Broadmountain Winery - 8 miles from Armstrong
Mollie Kelly - PSU
enologist

Controlling *Brettanomyces*

Wine yeast = Saccharomyces

Prof. Charles G. Edwards
Viticulture/Enology Team
Washington State University
Pullman, WA, USA

* Always rehydrate yeast.
Add yeast to ~~water~~ must
~~Use~~
* Use a microscope to check wine.

What is "*Brett*"?

Dekkera
Sporulating form

Sewage
Money
Barnyard
Horsey

Saccharomyces (wine yeast) dies w/o oxygen. Non-Sacc (wild yeast) ~~use~~ must have oxygen.
Bad Yeasts:
Kloeckra
Brettanomyces ~~Bruxellensis~~
Controlled by SO_2 + filtration
comes from grape juice concentrate

What is "*Brett*"?

Dekkera
- **Sporulating form**

Brettanomyces
- **Non-sporulating form**
- **Yeast does not form spores in wine**

Different names → SAME YEAST!

Zygosaccharomyces - Very Bad
Creates explosions. Can't be controlled.

Buy new met bisulfite every year

Sources

A. Used barrels.

B. Unfiltered wines brought into winery.

C. Droplets in air.

1. Found in winery processing areas.
(Connell et al., 2002)

D. Found in vineyards?

1. "...grape berry is the primary source..."
(Renouf and Lonvaud-Funel, 2007)

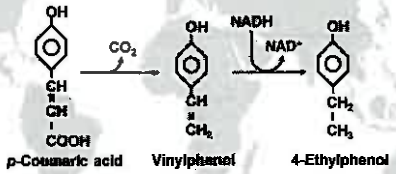
***Brettanomyces* is nowhere...**

...yet everywhere ...

at the same time.

Linda Bisson (2018)

Synthesis of 4-Ethylphenol



Bacteria:

Oenococcus oeni

Conducts malolactic ferm.

Desireable when expected

Undesirable when unexpected

Pediococcus

- Several species (4)
- Spoilage issues
  - Biogenic amines or ropiness
  - Bitterness
  - Excessive diacetyl (butter)
  - Dirty Socks

SO_2 & filtration can control

A- ~~Lactobacillus - 12 species~~

B- Many Spoilage issues

1. Volatile acidity
2. Mousiness
3. Cotton-lobe fibers
4. "Stuck" fermentation"

C- Grow rapidly

Acetobacter

A. ~~Several Species~~

B. Volatile acidity

C. Needs Oxygen for growth

What have we learned about controlling *Brettanomyces*?

- Role of SO_2?
- Ethanol x temperature?
- Removal by filtration?
- Impact of must nutrition
- Survival in oak barrels?

Impact of SO_2

A. Can SO_2 cause "VBNC"?
   1. "Viable but not culturable."
   2. Yeast alive but will not grow on media.
   3. If valid → yeast could be undetected.

B. How to detect VBNC?
   1. Measure metabolic activity or qPCR(?).
   2. Fluorescence microscopy.

Green = viable Orange/Red = dead

Brettanomyces

Active cell

Dead cell

Management

control spoilage by using multiple "hurdles"

~~0.8 mg/L SO_2 thought to inhibit~~ inhibit microorganism.

Minimize post-alcoholic nutrients

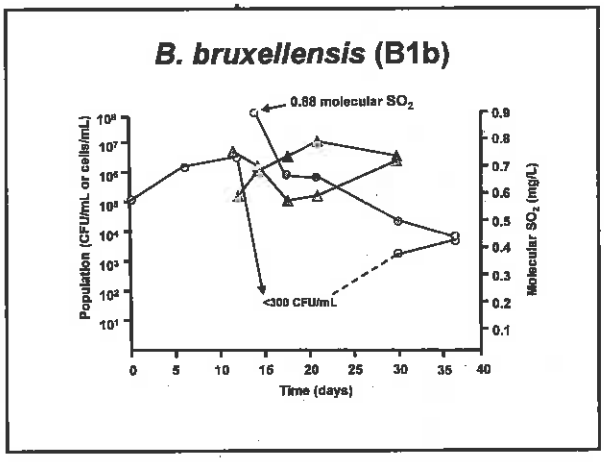
Sanitation Practices

+ Clean and sanitize equipment.
+ watch for dead spots in pipes
+ Be diligent - Monitor your wine! / Walk the cellar - sample wine often.
+ Run chemical + Microbiological tests

Book - Wine Microbiology

Part II

SO_2



A. SO_2 Inhibits browning

B. Binds acetaldehyde - use SO_2 (green apple, bandora)

C Some Microbes are resistant to SO_2 (2y.

D. Saccharomyces is susceptible to SO_2 so don't add too much

Decreasing Ph make the sulfite more effective

Is there a relationship between ethanol and temperature that could be useful to control *Brettanomyces*?

Forms of SO_2:

① Free vs. Bound

② Free molecular vs Sulfite vs Bisulfite

High Ph will cause spoilage. Add "tartaric acid".

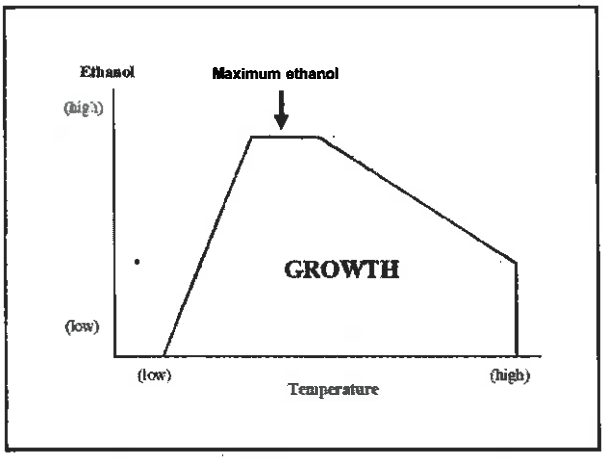
.8 mg/l of molecular SO_2

Free SO_2

Impact of Ethanol

A. Many wine microbes can grow in 12 to 14%.

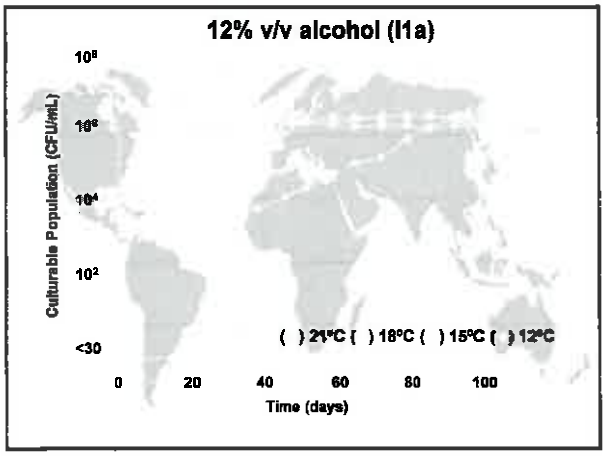
1. ***Lactobacillus fructiverans* can tolerate higher amounts, approaching 16-18%.**
2. **Non-*Saccharomyces* yeasts have lower ethanol tolerance than wine yeast *Saccharomyces*.**
   a. **4 to 5% ethanol.**
   b. **Temperature and pH greatly influence alcohol tolerance.**
3. **What about *Brettanomyces*?**

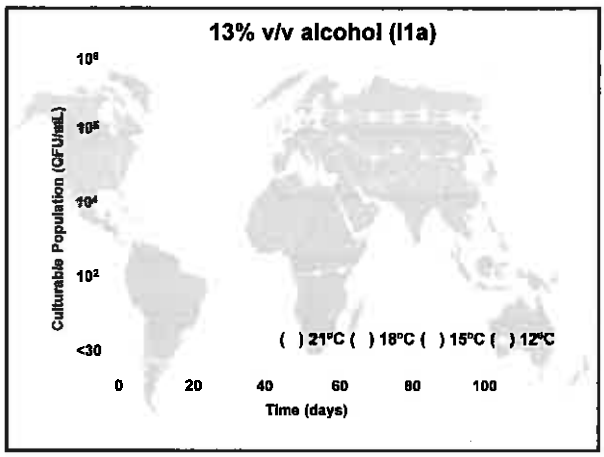


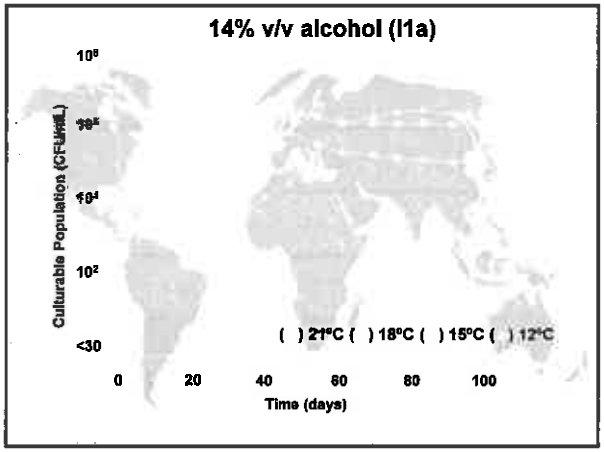
Research Focus
(Ethanol x Temperature)

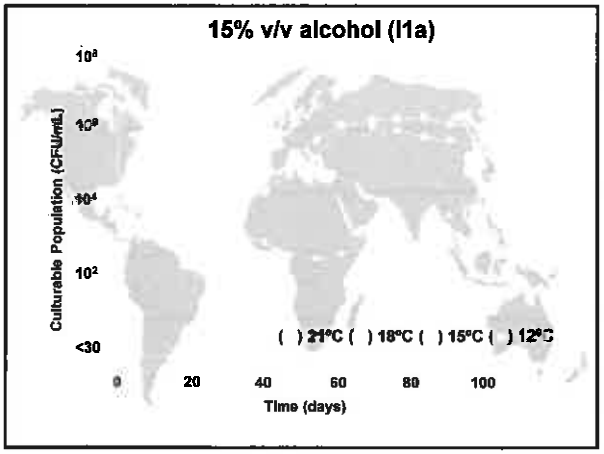
A. General protocol.

1. Inoculate Merlot wine at high populations (>10^4 cfu/mL).
2. Experimental design.
   a. Ethanol = 12, 13, 14, 15, or 16 % v/v
   b. Temperature = 12° , 15° , 18° , or 21° C.
   c. Strains = F3 and I1a
3. Weekly sample wines for 100 days.
4. Measure 4-EP after 100 days.

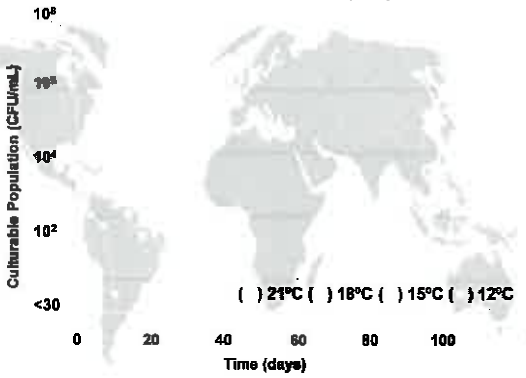



13% v/v alcohol (l1a)
Culturable Population (CFU/mL)
10^6
10^4
10^2
<30
( ) 21°C ( ) 18°C ( ) 15°C ( ) 12°C
0
20
40
60
80
100
Time (days)


14% v/v alcohol (l1a)
Culturable Population (CFU/mL)
10^6
10^2
<30
( ) 21°C ( ) 18°C ( ) 15°C ( ) 12°C
0
20
40
60
80
100
Time (days)


15% v/v alcohol (l1a)
Culturable Population (CFU/mL)
10^2
<30
( ) 21°C ( ) 18°C ( ) 15°C ( ) 12°C
20
40
60
80
100
Time (days)

16% v/v alcohol (I1a)



Production of 4-Ethylphenol (µg/L)

°C	Ethanol (%)	Strain F3	Strain I1a
21°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(+)
	16	(-)	(-)
18°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(+)
	16	(-)	(-)

(-) <30 µg/L; (±) 31 to 1000 µg/L; (+) >1300 µg/L

Production of 4-Ethylphenol (µg/L)

°C	Ethanol (%)	Strain F3	Strain I1a
15°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(-)
	16	(-)	(-)
12°	12	(-)	(±)
	13	(-)	(-)
	14	(-)	(-)
	15	(-)	(-)
	16	(-)	(-)

(-) <30 µg/L; (±) 31 to 1000 µg/L; (+) >1300 µg/L

What about filtration?

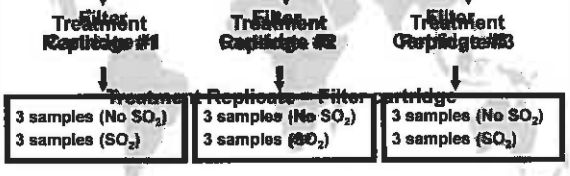
Filtration

A. Desirable to minimize filtration
   1. Expensive, quality concerns.

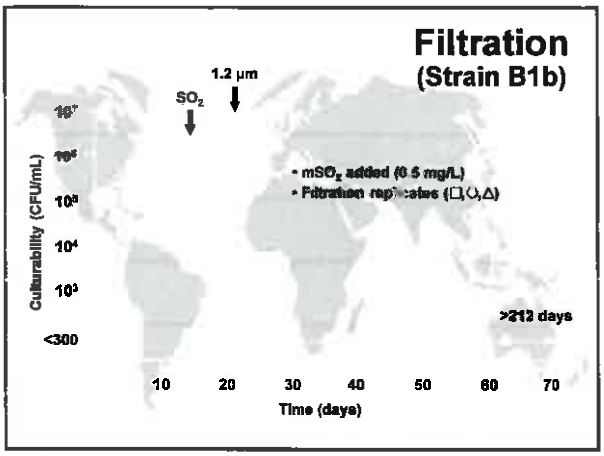
B. "VBNC" cells can exhibit dwarfing.
   1. Cells smaller than normal.
   2. Problem = filtered wines depending on pore size of membrane.

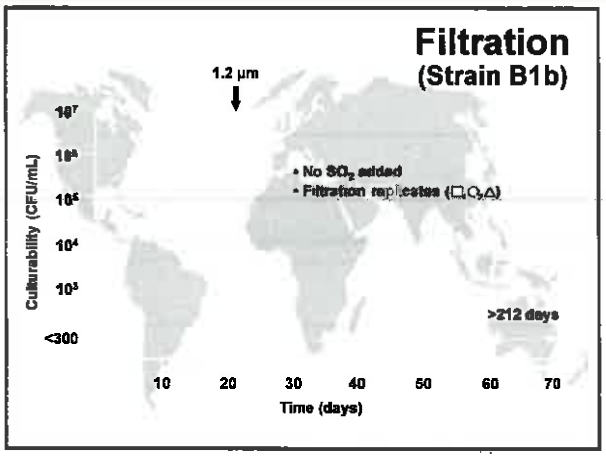
Research Focus
(Filtration)

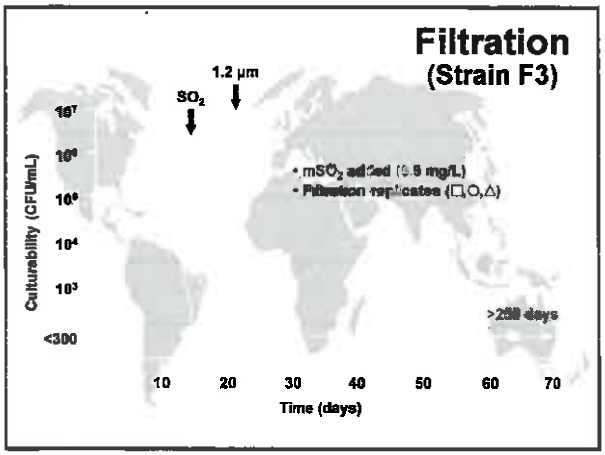
Red wines (0 or 0.5 mg/L mSO_2)

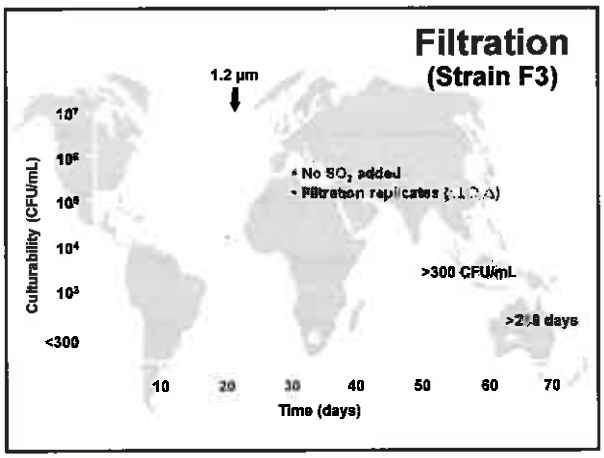


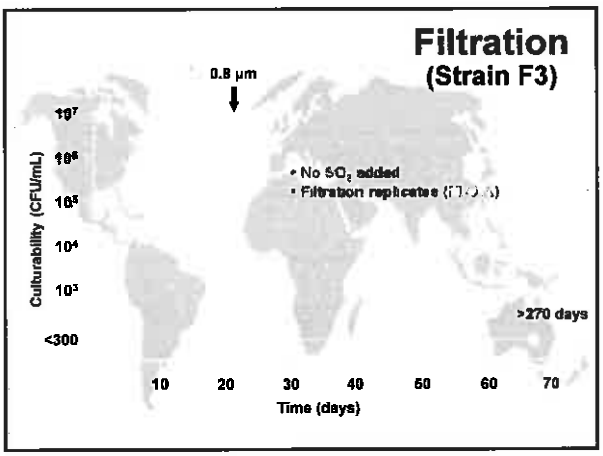
• Multiple ferments incubated >150 days.


Filtration
(Strain B1b)
1.2 µm
SO_2
• mSO_2 added (0.5 mg/L)
• Filtration replicates (□,○,△)
Culturability (CFU/mL)
10^7
10^6
10^5
10^4
10^3
<300
>212 days
10
20
30
40
50
60
70
Time (days)


Filtration
(Strain B1b)
1.2 µm
• No SO_2 added
• Filtration replicates (□,○,△)
Culturability (CFU/mL)
10^7
10^6
10^5
10^4
10^3
<300
>212 days
10
20
30
40
50
60
70
Time (days)


Filtration
(Strain F3)
1.2 µm
SO_2
• mSO_2 added (0.5 mg/L)
• Filtration replicates (□,○,△)
Culturability (CFU/mL)
10^7
10^6
10^5
10^4
10^3
<300
>288 days
10
20
30
40
50
60
70
Time (days)





Impact of Must Nutrition

1. If more N is added to grape must → is more N present in resultant wines?

2. Does higher N in wines encourage infections?

   • What amount of N is needed to support growth?

Residual nitrogen in "synthetic" wines

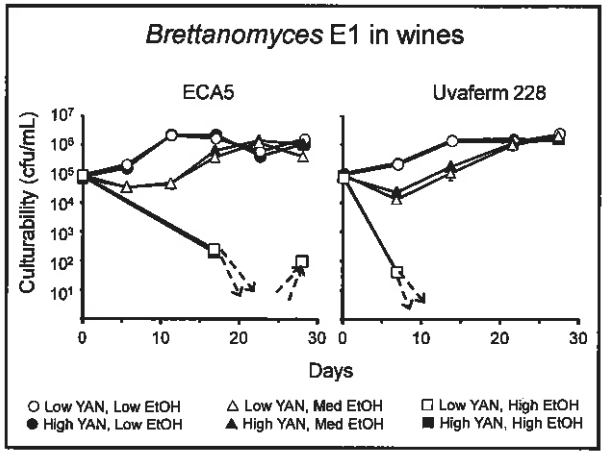
	Must treatment		Wine	
Yeast strain	YAN (mg N/L)	Sugar (g/L)	α-Amino nitrogen (mg N/L)	Ammonia nitrogen (mg N/L)
ECA5	150	230	50[b]	1[b]
		250	48[bc]	1[b]
		270	45[b]	1[b]
	250	230	85[a]	60[b]
		250	84[a]	60[b]
		270	86[a]	60[b]

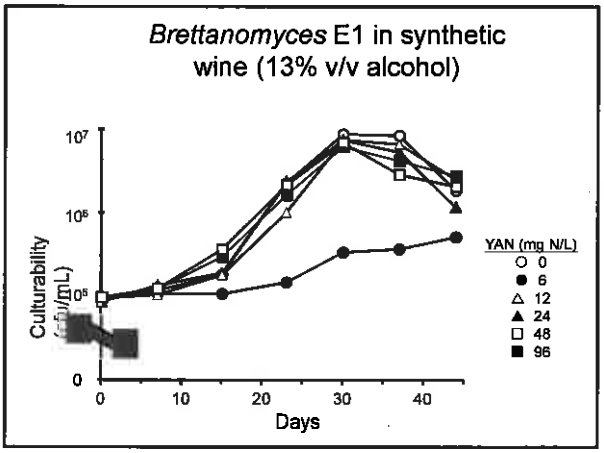
Residual nitrogen in "synthetic" wines

	Must treatment		Wine	
Yeast strain	YAN (mg N/L)	Sugar (g/L)	α-Amino nitrogen (mg N/L)	Ammonia nitrogen (mg N/L)
Uvaferm	150	230	45[b]	3[a]
228		250	44[b]	2[b]
		270	53[b]	3[b]
	250	230	90[a]	30[b]
		250	87[a]	30[b]
		270	89[a]	50[a]

Ethanol concentrations in wines

Sugar in musts (g/L)	Ethanol in wines (% v/v)	
230	**13.5 to 14.1**	**"low"**
250	**14.7 to 14.9**	**"medium"**
270	**15.6 to 15.9**	**"high"**





Survival in Oak Barrels

Methods to eradicate *Brettanomyces* from oak?

Given chemical methods can only penetrate wood few mm, what about use of heat?

1. Important factors affecting yeast penetration.

a. Oak species, toasting, stave location

2. Use of steam and hot water.

Research Focus
(Survival in Oak Barrels)

A. Heat destroys microbes including *Brettanomyces*.

1. Inactivation begins at 50°C.

B. Time at °C for 90% reduction in population (D-value).

1. At 55°C → 12 to 86 seconds.
   - Time and temperature critical.
2. Effect of initial population.
   - Higher population →
     longer time and/or
     higher temperature.

Experimental Design

16L Barrels (new)

American oak (light vs. heavy toast)

French oak (light vs. heavy toast)

225L Commerical Barrels (3 year old)

American oak (2 of medium-heavy toast)

French oak (2 of medium-light toast)

Experimental Design
(16L Barrels)

Research Focus
(Yeast Penetration)

Table saw

- Cut staves into 3 x 10 cm blocks

Band saw

- Saw cross-sections (4 mm thick)

Incubate cross-sections (up to 90 days)

- EBB recovery medium
- Sterilized low alcohol red wine
- Advantage → large surface area

Recovery of *Brettanomyces*
(225L barrels)

Research Focus
(Yeast Penetration)

Forstner drill bit

- Remove 2 mm layers.
- Collect wood shavings.
- Incubated in recovery medium.

Research Focus
(Penetration into Oak Staves)

Research Focus
(Survival in Oak Barrels)

Methods to eradicate *Brettanomyces* from oak?

Given chemical methods can only penetrate wood few mm, what about use of heat?

1. Important factors affecting yeast penetration.
   a. Oak species, toasting, stave location
2. Use of steam and hot water.

Research Focus
(Survival in Oak Barrels)

A. Heat destroys microbes including *Brettanomyces*.

1. Inactivation begins at 50°C.

B. Time at °C for 90% reduction in population (D-value).

1. At 55°C → 12 to 80 seconds.
   - Time and temperature critical.
2. Effect of initial population
   - Higher population → longer time and/or higher temperature.

Research Focus
(Survival in Oak Barrels)

Cross-Sectional Layer
(transfer into sterile wine)

Recovery of *Brettanomyces*
(16L barrels; top staves)

Recovery of *Brettanomyces*
(16L barrels; bottom staves)

Research Focus
(Destruction by Heat)

- Cut staves into 3 x 10 cm blocks/place into SS plate
- Thermocouples inserted every 4 mm
- Place plate over a steam kettle to record °C
- Periodically remove blocks; determine yeast recovery

→ →

Oak barrel staves (16L)

American (light toast)

American (heavy toast)

Oak barrel staves (16L)

French (light toast)

French (heavy toast)

What can we conclude?

A. *Brettanomyces* penetrates into oak staves.

- Approximately 8 mm.
- Affected by oak species and toasting.
- Difficult to reach with antimicrobial chemicals.

B. Steaming for 9 to 12 minutes needed to remove.

- Wineries will not know depth of penetration in any given barrel.

Research Focus
(Use of hot water)

Equivalent results to steaming if...

0 to 4 mm cross-section

- 70°C for 20 minutes
- 80°C for 15 minutes

0 to 9 mm cross-section

- 70°C for 30 minutes
- 80°C for 20 minutes

How do we *REALLY* control *Brettanomyces*?

Conclusions

Incorporate as many hurdles as is REASONABLE for the winery

Too few hurdles = increased risk of spoilage.

Too many hurdles = expense higher than benefit.

KEY → *Hurdle interactions exist!*

If SO_2, filtration, heat, or chitosan does not control *Brettanomyces*…


Thank you for your support!
• WAGrape/Wine Research Group
• Regional wineries
• Scott Laboratories
• Lallemand Inc.
• GusmerEnterprises
• Graduate students
Pennsylvania Industry
Washington State University