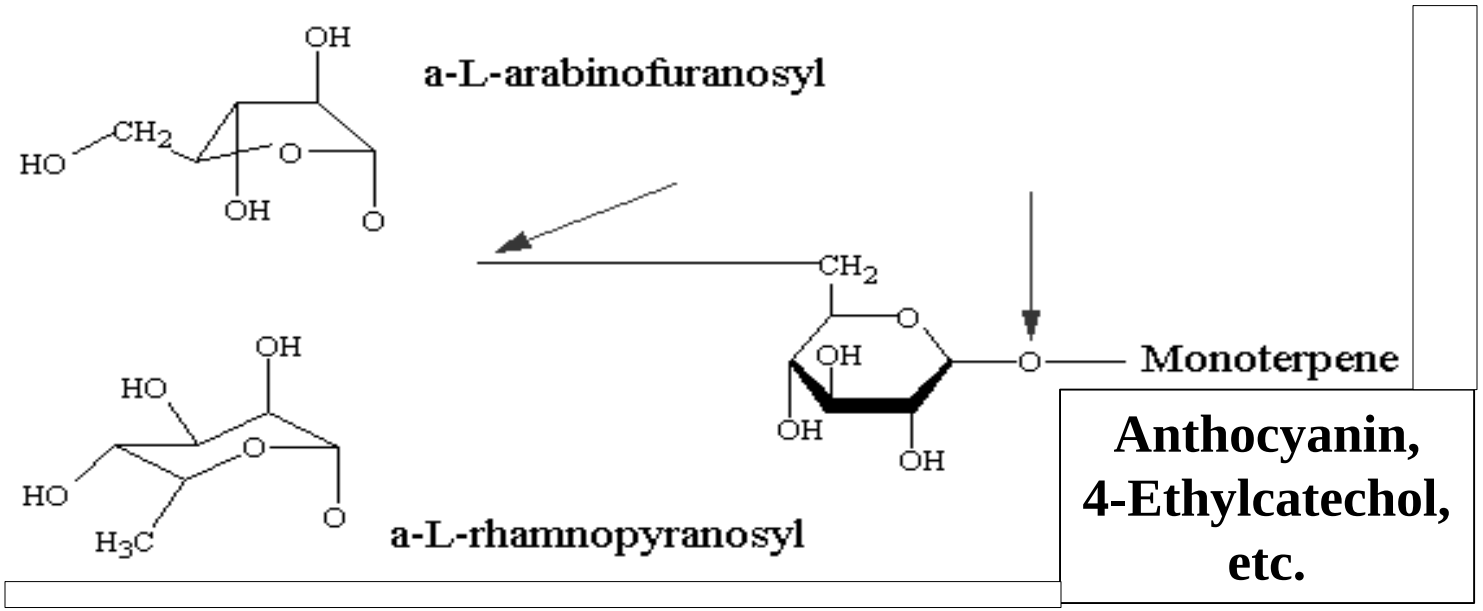
- **Significant strain differences in length of growth cycle and peak population densities.**
- **Blooms may be explained by VNC.**
- **Large range of 4-ethylphenol (4-EP).**
- **Large range of 4-ethylguaicol (4-EG).**
- **4-EP and 4-EG correlated.**
- **4-EP and 4-EG not correlated to isovaleric acid (IVA).**

Conclusions

- **With the exception of one strain, most 4-EP was produced after the population reached maximum cell density.**
- **The correlation between 4-EP and viable cell density was not as strong as the correlation with cumulative cell density.**
- **There were significant sensory differences among strains.**
- **4-EP correlated to low glucose/fructose.**

Important Enzymes: Esterases, Glucosidases

- **Glycosidases**



Glycosidase Activity in *Brettanomyces bruxellensis*, other Yeasts, and *Oenococcus oeni*

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.**

B. bruxellensis strain	Whole Cell	Permeabilized	Supernatant
211	27 c	142 e	11 bcd
212	5 d	341 a	9 bcd
213	34 c	105 f	14 bcd
214	19 c	110 f	6 cd
215	< LOD	74 g	11 bcd
216	59 b	321 b	24 a
Brux	26 c	182 d	11 bcd
Souche ‘Ave’	14 c	138 e	7 cd
Souche ‘O’	< LOD	< LOD	4 d
Souche ‘M’	82 a	179 d	9 bcd
Vin 1	32 c	14 g	< LOD
Vin 3	22 c	232 c	9 bcd
Vin 4	65 b	25 g	4 d
Vin 5	4 d	21 g	11 bc

Conclusions

- **Large variation among strains in total enzyme activity.**
- **Eight strains of *Brettanomyces bruxellensis* had high *beta*-glucosidase activity (670-2,650 nM/mL/g dry cells).**
- **Large variation in supernatant and permeabilized activity.**

Results of Physiological Tests

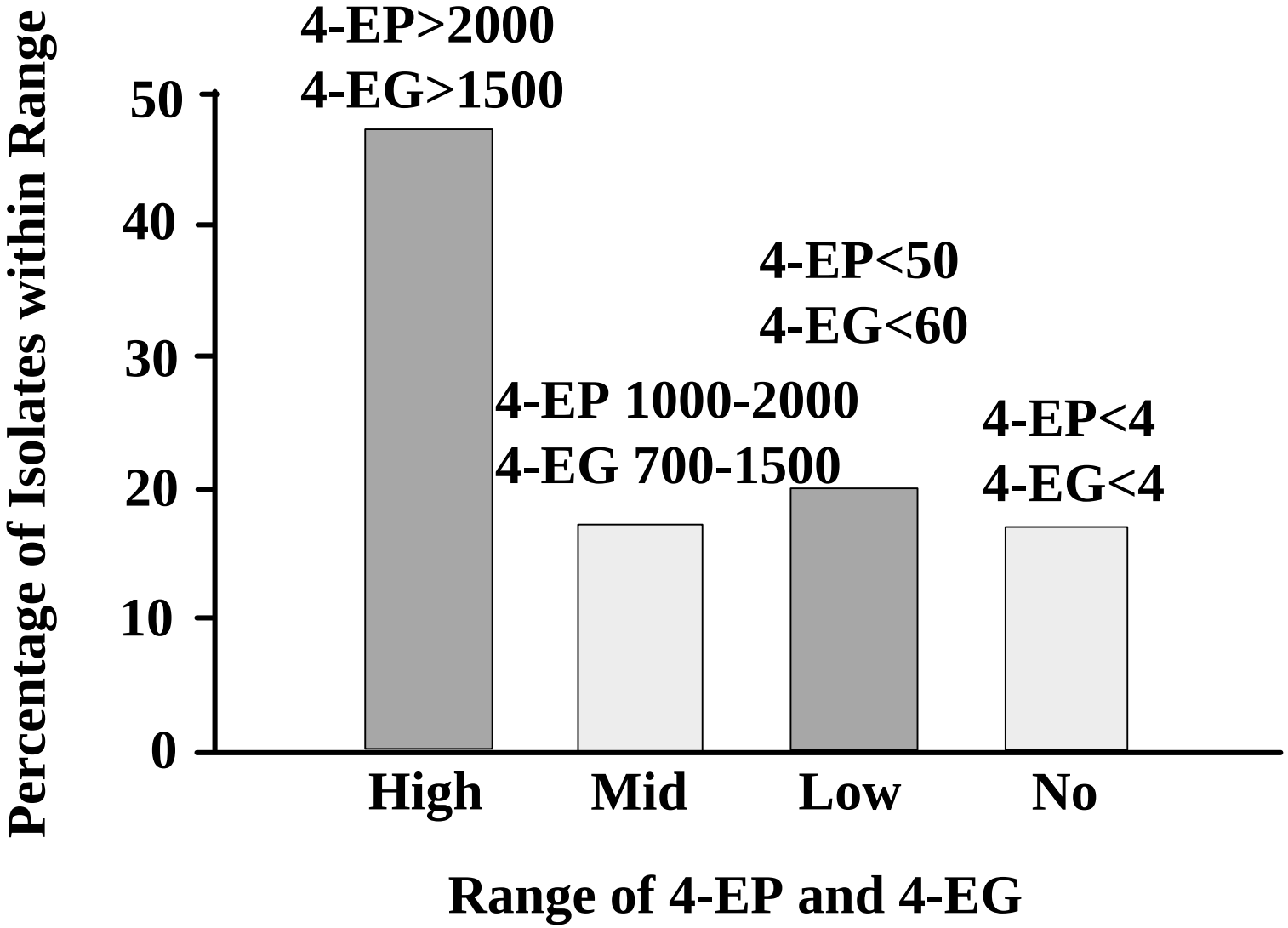
L. Joseph, T. Henick-Kling, L. Conterno

- **Regional differences in metabolism**
  - **75% of European strains used malic acid, 12% CA strains did**
  - **All CA strains used nitrate, < 30% of European strains did**
  - **63% of European strains used ethanol, 18% CA strains did**
  - **Most CA strains grew at 37 C, no European strains did**

Physical Characteristics

- **All isolates tolerant to 10% ethanol or higher.**
- **33 isolates grew well at pH 2.**
- **More than 30% of isolates grew at 10^{o} C.**
- **More than 35% of isolates grew at 37^{o} C.**
- **3 isolates (about 10%) grew at both temperature extremes.**
- **Almost 50% showed tolerance to 30 mg/L or greater free SO_2 at pH 3.4.**

4-EP and 4-EG Production



Climate Impact on *Brett* Metabolites

Henschke, 2004

- **4-EP / 4-EG decrease in cool regions**
- **Malvidin-3-*p*-coumaryl glucoside may be precursor to 4-EP**

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_2 gas**
  - **Sediment in bottle**
- **Sensory effects**
  - **Reduced varietal character**
    - **Esterase activity degrades some fruity aromas**
    - **Floral aromas are also reduced**
  - **Aromatic compounds**
  - **Bitter/metallic finish**
  - **Sometimes: mousy taint (ACPY/ACTPY)**
- **Lots of strain variation**

Brettanomyces Detection

- **Sensory**
  - **Train lab and production crew to recognize danger signals using standards**
  - **When sensory effects are noticeable, it may be too late**
- **Matrix effect**

Brett Standards

- **Components of FlavorSense, San Rafael, CA, *Brett* standard**
  - **4-Ethylphenol**
  - **4-Ethylguaiacol**
  - **Furfural**
  - **3-methyl-2-buten–1-ol**
  - **Guaiacol**
  - **Isobutyl alcohol**
  - **Isobutryic acid**
  - **Isovaleric acid**
  - **Propionic acid**

Brettanomyces Detection

- **Direct Microscopic Examination**
  - **Difficult when < 1000 cells/ml**
  - **Requires skill in identifying cells**
- **Culturing**
  - **Sampling method is very important**
  - **Detects only microbes that are present and alive**
  - **Disadvantages:**
    - **Must select and prepare media properly**
    - **False negatives (VNC)**
    - **Takes time for growth (3-7+ days)**
    - **Requires skill in identifying colonies**

Brettanomyces Detection

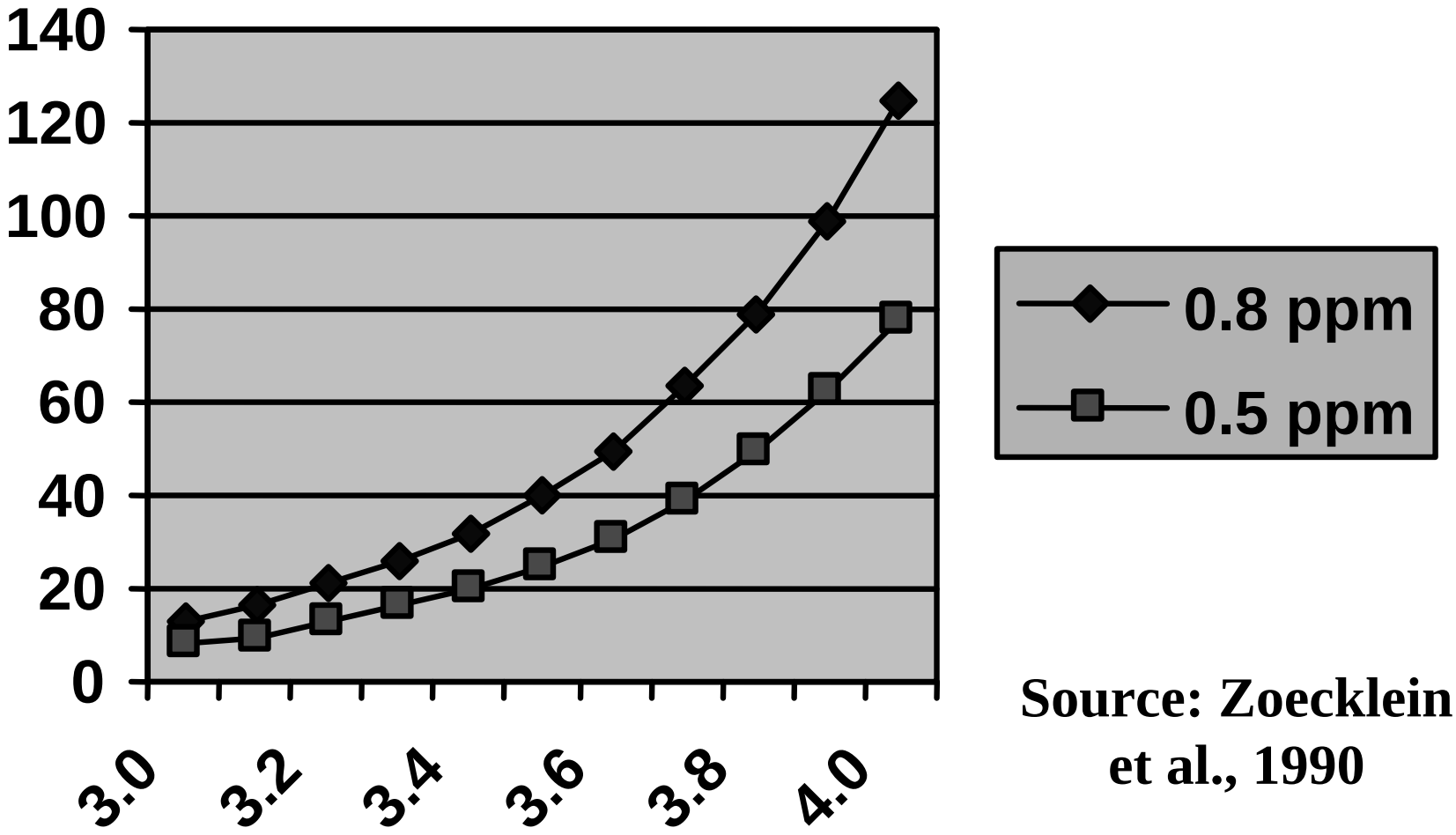
- **Chemical analysis**
  - **‘Marker’ compounds: 4-EP, 4-EG, fatty acids**
  - **Tests metabolites, not activity itself**
    - **Not necessarily directly related (e.g., 4-EP vs. culturing)**
- **Antibody-based methods**
  - **ELISA**
    - **A. Kuniyuki et al. *Am. J. Enol. Vitic*. 35:143-145**
- **Nucleic Acid-based methods**
  - **Polymerase Chain Reaction (PCR)**
    - **J. Ibeas et al. *Appl. Environ. Microbiol.* 62:998-1003**
    - **L. Cocolin et al. *Appl. Environ. Microbiol.* 70: 1347-1355**
    - **T. Phister and D. Mills *Appl. Environ. Microbiol.* 69:7430-7434**
- **Problems: False positives, expensive, hand-held ‘field’ unit**

Keys to *Brettanomyces* Management

Generally find only 1 biotype in a wine

- **Wine composition**
  - **Minimize substrates for growth**
    - **N (Formol titration, www.vtwines.info or *Am. J. Enol. Vitic.* 53:325-329.)**
  - **SO_2 management**
  - **pH management**
- **Winemaking operations**
  - **Cellar temperature**
  - **Population monitoring and control**
  - **Cellar and barrel sanitation / hygiene**
  - **Preparation for bottling**

Free SO_2 Needed to Achieve 0.5 and 0.8 ppm Molecular SO_2, at Different pHs



Source: Zoecklein et al., 1990

Encourage/Discourage *Brett*

- **To ENCOURAGE**

  <u>Wine composition</u>
  - **Red wine**
  - **pH > 3.6**
  - **Molecular SO_2 < 0.2 mg/L**
  - **Alcohol 13% or below**
  - **Residual hexose sugars**
  - **Biotin, thiamine**
  - **Amino acids**
  - **Yeast lees present**

- **To DISCOURAGE**

  <u>Wine composition</u>
  - **White wine**
  - **pH < 3.6**
  - **Molecular SO_2 0.4 mg/L or greater**
  - **Alcohol > 13%**
  - **RS < 0.2 g/L**
  - **Vitamins depleted**

    "Nutrient desert"
  - **Clarified**

Source: Lisa Van de Water

Encourage/Discourage *Brett*

- **To ENCOURAGE**

  <u>Winemaking operations</u>
  - **Temperature 25-30 C**
  - **Oxidative conditions**
  - **New barrels**
  - **Poor sanitation**
  - **Cross-contamination**
  - **Barrels washed in cold water**
  - **No aggressive barrel sanitation**

- **To DISCOURAGE**

  <u>Winemaking operations</u>
  - **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**

Source: Lisa Van de Water

Effect of Barrique Sanitation Procedures -

Manuel Malfeito-Ferreira, 2004

- **Barrel sanitation experiment**
  - **Cold rinse, then hot water rinse 3x 70 C**
  - **Same as above plus SO2 1 month (200 ppm pH3)**
  - **Cold rinse, fill with 90 C water 15 min**
  - **Cold rinse, 70 C rinse, steam low pressure 10 min**
    - **Most effective treatment**

- ***Brett / Dekkera* was found 8 mm deep in staves.**

Barrels cannot be "sterilized" with SO_2, rinsing, or ozone.

Isolate *Brett*+ barrels.

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**

Source: Lisa Van de Water

Brett and Biofilms

- **Liquid / solid interface**
- **17 / 35 strains form biofilms (Joseph, 2004)**
- **pH effect**
- **Impact of cleaning compounds on biofilms**

Monitoring *Brett*

- **Have a HACCP-like plan (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**

Acknowledgements

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- Virginia Grape Growers Association
- Vino Farms
- Roy Thornton
- Lisa Van de Water