GRAPE & WINE ROADSHOW

Avoca/Pyrenees Seminar

Thursday 15th August, 2013

Did you know that DAP can strongly affect the flavour profile and style of wine

Paul Henschke

Practical management of Brett in the winery

Eric Wilkes

Morning Tea

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VESDA – The new risk assessment tool for smoke taint
Ricky James, DEPI Victoria

Strategies for successful MLF
Eveline Bartowsky

Causes and management of slow and stuck fermentations
Paul Henschke

Lunch

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Avoca/Pyrenees Seminar

Thursday 15th August, 2013

Rotten egg, cabbage and rubber:
compounds responsible for reductive off-flavours in wine
Leigh Francis

Afternoon Tea

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Avoca/Pyrenees Seminar

Thursday 15th August, 2013

Saving time and money:
Automated methods for juice and wine analysis
Eric Wilkes

Using MLF to accentuate wine aroma and flavour
Eveline Bartowsky

Features of the AWRI website and closing comments
Mark Krstic

Did you know that DAP can strongly affect the flavour profile and style of wine?

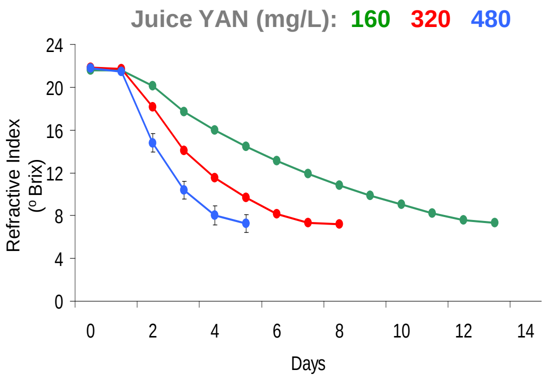
Paul A Henschke
Principal Research Microbiologist

Simon Schmidt, Radka Kalouchova, Maurizio Ugliano*, Cristian Varela, Sally-Jean Bell*, Leigh Francis
*formerly AWRI

Roles of NITROGEN in fermentation

The Australian Wine Research Institute

- **Key yeast nutrient**
  - determines biomass formation



- **Metabolic regulation**
  - regulates carbon metabolism
    ≡ alcoholic fermentation
- **Aroma/flavour compound formation**
  - esters, alcohols, carbonyls, acids, etc
- **Sulfur metabolism regulation**
  - H_2S, mercaptans
- **Arginine-Urea metabolism regulation**
  - ethyl carbamate

Yeast Assimilable Nitrogen (YAN)

The Australian Wine Research Institute

$$YAN = FAN + NH_3\text{-}N$$

Yeast assimilable nitrogen

Free amino nitrogen ≡ α-amino N

Ammonia nitrogen

(Primary amino acids)
(excludes secondary)
(amino acids, eg Proline)

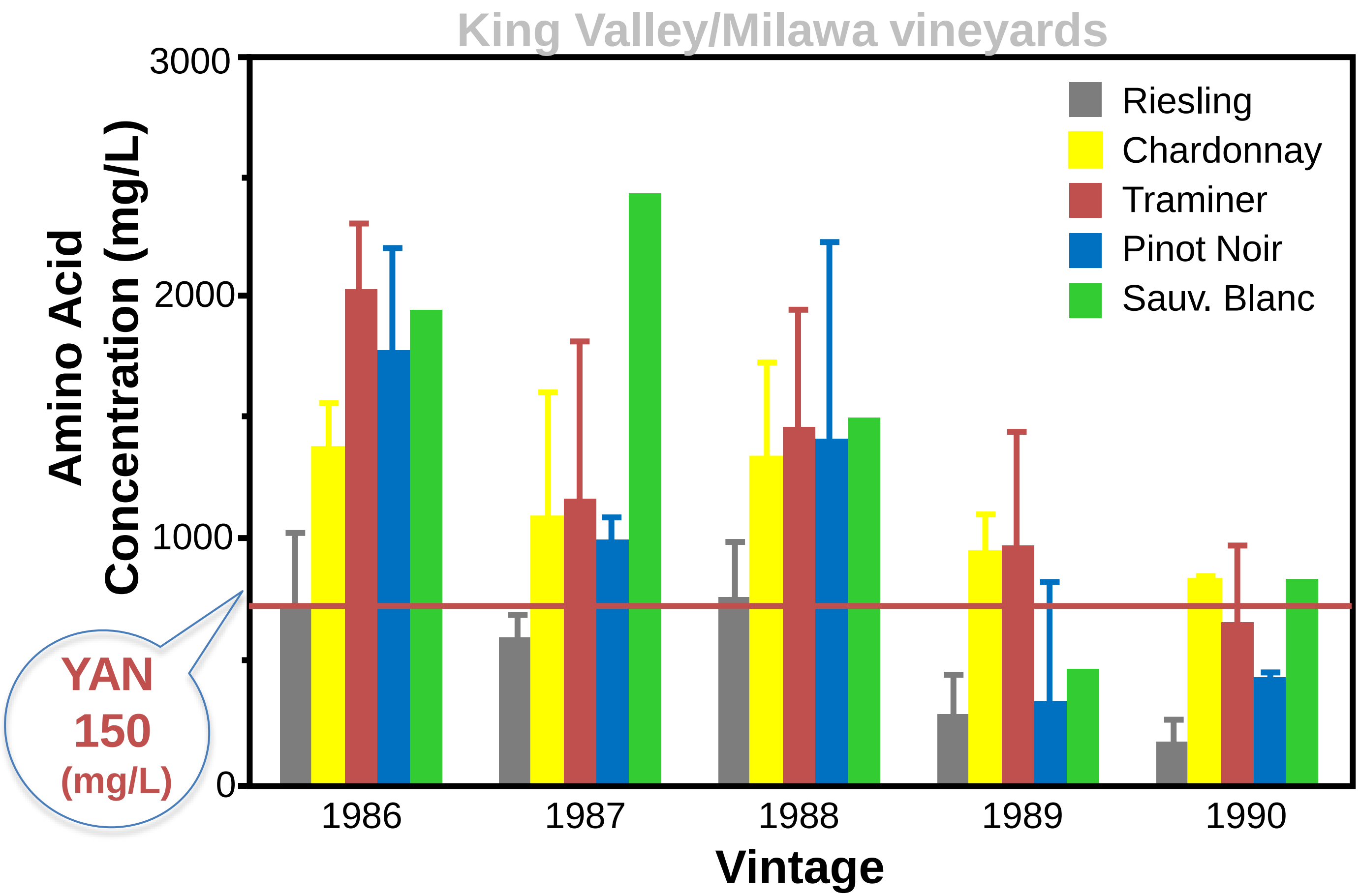
FAN quantitation
- NOPA assay (AWRI)
- NIR

NH_3 quantitiation
- Enzyme kit (AWRI)
- Electrode

AWRI Commercial Services provide YAN analysis service

Effect of vintage year and grape variety on juice nitrogen content

The Australian Wine Research Institute



YAN varies from year to year, being affected by the season and vineyard management practices

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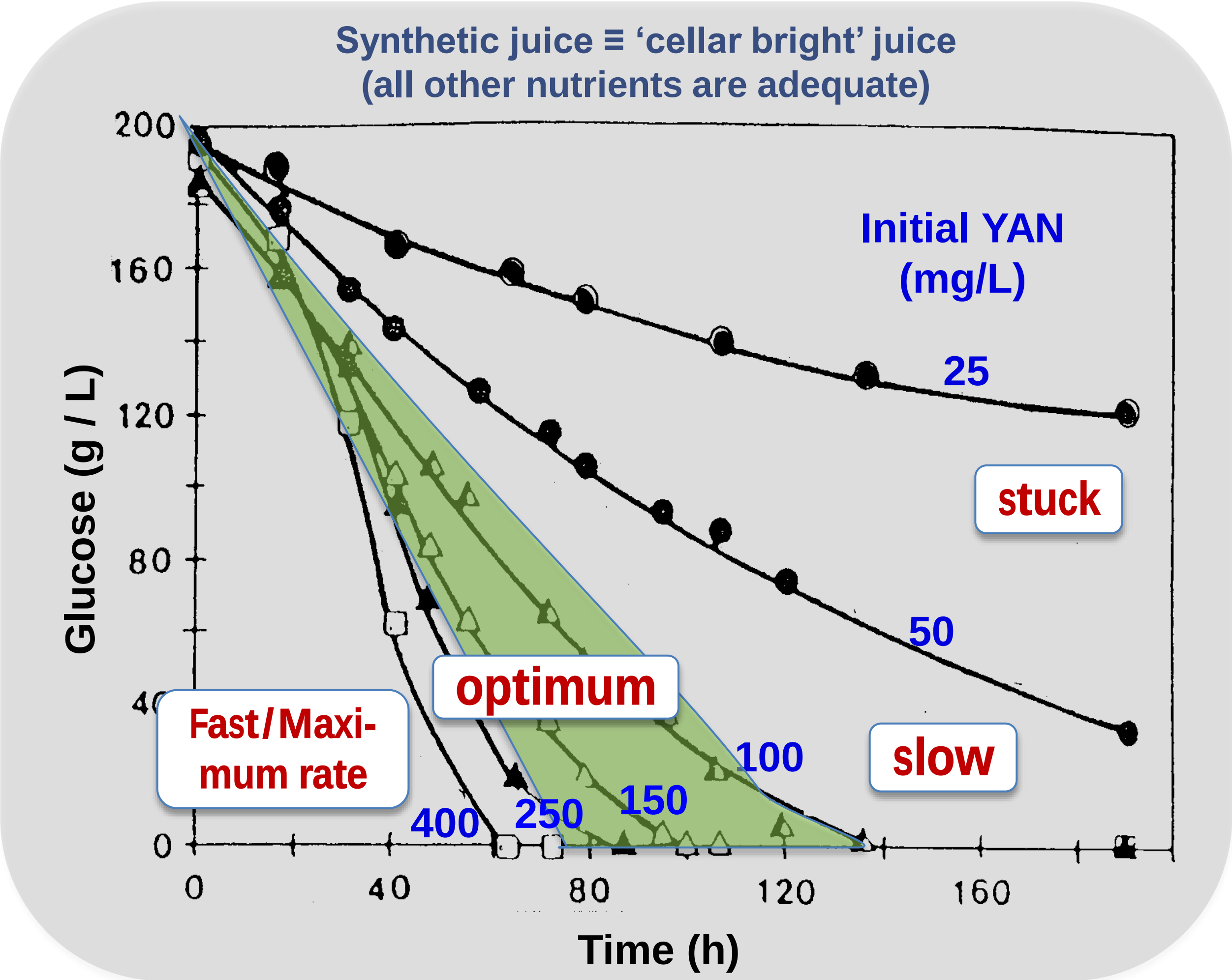
YAN analysis should be carried out over, at least 2 consecutive seasons, on vineyards that give fermentation problems

Source: Gockowiak & Henschke (1992)
Aust. NZ Grapegrower Winemaker (340):131-138

Fermentation response to initial [YAN] in clarified must

The Australian Wine Research Institute



Grape juice YAN typically varies between 50 – 400 mg/L, producing fermentation rates at risk of being slow or stuck, at one extreme, to too fast, which can cause rapid temperature increase and loss of aromas

----

Optimum fermentation rate depends on winemaking objectives but YAN should typically fall between 150 – 350 mg/L, using cooling to control required rate

Source: Salmon (1989)
Appl. Environ. Microbiol. 55:953-958

YAN management

The Australian Wine
Research Institute

Yeast demand for YAN

YAN depend on strain/fermentation conditions
(eg grape solids, aeration, warmer conditions, etc affect demand)

- **Minimum YAN = 100 – 150 (mg/L)**
  lower for reds / higher for whites
- **Optimum YAN = 150 – 350**
  depends on w/m objectives
- **Maximum YAN = 350 – 450 (mg/L)**
  strain dependent

What are the flavour implications

of DAP added to juice/must YAN?

Case studies

The Australian Wine Research Institute

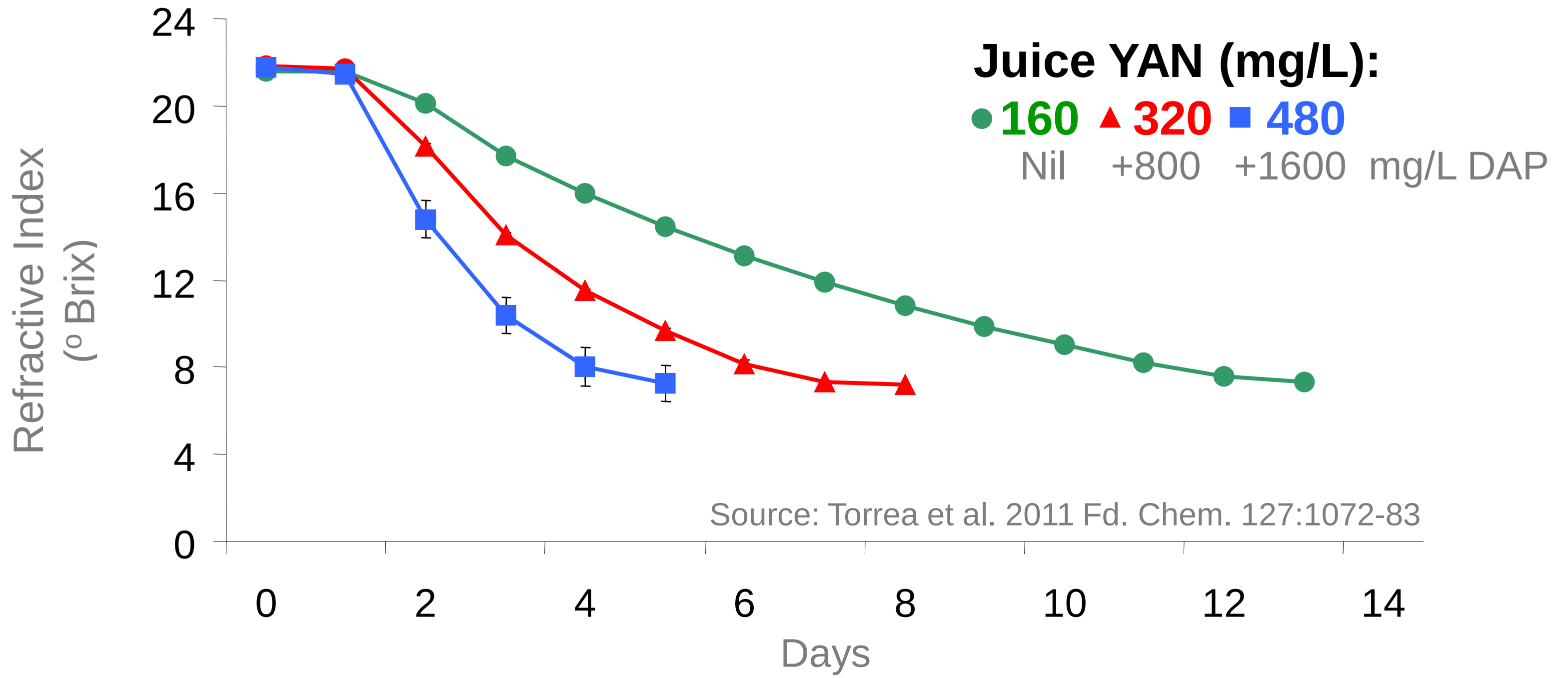
Aim: to establish relationships between

- **Wine type (Chardonnay, Shiraz)**
- **Yeast strain**
- **YAN (initial, adjusted with DAP)**
- **Basic wine chemistry**
- **Wine flavour**

Effect of DAP addition in Chardonnay – fermentation kinetics

The Australian Wine Research Institute

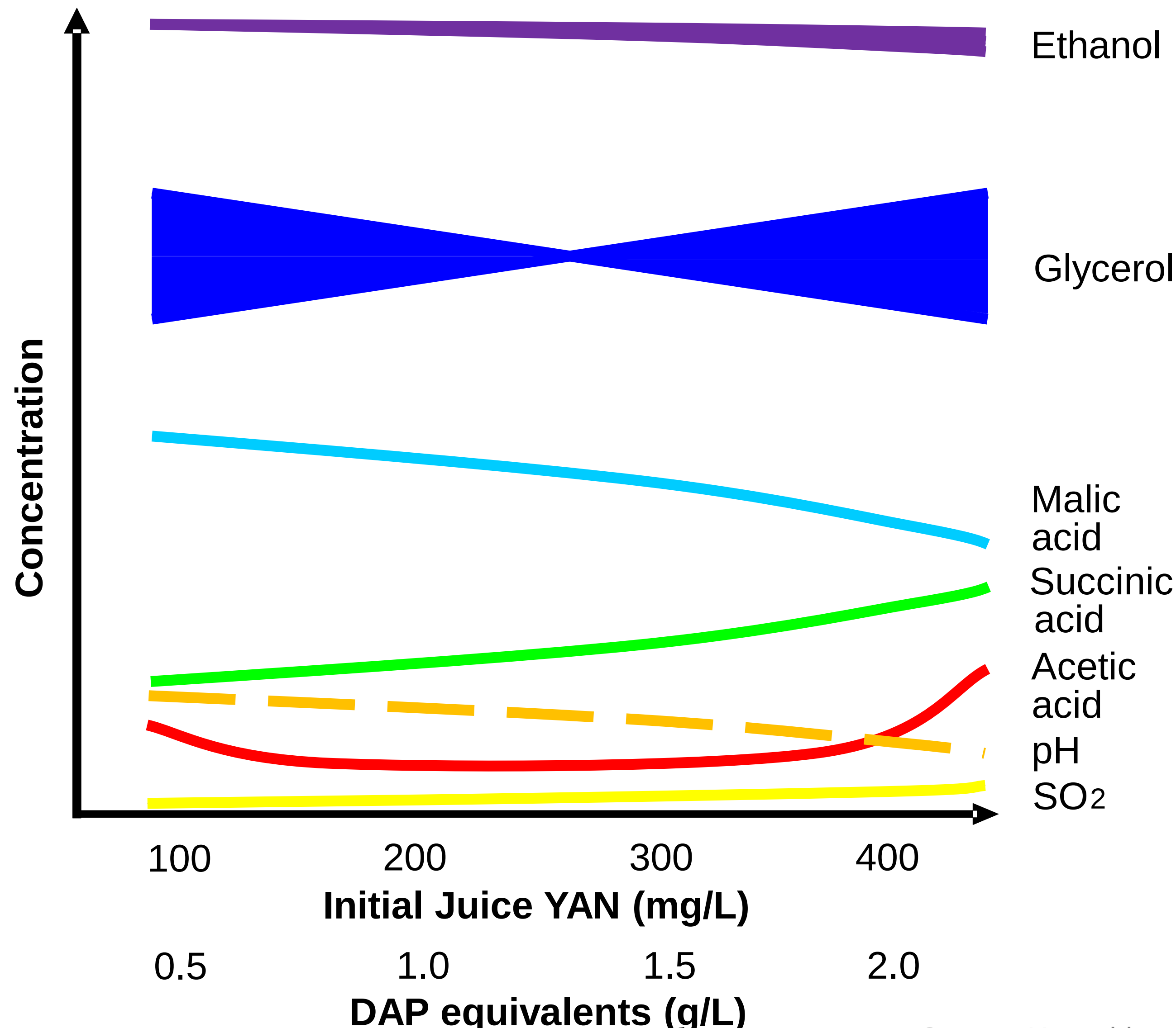
Juice: Chardonnay (cold settled, filtered)
YAN: 160 mg/L, adjusted to 320 and 480 mg/L with DAP
Yeast: AWRI 796, 18°C



Juice YAN affects:
- yeast growth (biomass)
- fermentation rate and
- fermentation duration (up to 50% reduction)

Chardonnay – impact of DAP – YAN on Basic Wine Chemistry

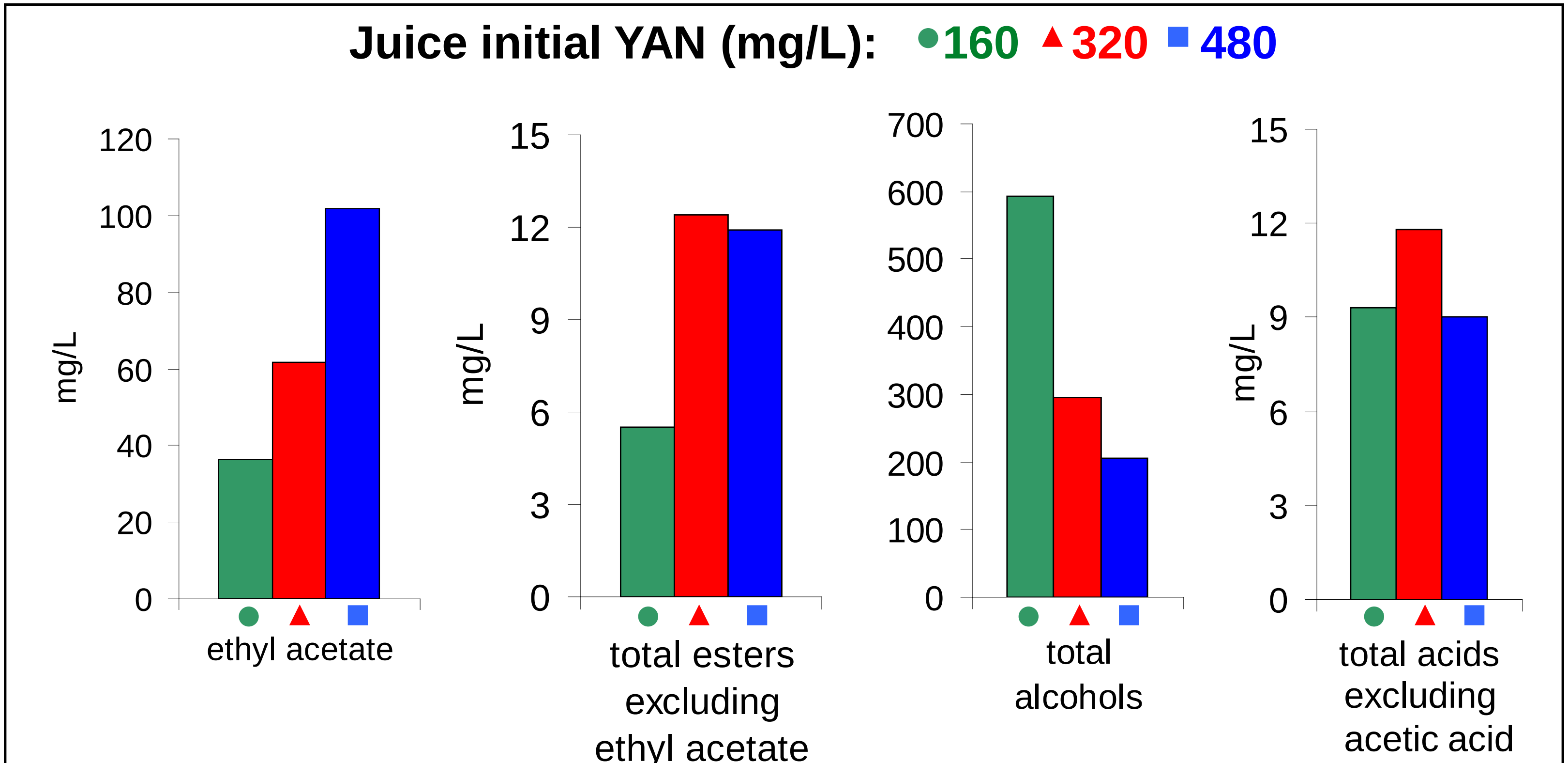
The Australian Wine Research Institute



Source: Henschke et al. Wine Viti J (2012)

Effect of DAP addition in Chardonnay – yeast aroma compounds

The Australian Wine Research Institute

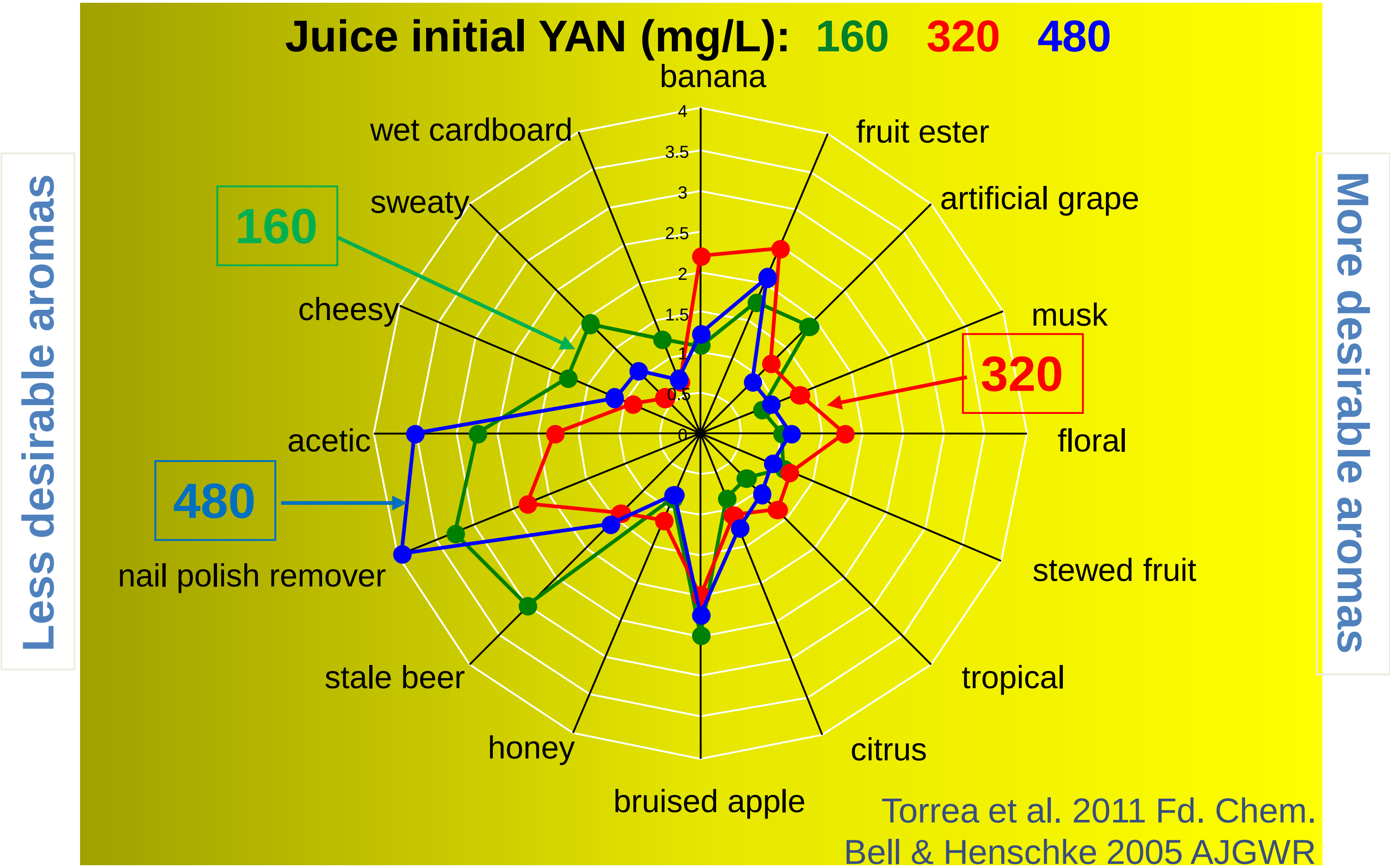


Source: Ugliano et al. Dec 2007 A&NZ Wine Ind.J.

- **Esters**: Increase with YAN, then plateau at high YAN
- **Higher alcohols**: Inverse relationship with YAN
- **Volatile acids**: Complex relationship with YAN

Effect of DAP addition in Chardonnay – wine aroma profile

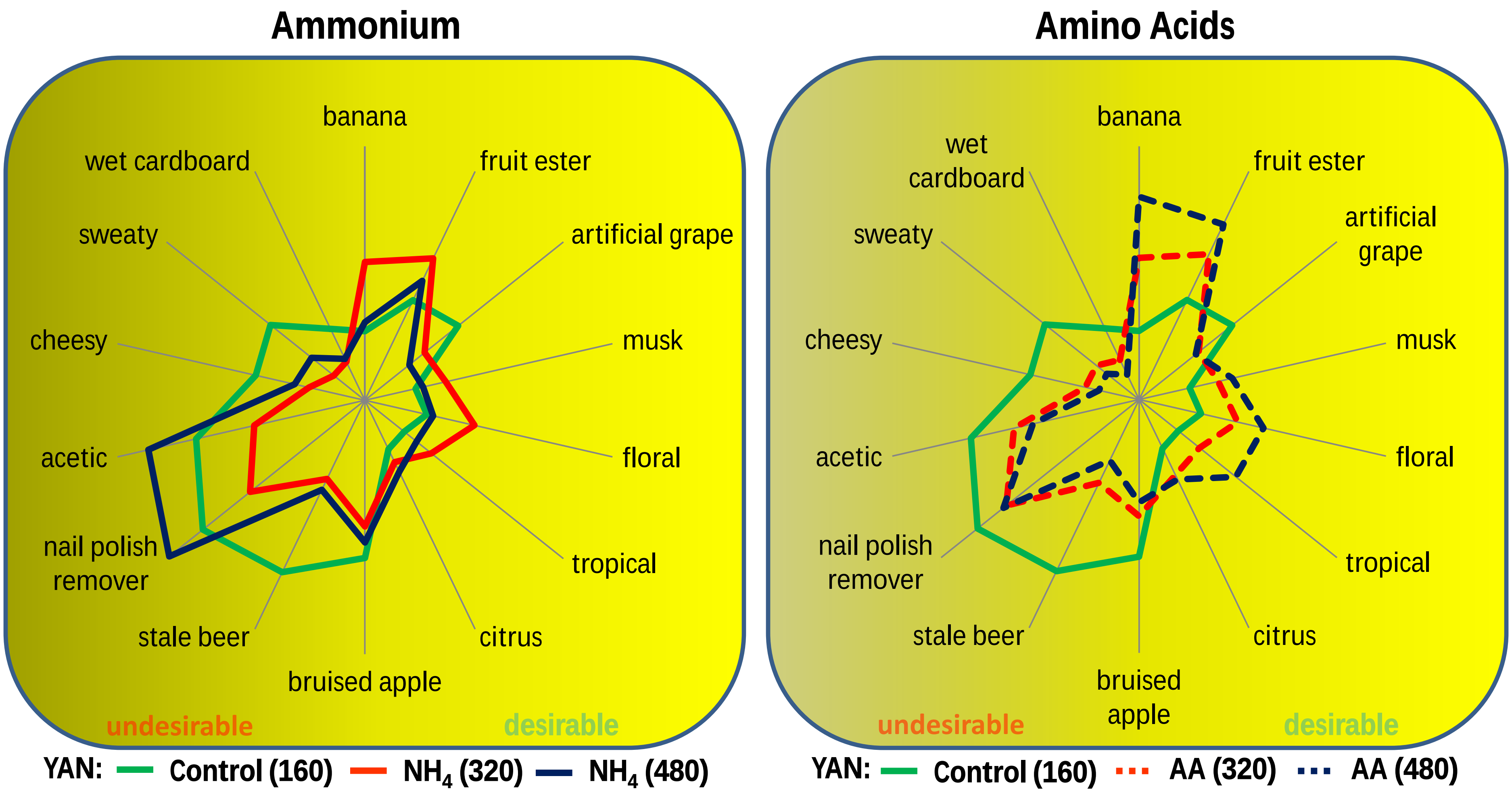
The Australian Wine Research Institute



Low YAN (160 mg/L): Complex (enhanced sweaty, cheesy, VA, stale beer, grape)
Moderate DAP-YAN (320 mg/L): Enhanced fruity-floral aromas
High DAP-YAN (480 mg/L): Volatile (estery/solvent taint)
Risk of large DAP additions: can lead to masking varietal character

Comparison of Organic N (Amino acids) vs Inorganic N (NH_4^+) addition on Chardonnay Wine Aroma Profile

The Australian Wine Research Institute



Profiles with Amino Acids similar excepting no ester/solvent char. at high YAN

Amino acids (simulates grape YAN) increases aroma intensity

Effects on other white varieties/yeast combinations similar

Source: Torrea et al. (2011) Fd. Chem.

Chardonnay – impact of DAP – YAN

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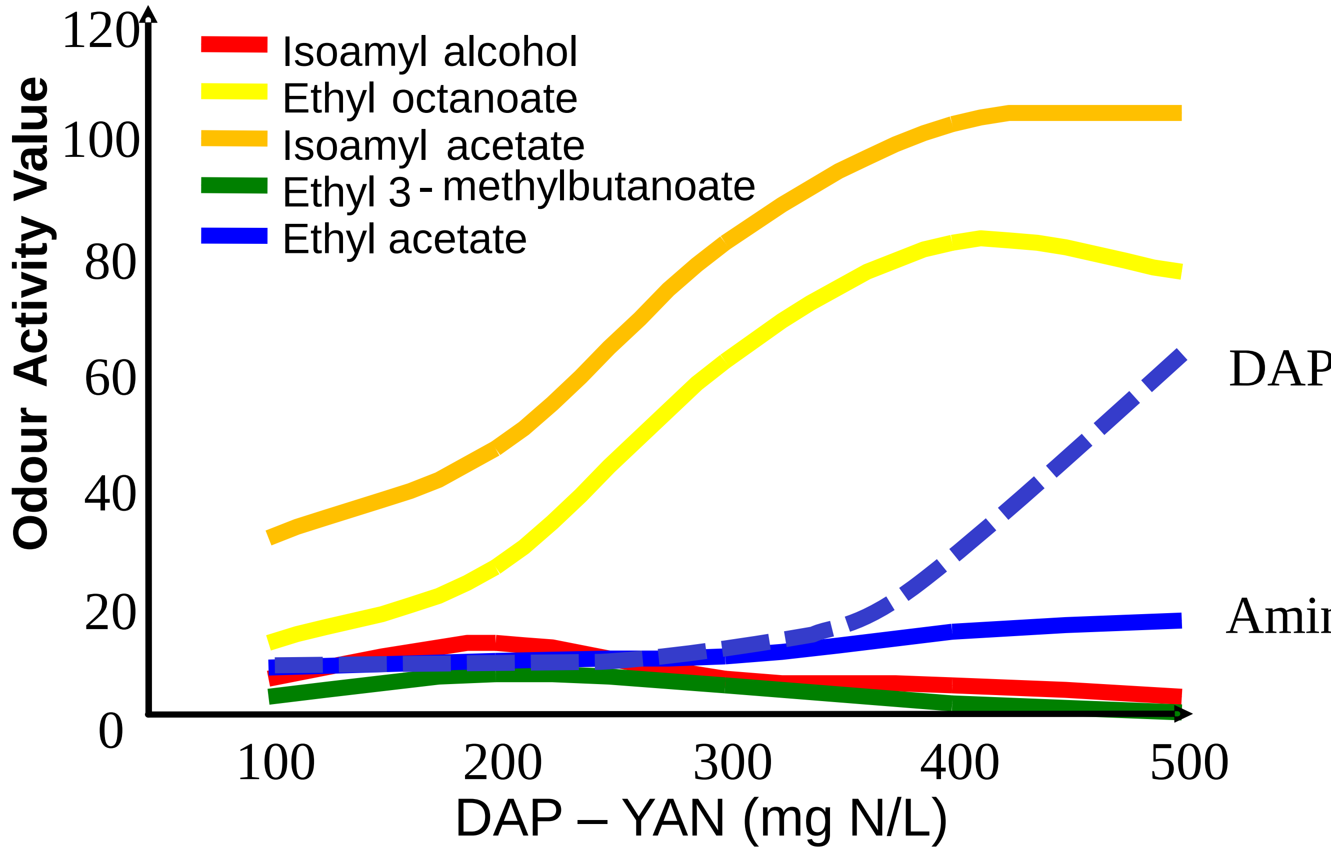

Source: Ugliano et al. Dec 2007 A&NZ WIJ

Principle effects of DAP:

- Increases fruity-floral wine esters
- Increases aroma intensity
- Excessive DAP (>1 g/L) can give solvent-ester taint

Effect of DAP addition in Shiraz – fermentation kinetics

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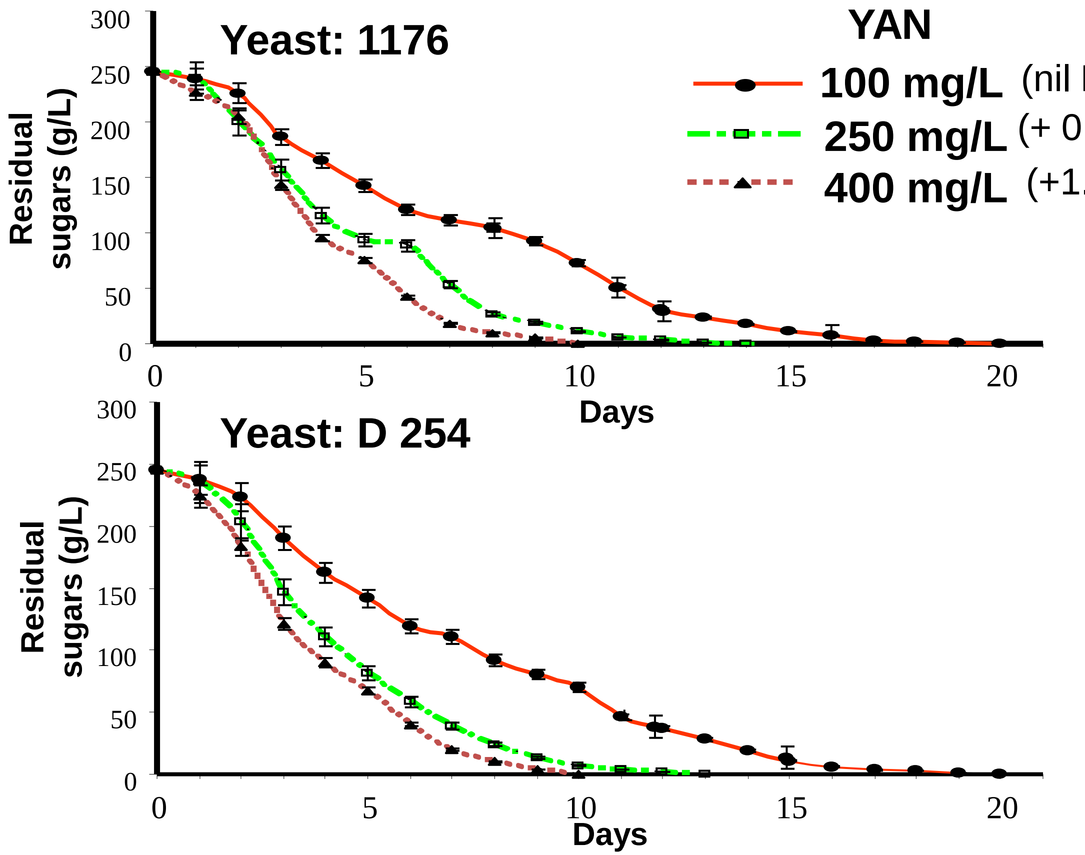
Shiraz must (macerated), 22°C

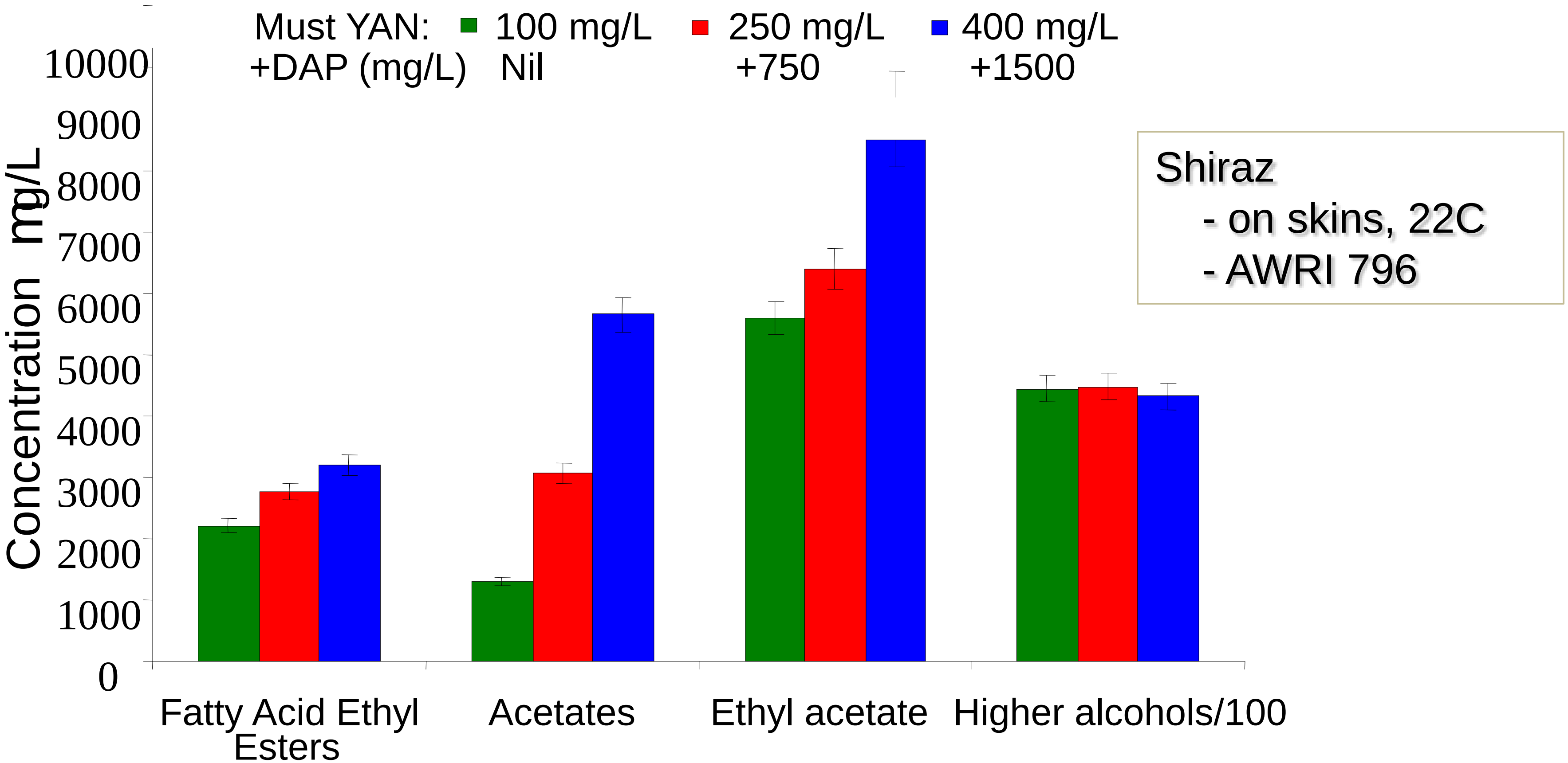


Source: Ugliano et al. (2008) J AgFdChem

Juice YAN affects: yeast growth (biomass)
fermentation rate and
fermentation duration (~50% reduction)

Effect of must YAN on Shiraz – yeast aroma compounds

The Australian Wine Research Institute



- Esters (fruity and floral) increase with increasing YAN
- Higher alcohols largely unaffected by YAN

Source: Ugliano et al. Dec 2007 A&NZ WIJ

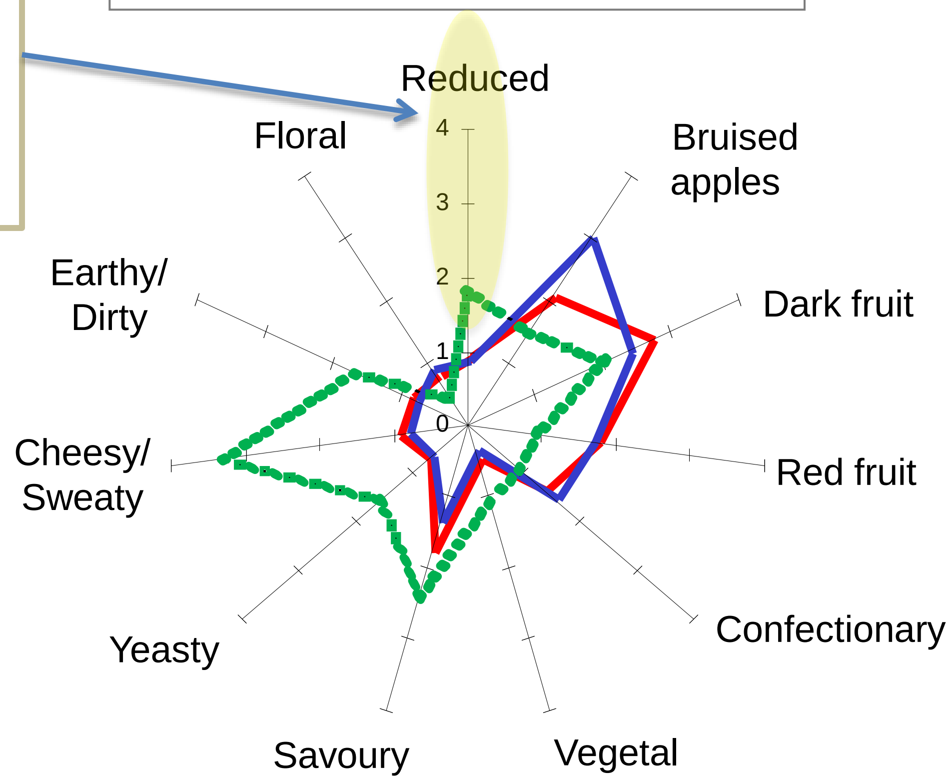
Effect of DAP on Shiraz wine flavour

The Australian Wine Research Institute

Classical H_2S response to N

Higher DAP = lower H_2S

Saccharomyces bayanus
AWRI 1176



YAN

- **100** (nil DAP)
- **250** (+ 0.75 g/L DAP)
- **400** (+1.5 g/L DAP)

Shiraz
- on skins, 22°C

Source: Ugliano et al. (2010) JAgFdChem

- **Low YAN** – complex savoury, earthy, cheesy, reduced wine
- **Moderate DAP-YAN** – enhanced dark and red fruit
- **High DAP-YAN** – dark and red fruit with bruised apple

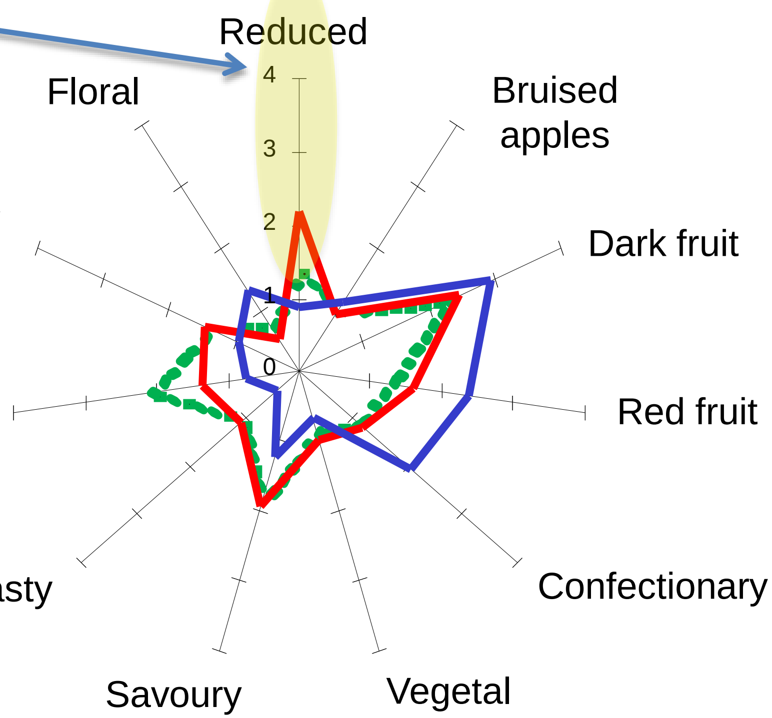
Effect of DAP on Shiraz wine flavour

The Australian Wine Research Institute

Non-Classical H_2S response to N

Moderate DAP = higher H_2S

Saccharomyces cerevisiae
D254



YAN

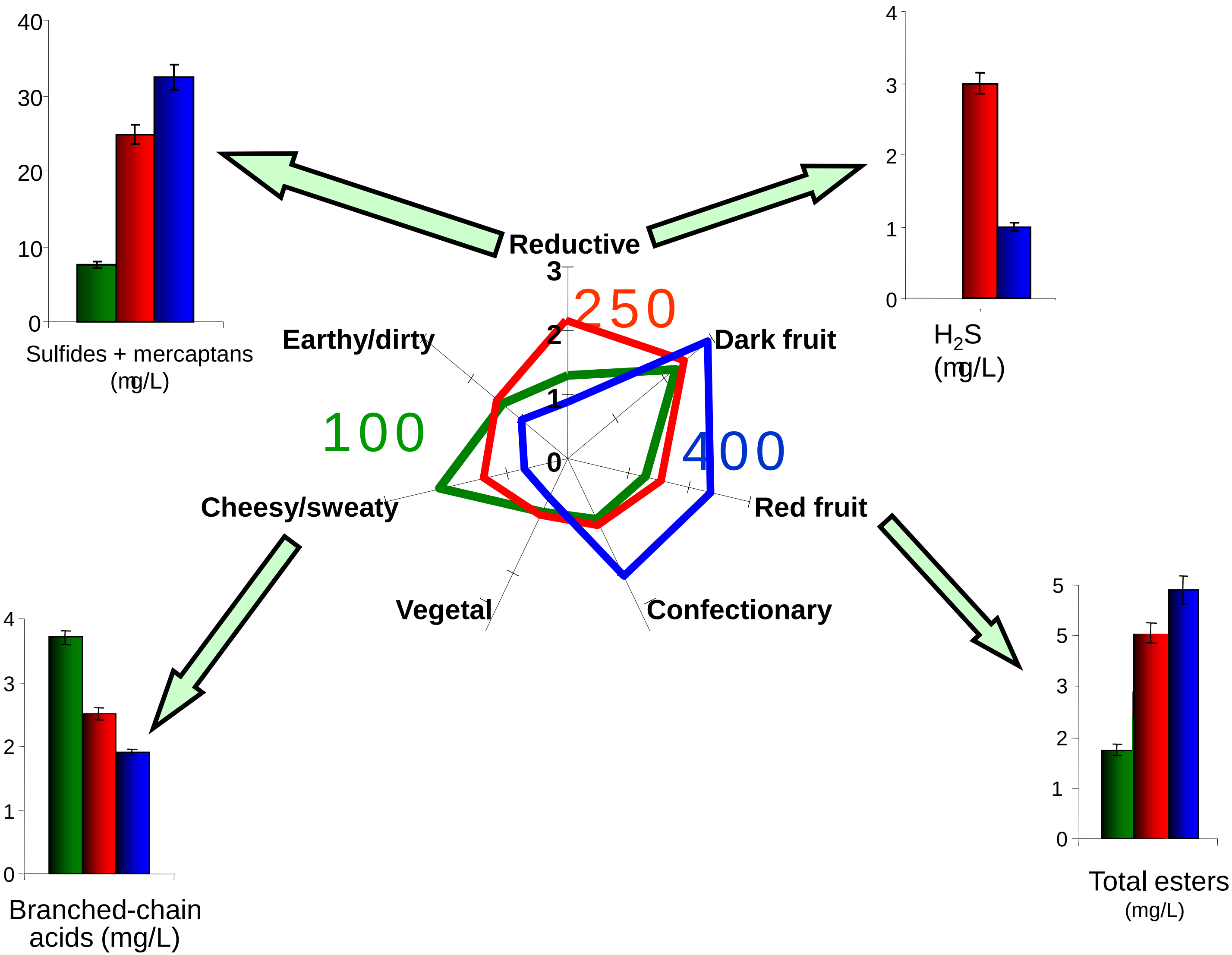
- **100** (nil DAP)
- **250** (+ 0.75 g/L DAP)
- **400** (+1.5 g/L DAP)

Shiraz
- on skins, 22°C
Source: Ugliano et al. (2010) JAgFdChem

- **Low YAN** – complex savoury, cheesy with dark fruit
- **Moderate DAP-YAN** – similar but reductive
- **High DAP-YAN** – enhanced floral/estery dark and red fruit

DAP and Shiraz volatile compounds

The Australian Wine Research Institute



>> For some yeasts, moderate DAP supplement-ation of low YAN musts can result in higher H_2S and reductive character

But DAP can increase esters, which can mask reductive aromas, so H_2S may not be apparent in young wine

YAN and wine aroma

Timing of H_2S depends on yeast strain

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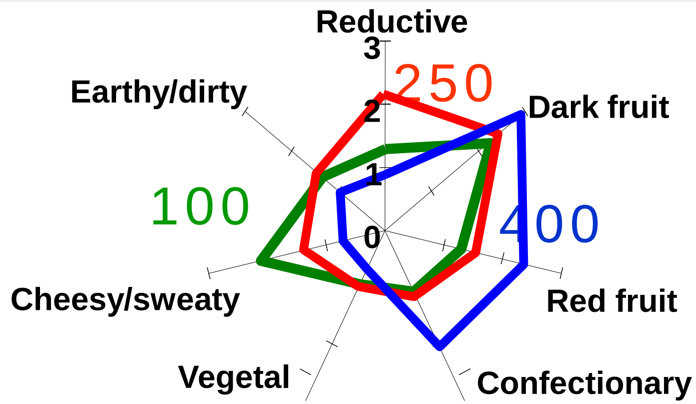

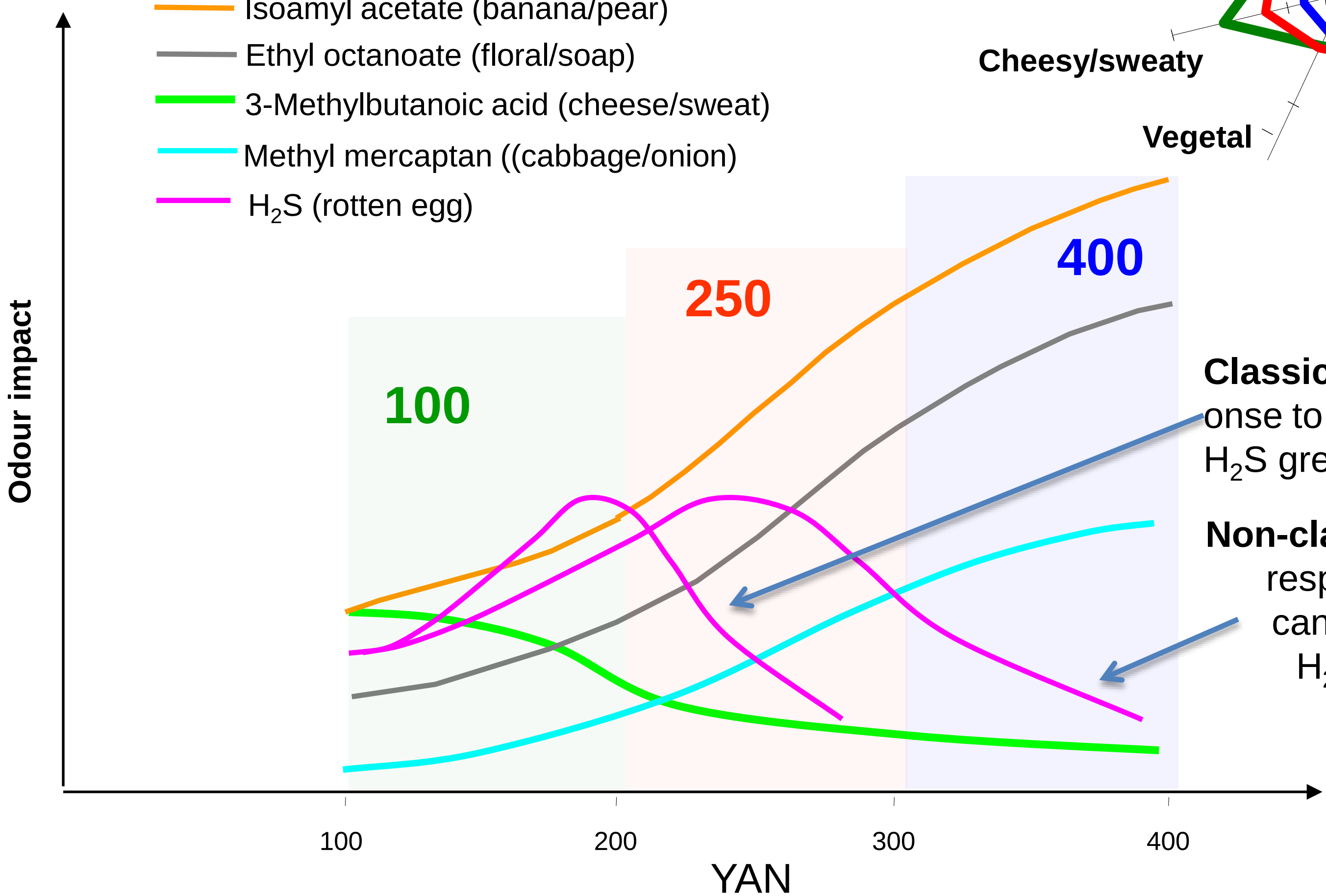

Classical yeast H_2S response to DAP; risk of wine H_2S greatest at low YAN

Non-classical yeast H_2S response to DAP can lead to residual H_2S in wine post fermentation

Summary

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- Yeast Assimilable Nitrogen (YAN), as DAP, is a powerful tool to manage:
  > fermentation kinetics,
  > aroma formation (type & intensity) during fermentation,
  (high yeast esters can however mask varietal character)

  and

  > wine style
  (complex –> clean, fruity, estery –> solvent, ester taint)

- Addition of nitrogen **<u>does not systematically eliminate</u>** the occurrence of reductive off-flavours but the concommitant increase in esters tends to mask reduction.
  - Moderate DAP can increase reductive off-flavours in some combinations of juice/must and certain yeast strains

Acknowledgements

The Australian Wine Research Institute

- Project scientist – Dr Diego Torrea (Chardonnay) (formerly AWRI)
  – Dr Maurizio Ugliano (Shiraz) (formerly AWRI)
  – Dr Simon Schmidt (Yeast nutrients)
  – Dr Cristian Varela (Yeast development)
- Vineyard N trial site: Dr Sally-Jean Bell, Orlando - Brian White
- Sensory analyses: Dr Leigh Francis and team
- Fermentation and analysis: Radka Kalouchova
- Project supervisor – Dr Paul Henschke

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gwrdc GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Further reading

Bell, S.-J., Henschke, P.A. (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11: 242–295.

Gockowiak, H., Henschke, P.A. (1992) Nitrogen composition of grape juice and implications for fermentation: results of a survey made in N-E Victoria. Aust. Grapegrower Winemaker (340): 131, 133–138.

Henschke, P.A., Varela, C., Schmidt, S., Torrea, D., Vilanova, M., Siebert, T., Kalouchova, R., Ugliano, M., Ancin-Azpilicueta, C., Curtin, C.D., Francis, L. (2012) Modulating wine style with DAP: case studies with Albariño and Chardonnay. Aust. N.Z. Grapegrower Winemaker 581, 57–63.

Torrea, D., Varela, C., Ugliano, M., Ancin-Azpilicueta, C., Francis, I.L. Henschke, P.A. (2011) Comparison of inorganic and organic nitrogen supplementation of grape juice - effect on volatile composition and aroma profile of a Chardonnay wine fermented with *Saccharomyces cerevisiae*. Food Chem. 127, 1072–1083.

Ugliano, M., Henschke, P.A., Herderich, M.J., Pretorius, I.S. (2008) Nitrogen management – critical for wine flavor and style. Pract. Winery Vineyard (May/June), 6–25.

Ugliano, M., Fedrizzi, B., Siebert, T., Travis, B., Magno, F., Versini, G., Henschke, P.A. (2009) Effect of nitrogen supplementation and *Saccharomyces* species on H_2S and other volatile sulfur compounds in Shiraz fermentation and wine. J. Agric. Food Chem. 57, 4948–4955.

Ugliano, M., Siebert, T., Mercurio, M., Capone, D., Henschke, P.A. (2008) Volatile and color composition of young and model-aged Shiraz wines as affected by diammonium phosphate supplementation before alcoholic fermentation. J. Agric. Food Chem. 56, 9175–9182.

A W R I

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Practical management of ‘Brett’ in the winery

Eric Wilkes – Group Manager Commercial Services
Chris Curtin – Research Manager

‘Brett’ = spoilage?

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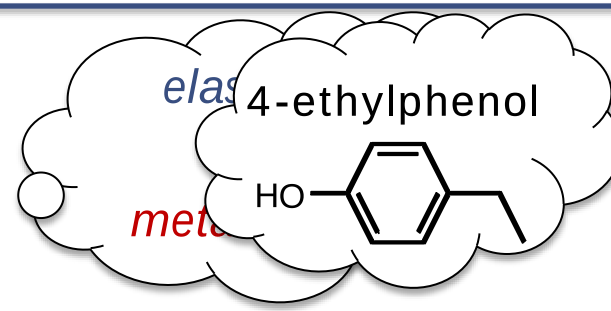

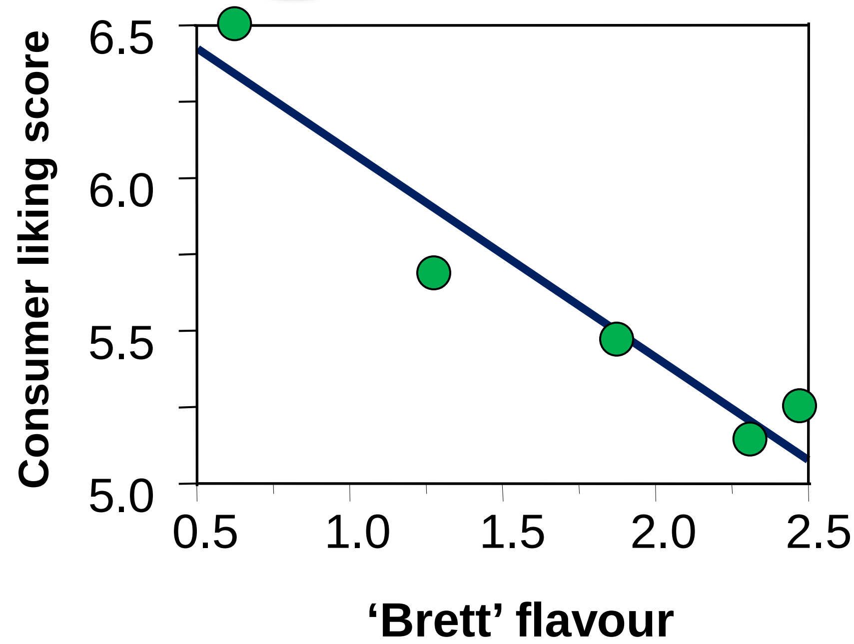

Dekkera bruxellensis, 'Brett', and red wine

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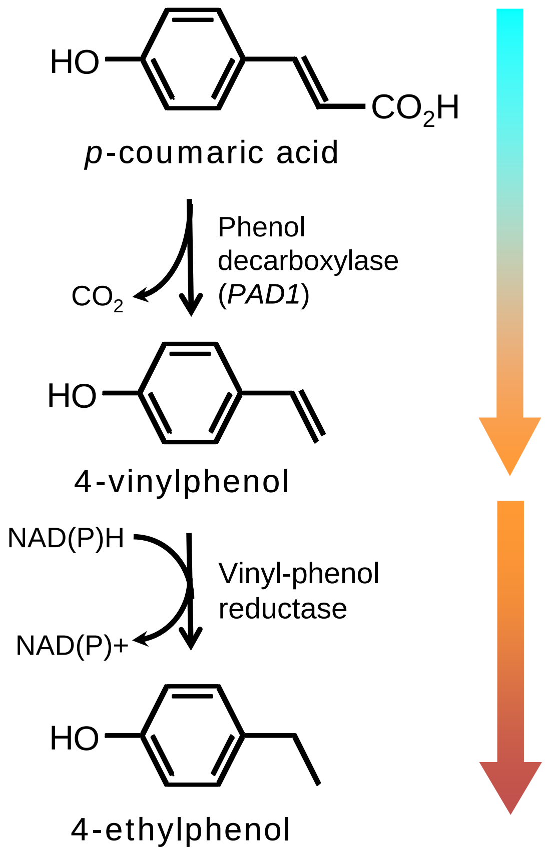
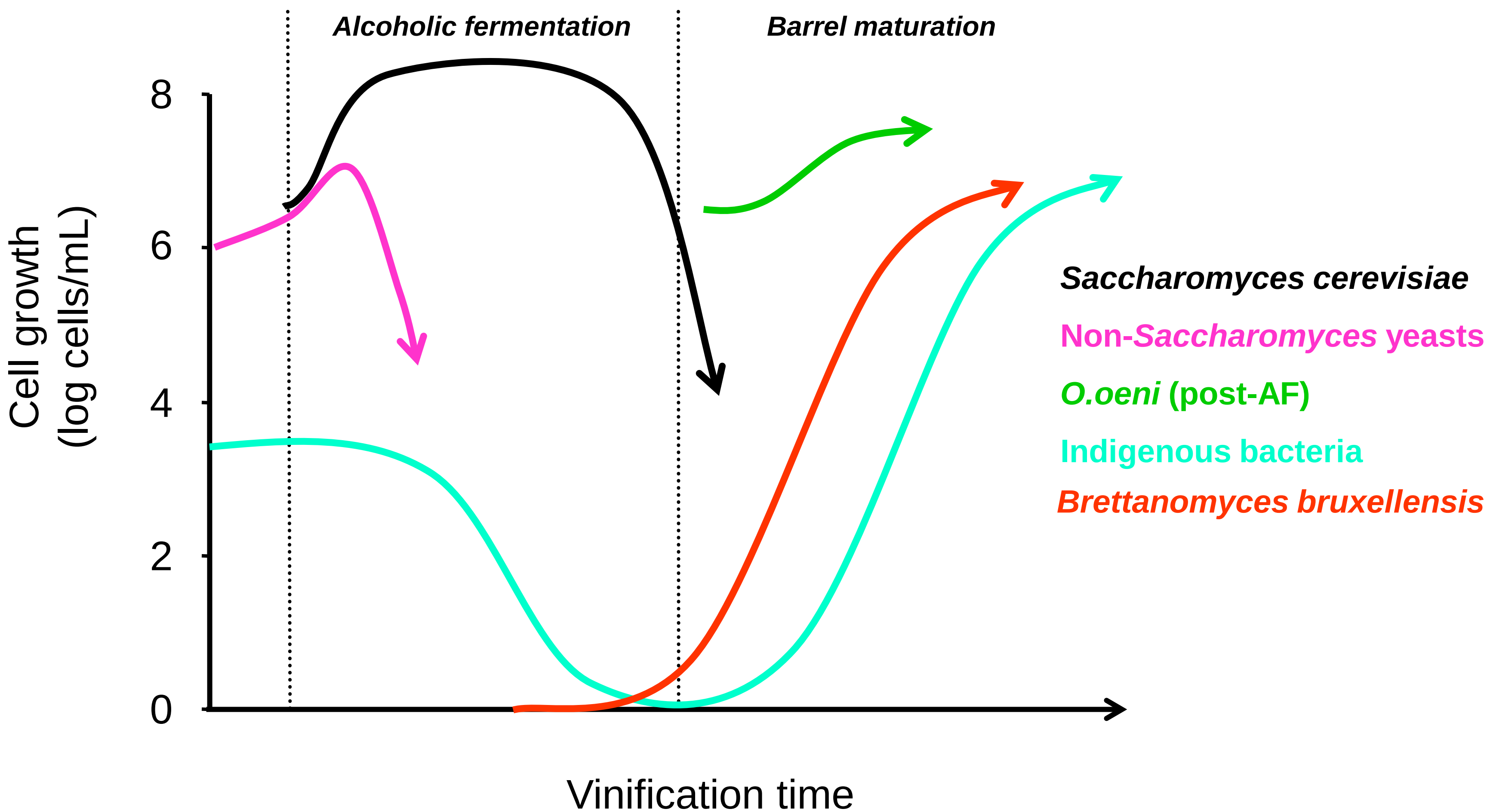


Various bacterial & yeast species (including *Saccharomyces*)

Dekkera (Brettanomyces) bruxellensis

Brettanomyces bruxellensis, ‘Brett’, and red wine

The Australian Wine Research Institute



‘Brett’ **IS** in your region, and your winery

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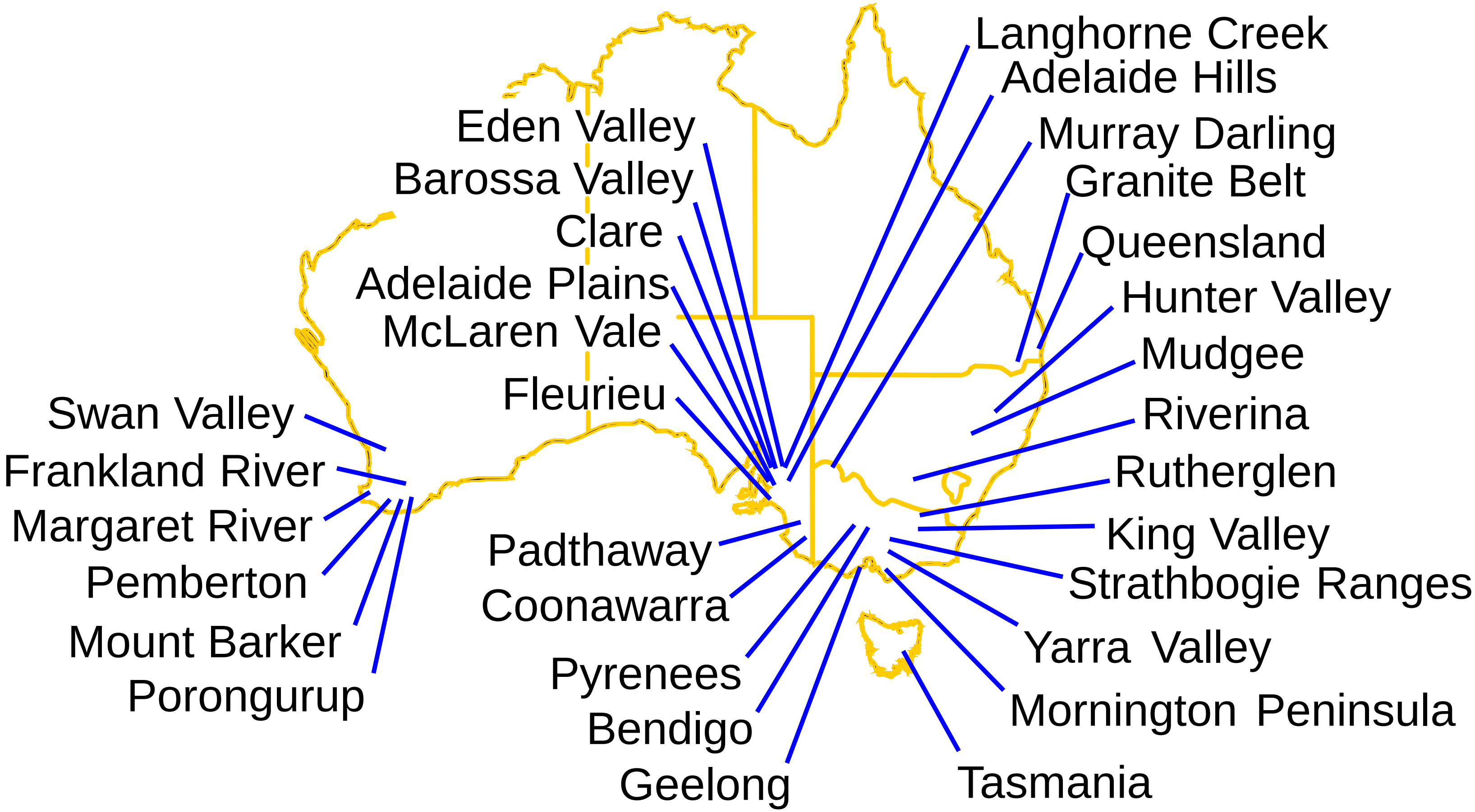
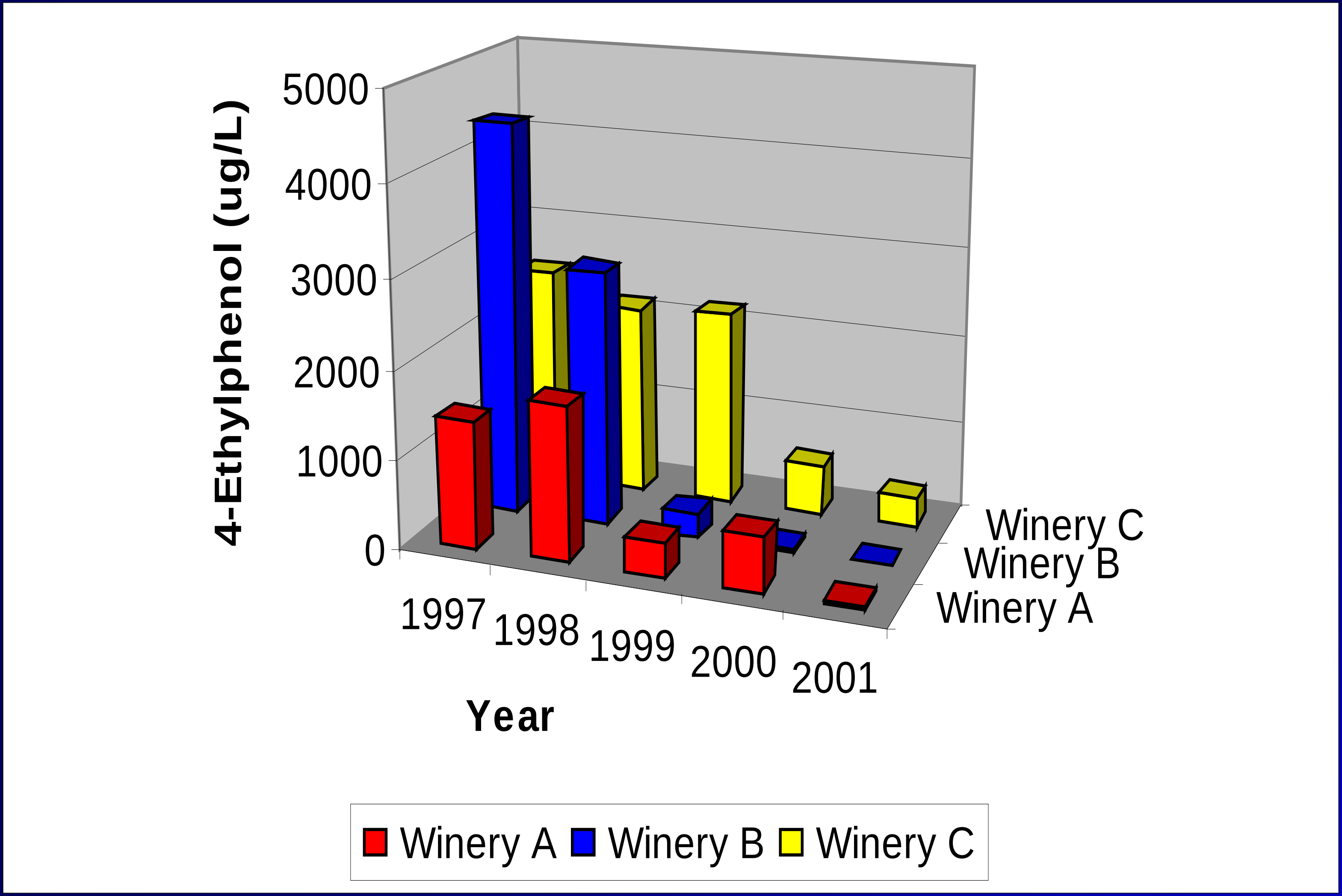


We isolated *Brettanomyces* from 31 winemaking regions of Australia

Don’t

You **CAN** manage the risk of ‘Brett’ spoilage in the winery

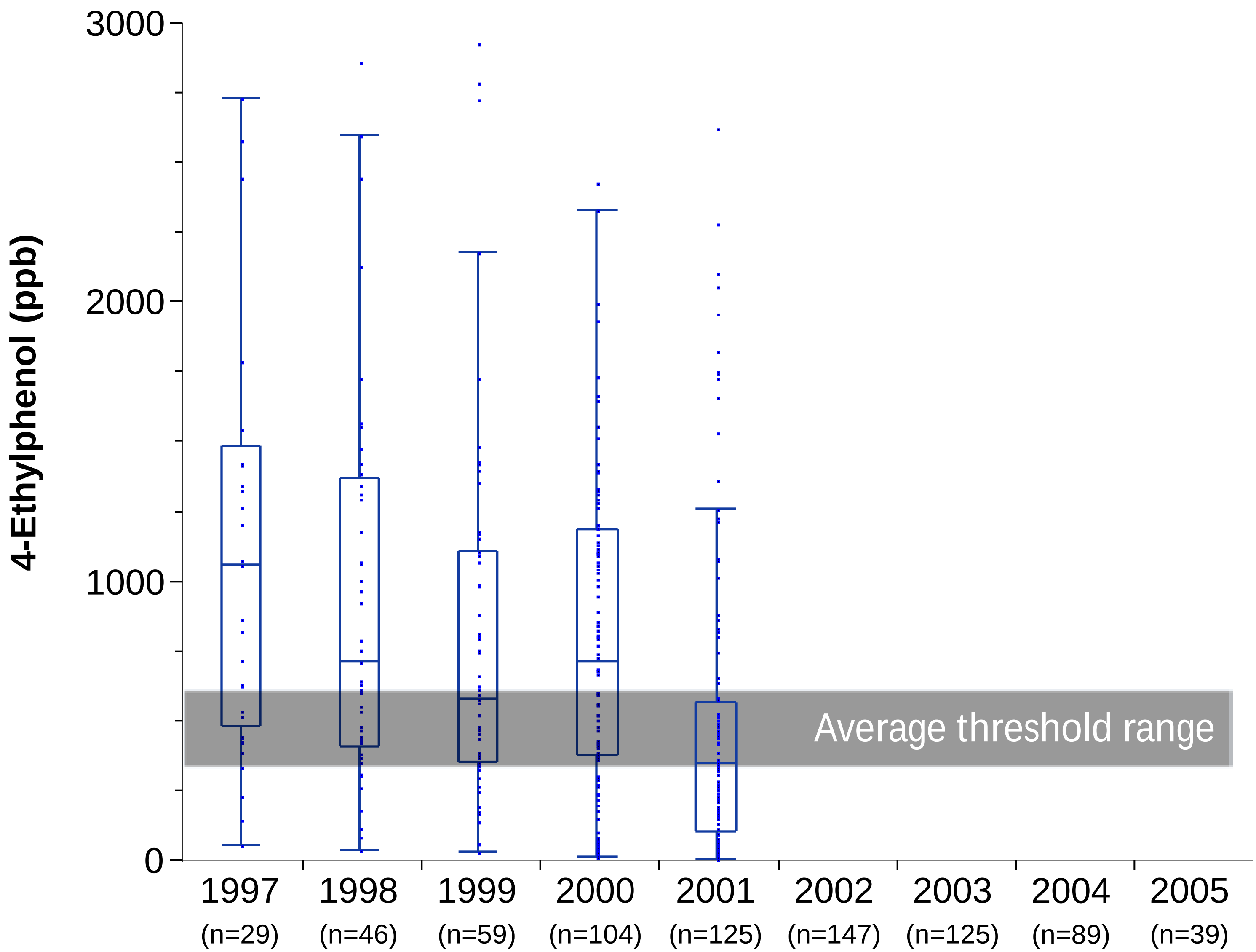
Trend in 4-Ethylphenol concentrations in Cabernet Sauvignon wines from three Australian producers 1997 - 2001



NOTE: Wineries A and B first contacted the Institute with regard to strategies for the control *Brettanomyces* in 1998, and winery C in 1999. Many other producers are seeing a similar trend from 2000 onwards

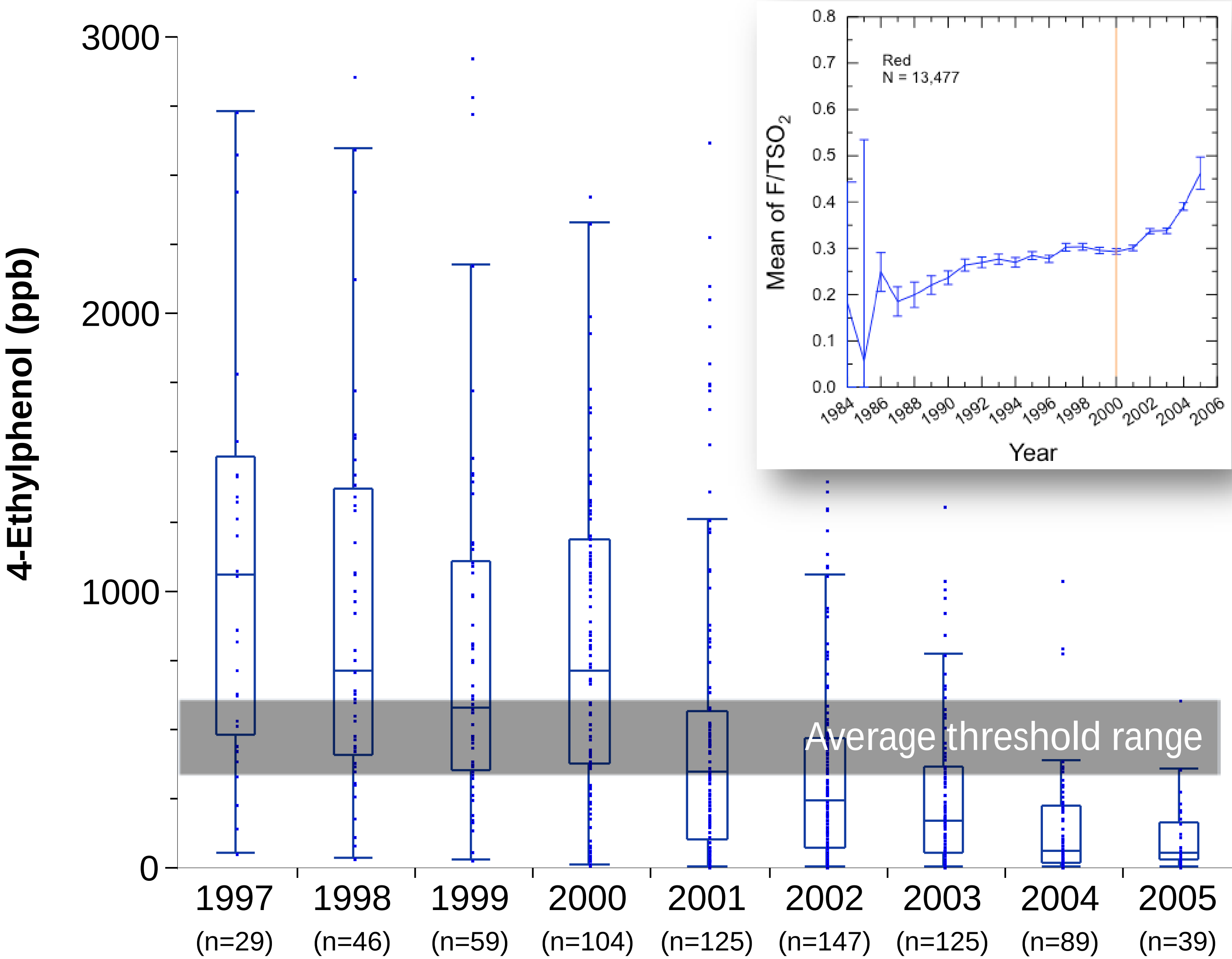
The incidence of 'Brett' in Australian wine

The Australian Wine Research Institute



The incidence of 'Brett' in Australian wine…

The Australian Wine Research Institute



What is the ‘Brett’ strategy based on?

Scientific literature

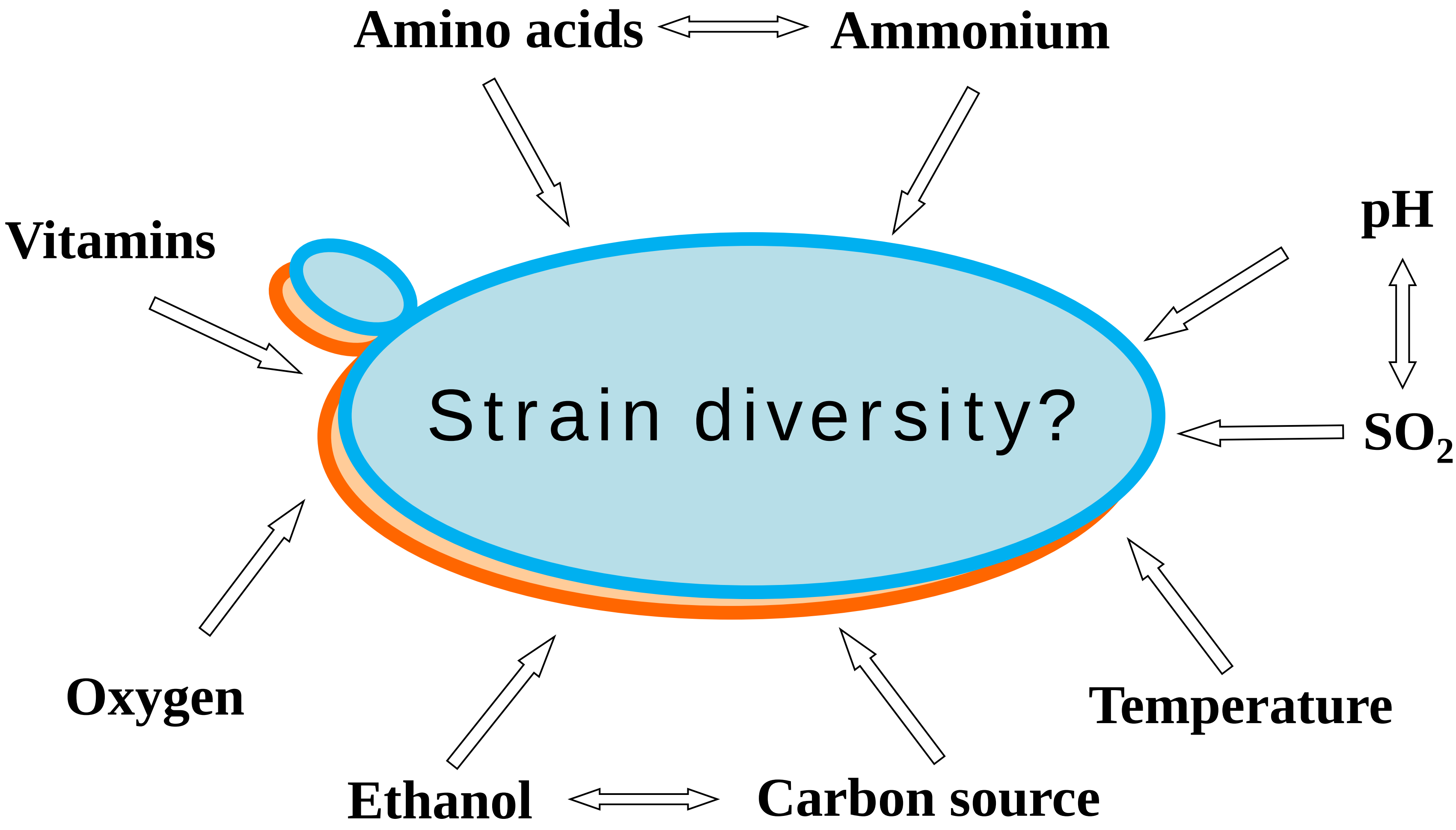
+

What worked for early adopters

+

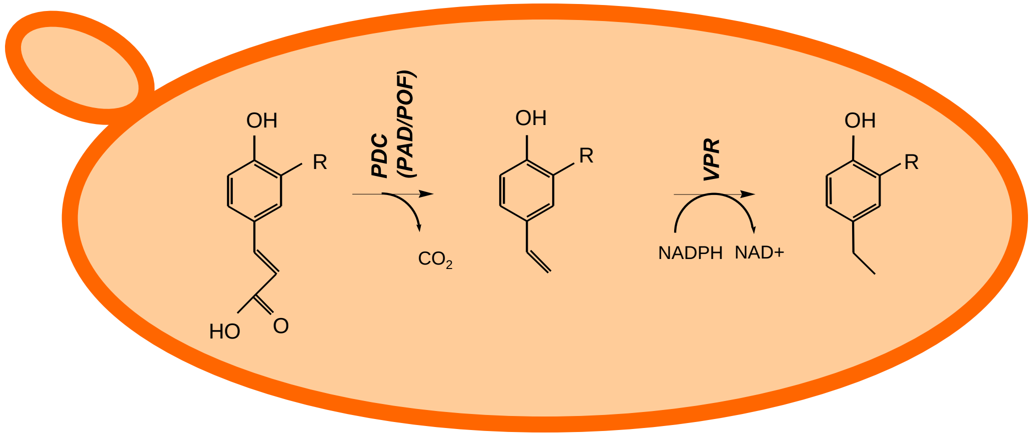
Research

What are the key factors?



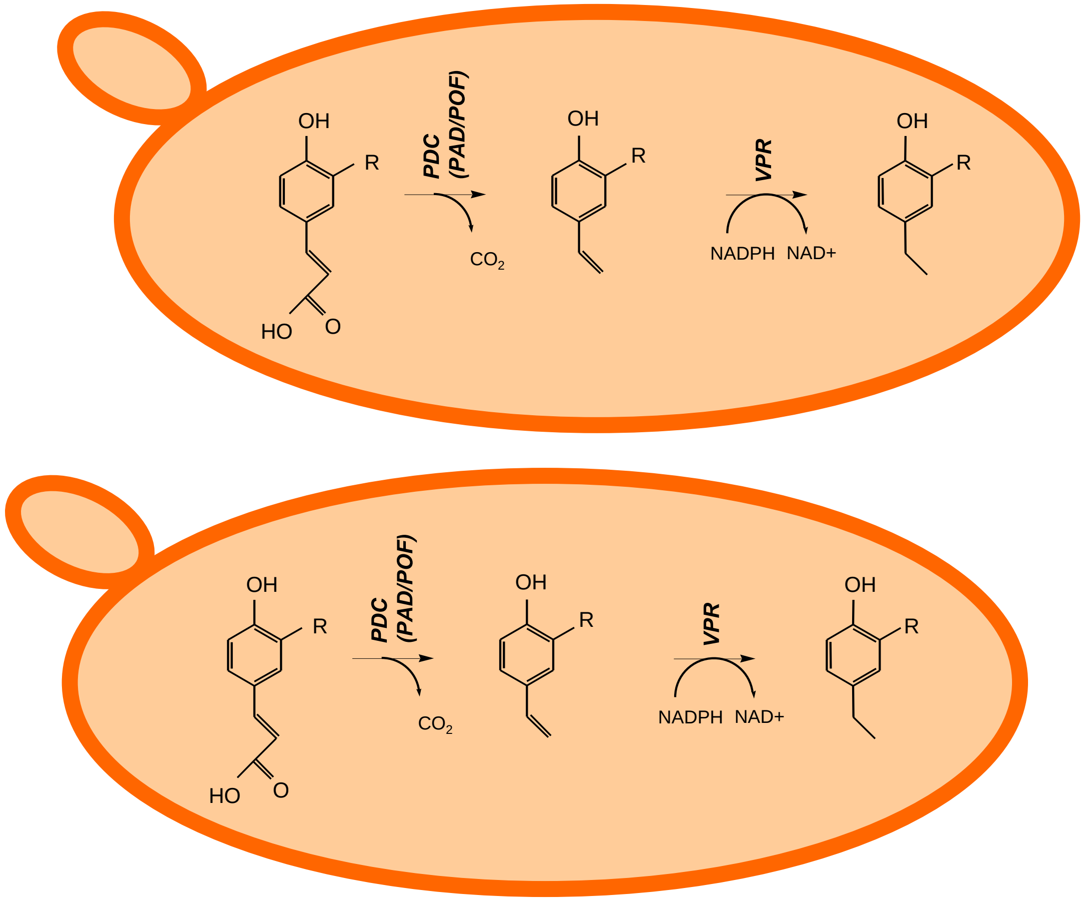
What are the key factors?

The Australian Wine Research Institute



What are the key factors?

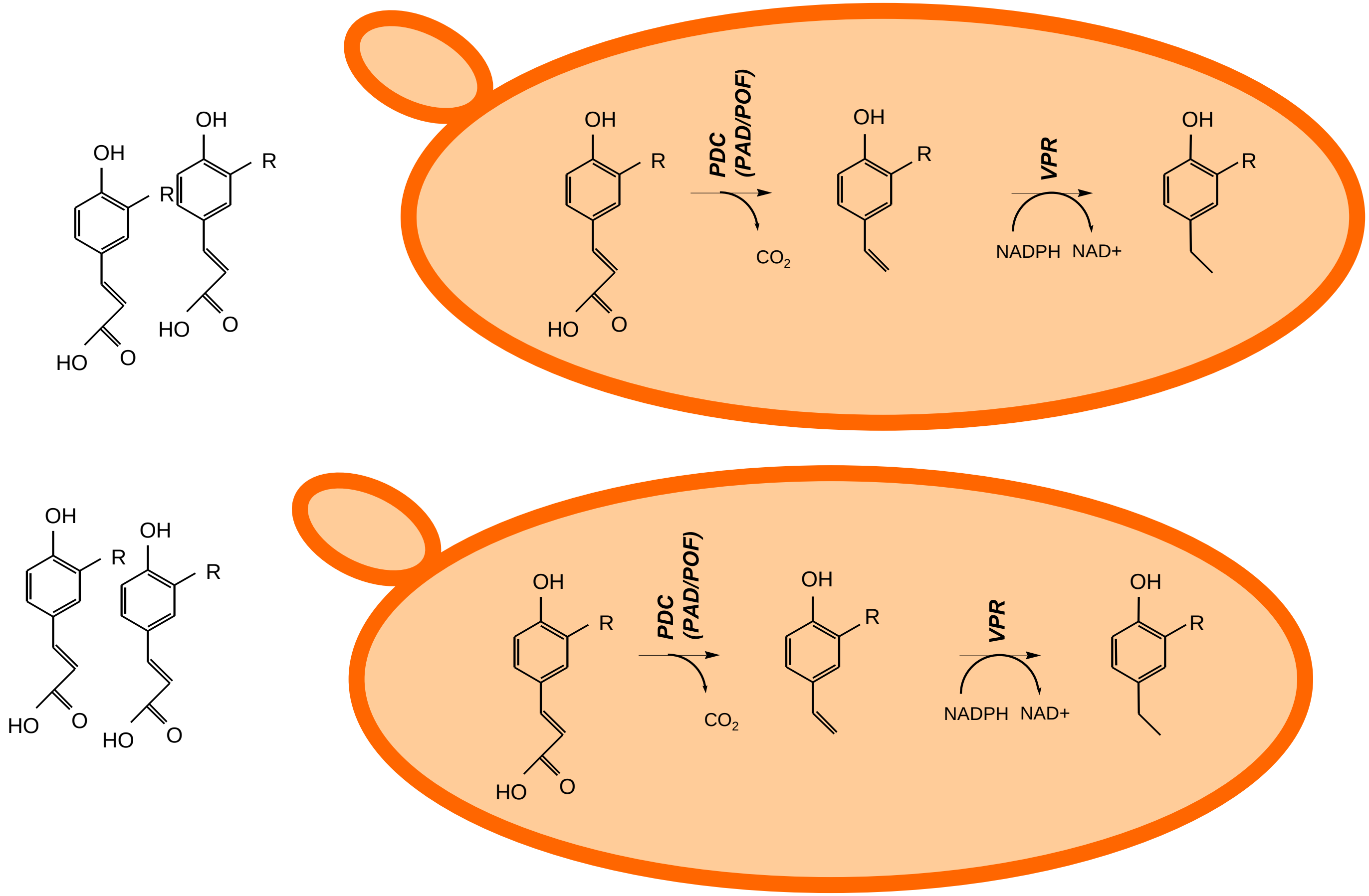
The Australian Wine Research Institute



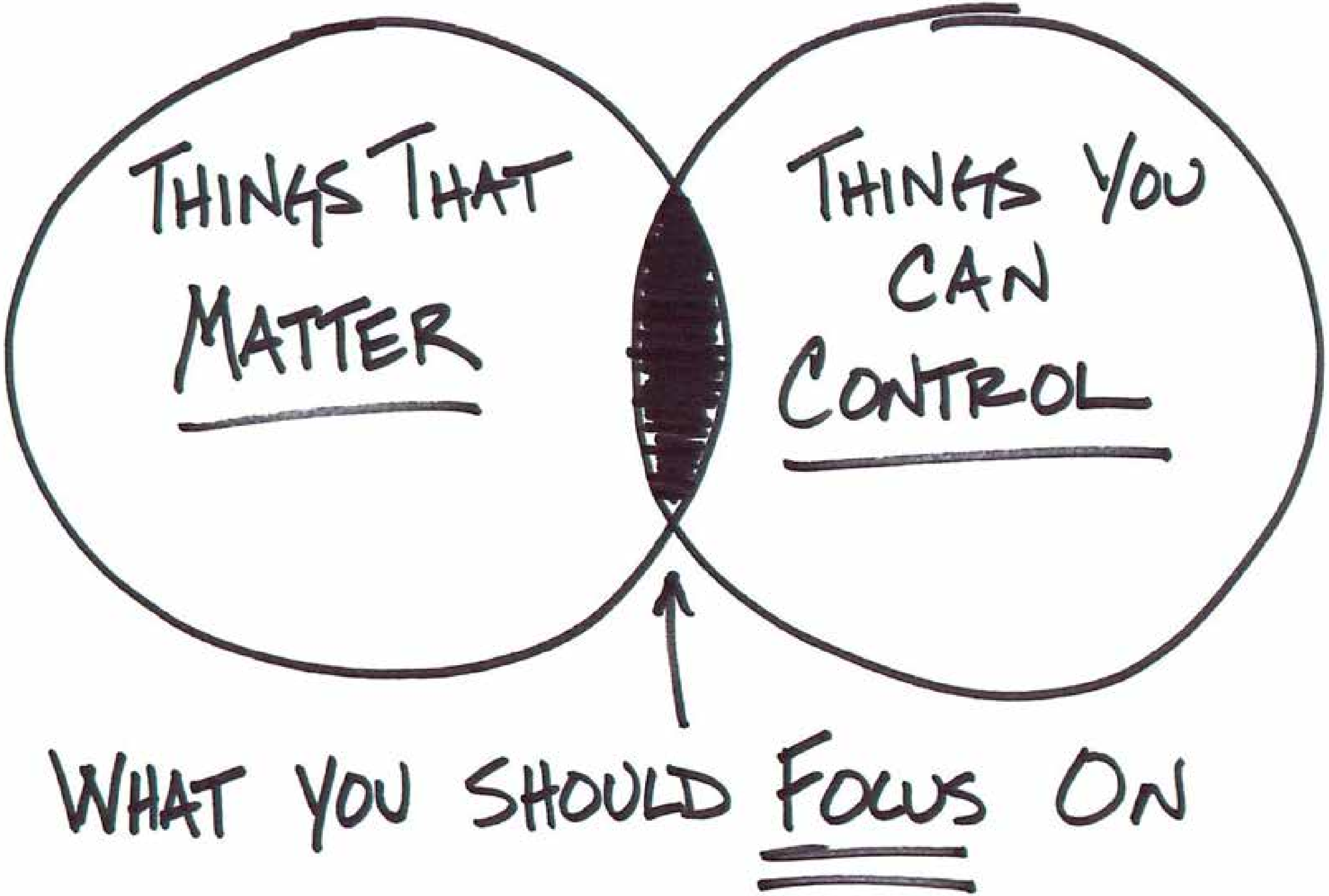
Now 4-EP is being formed at **twice** the rate

What are the key factors?

The Australian Wine Research Institute



Now 4-EP is being formed at **four times** the rate



The ‘Brett’ control strategy focuses on **minimising** Brettanomyces population size entering the winemaking process, **controlling** population growth, and where required, **removing** the population

How can you **minimise** population size?

The Australian Wine Research Institute

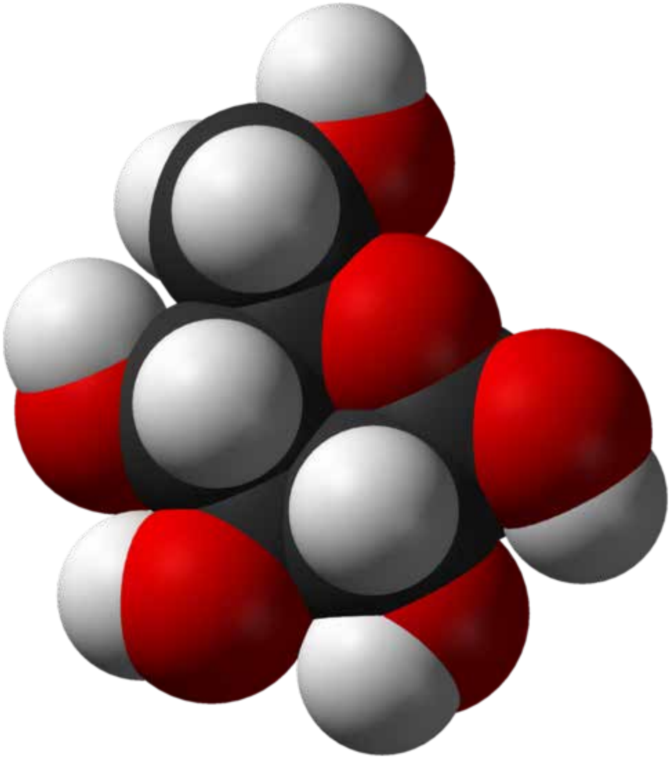
SO_2 @ crusher

General sanitation

Barrel sanitation

How can you **control** population growth?

- Avoid residual sugar:
  - Best practice fermentation management
  - Strong starter culture
  - Aerate when most active i.e. at least one aerative racking or rack & return
  - Keep temperature of wine within two degrees of fermenter during pressing & at least 12 hours afterwards



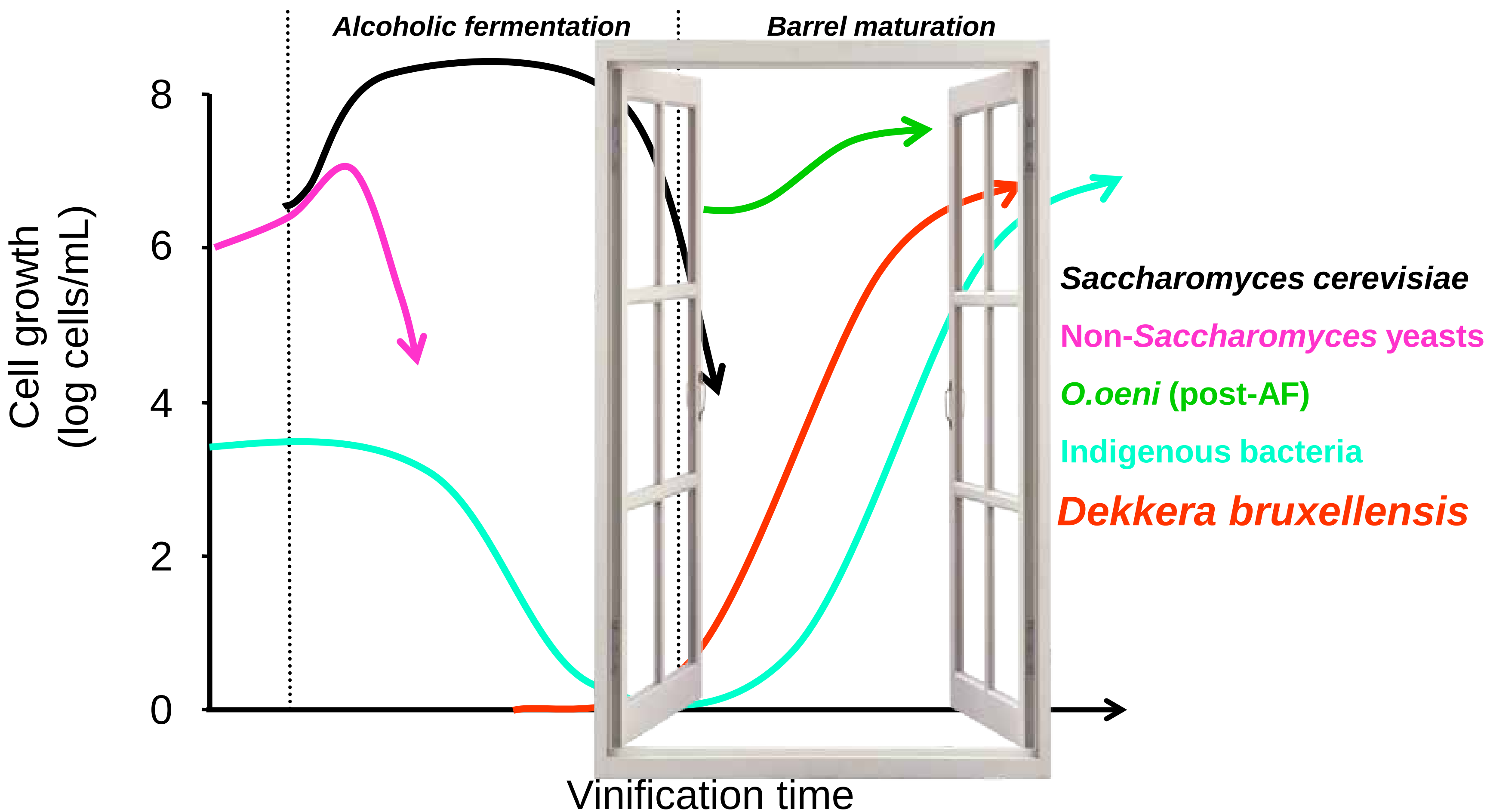
How can you **control** population growth?

The Australian Wine Research Institute

- Minimise residual nitrogen:
  - *Brettanomyces* uses a range of N-sources, including proline
  - Excess DAP ***might*** increase risk of ‘Brett’, or other microbial spoilage
  - Why do you need to add DAP? N-deficiency or other fermentation management issues?

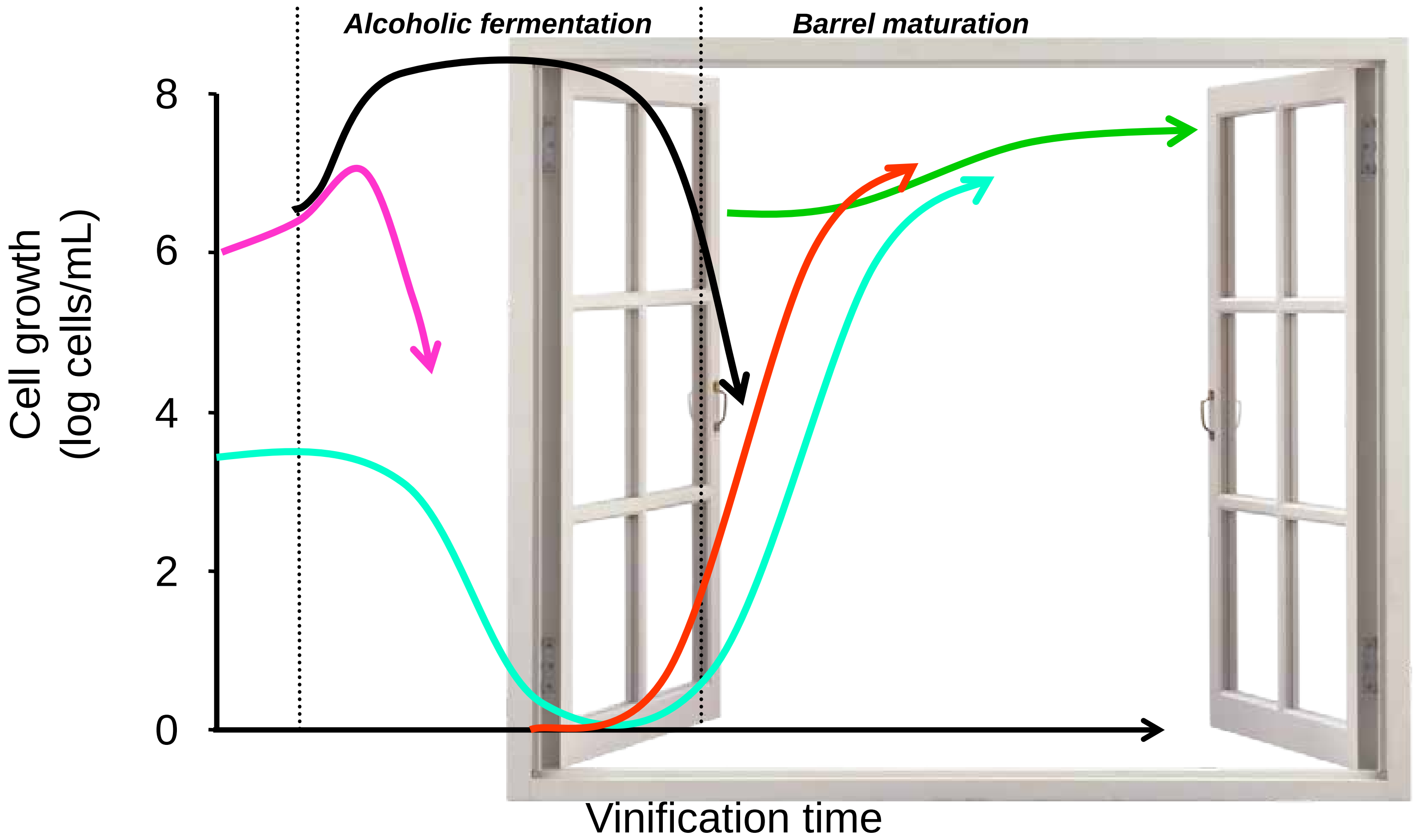
How can you **control** population growth?

The Australian Wine Research Institute



How can you **control** population growth?

The Australian Wine Research Institute



How can you **control** population growth?

The Australian Wine Research Institute

- Barrel management:
  - Monitor regularly
  - Top with good quality, non-'Bretty' wine kept at ~250-300ppm TSO_2
  - New barrels *can* be more prone to 'Brett'
  - Program to empty, clean, refill on the same day

How can you **control** population growth?

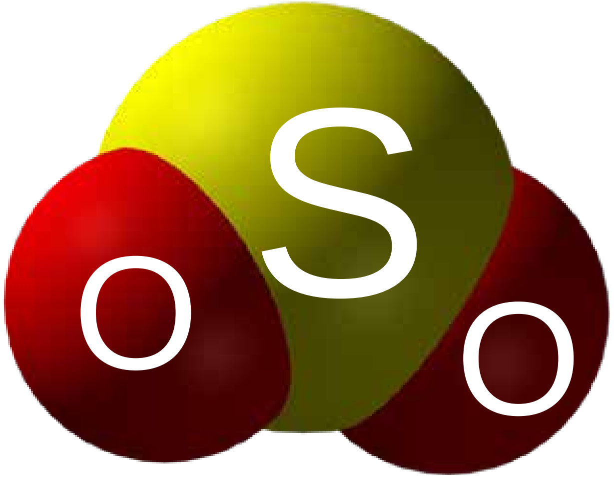
The Australian Wine Research Institute

- Minimise turbidity:
  - Turbidity = less effective SO_2
  - Delayed racking increases lees contact (++nutrients)
  - Delayed racking means greater loss of SO_2
  - When racking take care not to introduce lots of dissolved oxygen

How can you **remove** the population?

The Australian Wine
Research Institute

- Things to note about SO_2 use:
  - It's all about timing & magnitude!
  - Immediately after MLF, clarify and make a large SO_2 addition (60 mg/L)
  - Don't make small incremental additions
  - SO_2 is your friend, so don't be afraid to use it appropriately



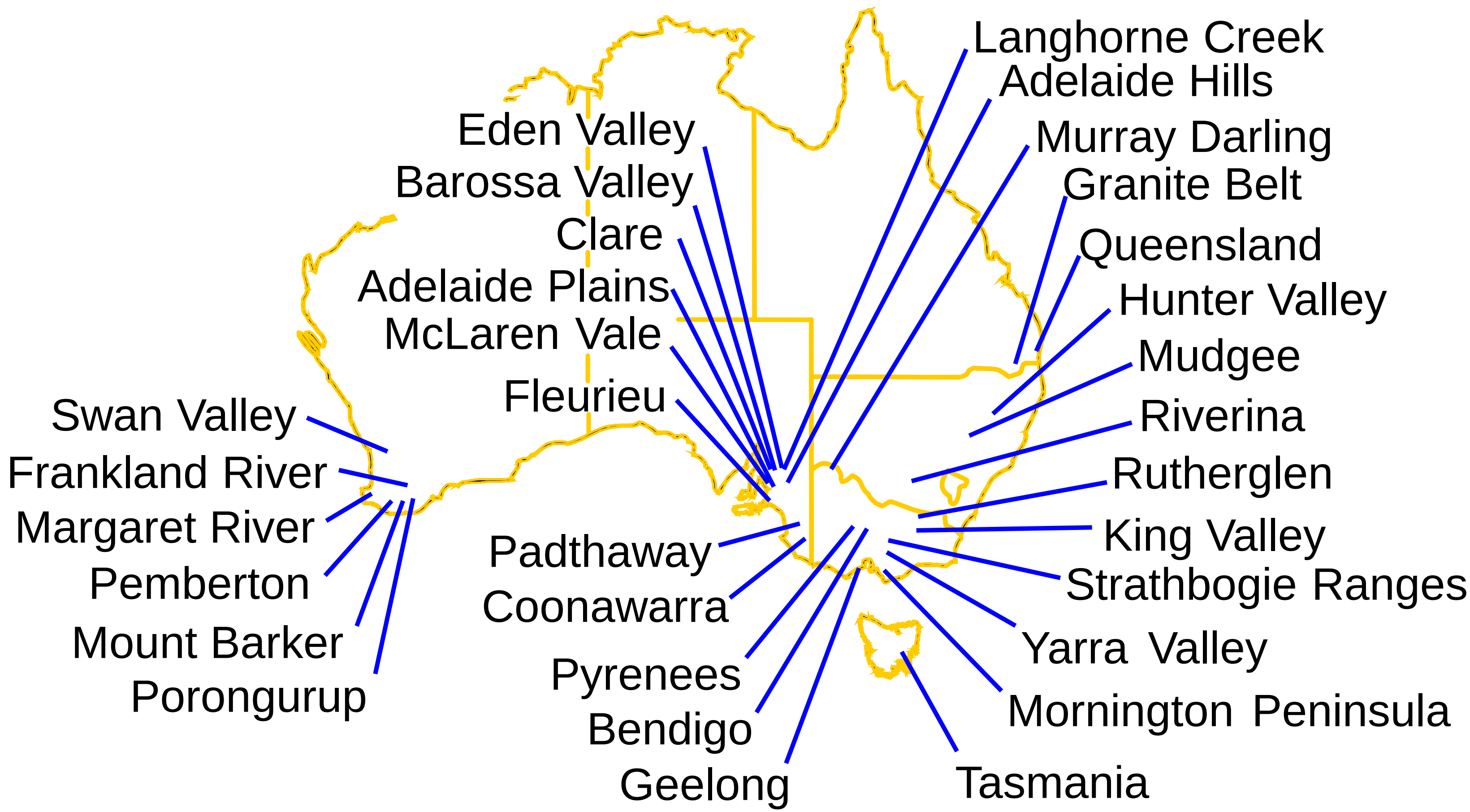
See Robinson & Godden, *Technical Review* 145 (Aug 2003)

How can you **remove** the population?

- Things to note about SO_2 use:
  - A low ratio of Free:Total SO_2 indicates microbial activity (and /or oxidation), while effective SO_2 management gives high ratios
  - Only 35-40% of any addition is yielded as free SO_2 and only the molecular SO_2 component is active
  - The higher the turbidity, the lower the free SO_2
  - At least 5mg/l SO_2 will be lost during careful transfers
  - Wine stored in new barrels loses SO_2 faster than wine stored in old barrels, and hot water or steam cleaned barrels lose SO_2 faster than rinsed barrels

SO_2 tolerance of Australian *Brettanomyces*

The Australian Wine Research Institute

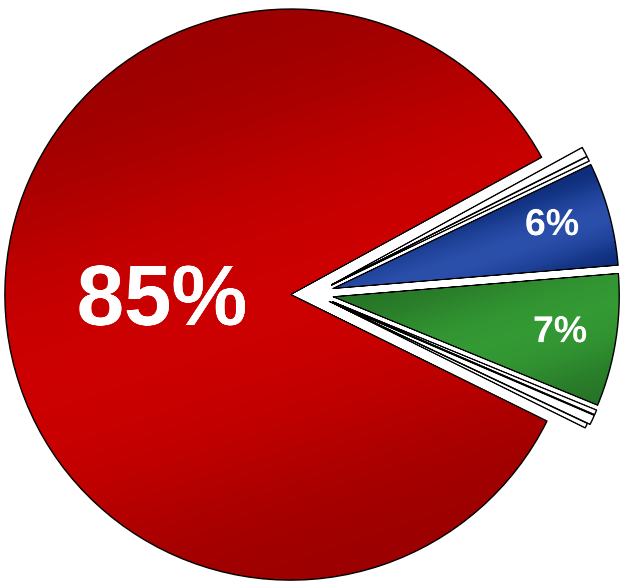


We isolated *Brettanomyces* from 31 winemaking regions of Australia

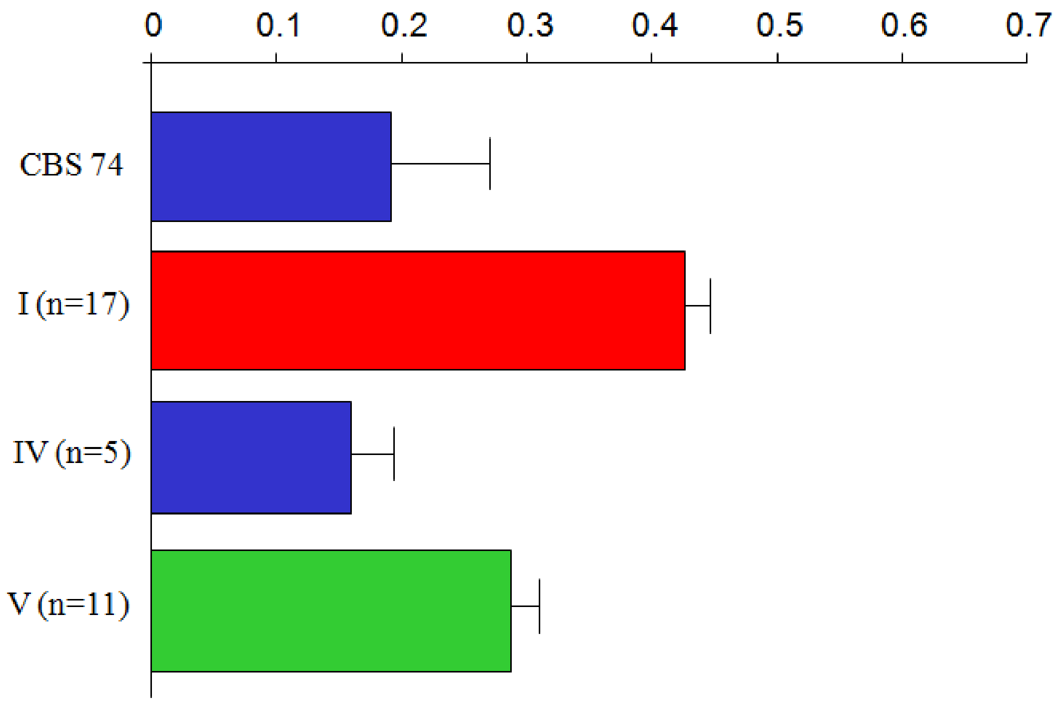
SO_2 tolerance of Australian *Brettanomyces*

The Australian Wine Research Institute

Three strains (DNA markers)



Maximum molecular SO_2 (mg/l) at which growth occurred within 7-days in laboratory conditions



What does this mean for past advice on SO_2 management?

The Australian Wine Research Institute

"In practice, a free SO_2 concentration of 30 mg/L always results in the total elimination of all viable populations after 30 days"

P.Ribereau-Gayon, Y.Glories, A.Maujean and D.Dubourdieu 2000

Handbook of Enology Volume 2

The Chemistry of Wine Stabilization and Treatments

Concentration of molecular SO_2 required to kill yeast:

Saccharomyces cerevisiae 0.825mg/l

Dekkera / Brettanomyces 0.825mg/l

Boulton 1998 (from Beech et al 1979)

How can you **remove** the population?

The Australian Wine Research Institute

- Filtration & clarification
  - If a haze is caused by viable microorganisms, filtration prior to bottling is highly recommended
  - *"A well performed filtration of the appropriate grade does not have a negative impact on wine quality"*
  - Even if there were a negative impact, it would be massively outweighed by the potential for wine spoilage

Take-home message

If you have a ‘Brett’ problem, don’t try to solve it by addressing one area only

‘Brett’ can be successfully managed during winemaking through implementation of a ***holistic*** *control strategy*

Some ‘Brett’ F.A.Q.’s

- Outside of the winery, where does *Brettanomyces* live? Does it come from the vineyard?
- Are there any strains of *Brettanomyces* that are…less bad…?
- Why can some strains tolerate higher levels of sulfite than others, and could we end up with a ‘super-bug’?
- Does SO_2 really kill *Brettanomyces*? Other than SO_2, what else can be used to control *Brettanomyces* populations?
- How can I monitor ‘Brett’ in the winery?

Acknowledgements

The Australian Wine
Research Institute

- Jenny Bellon, Toni Cordente, Adrian Coulter, Geoff Cowey, Miguel de Barros Lopes, Leigh Francis, Peter Godden, Matt Holdstock, Emma Kennedy, Robyn Kievit

- Many <u>anonymous</u> industry collaborators

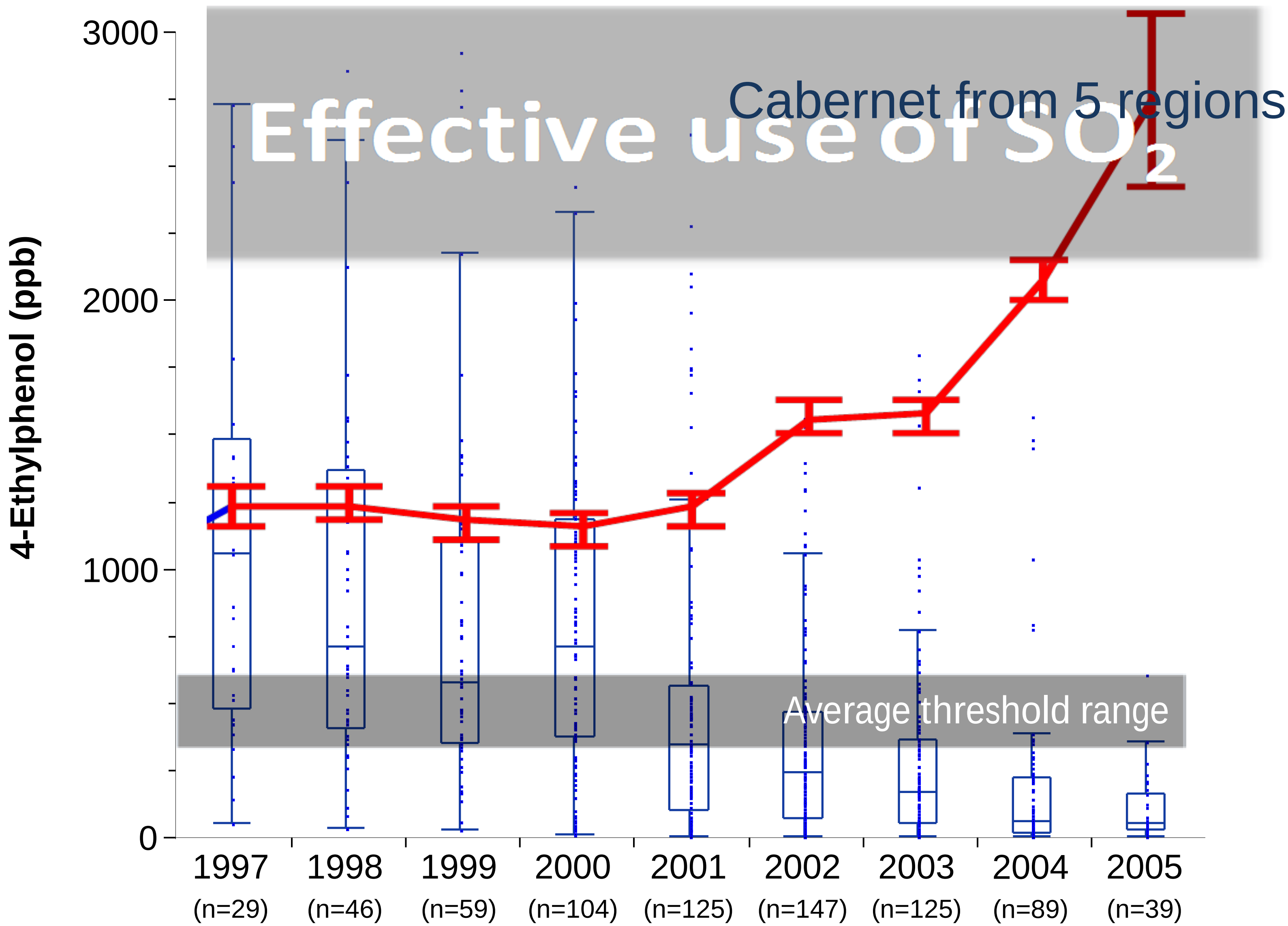
GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

This work was financially supported by Australia's grapegrowers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

The AWRI is part of the Wine Innovation Cluster

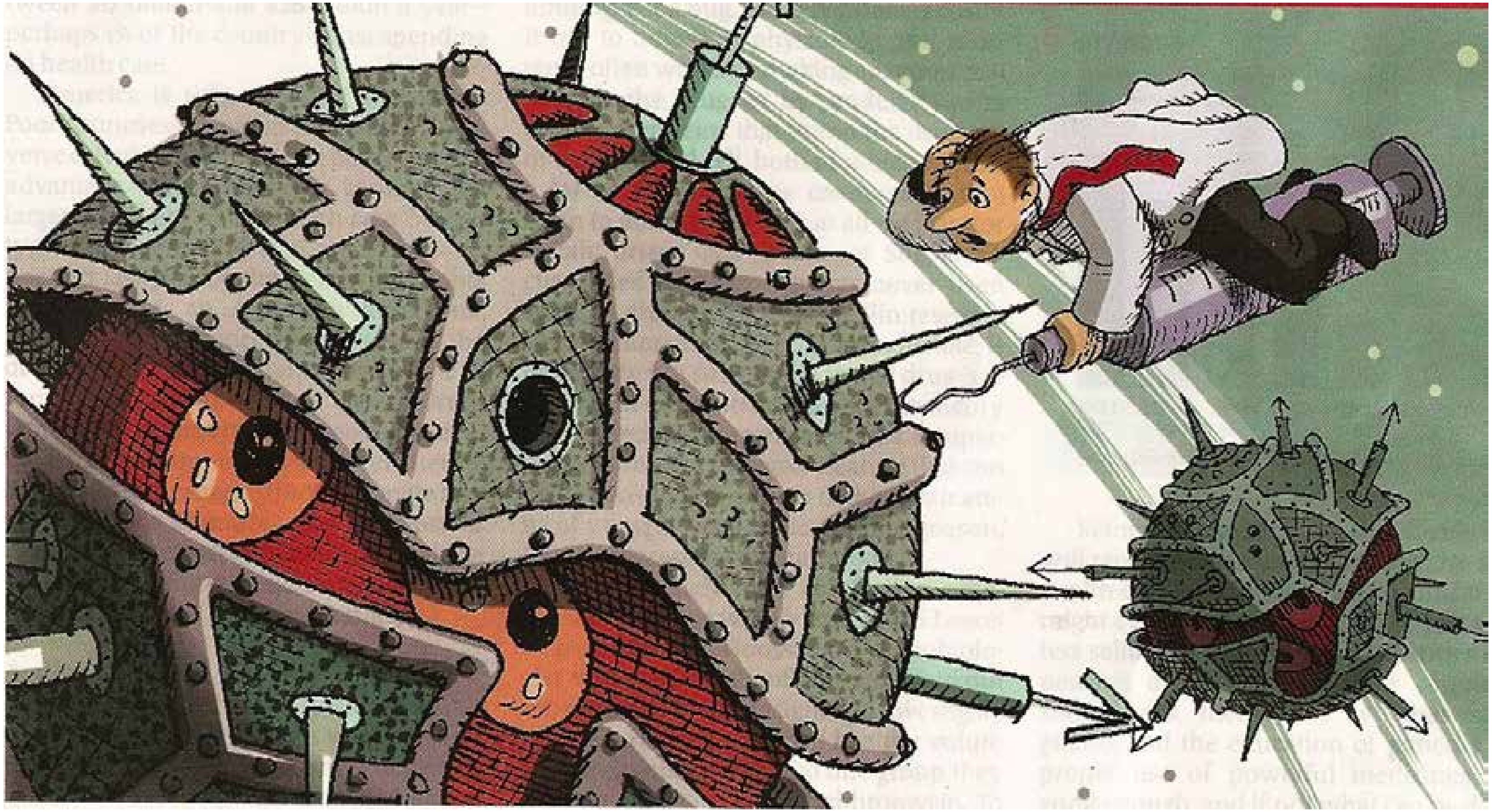
Incidence of ‘Brett’ in Australian wine

The Australian Wine Research Institute



Alarm bells were ringing

The Australian Wine Research Institute



Steven A.R. Murphy , MD
LIC #: 044806 • NPI #: 1962623165
2015 West Main Street
Stamford , CT 06902
Tel: 203-617-0742 • Fax: 212-918-9394

Name. Patient X DOB 101076

Address. Date 51010

℞

Whole Genome,
Lipids, SMA 20, CBCD

Dx: V70.0

MD

DO NOT REPEAT ☐ NO SUBSTITUTION ☐ PLEASE LABEL ☐

Repeat	1	2	3	4	5	NR

SCRIPT #1902

Order #

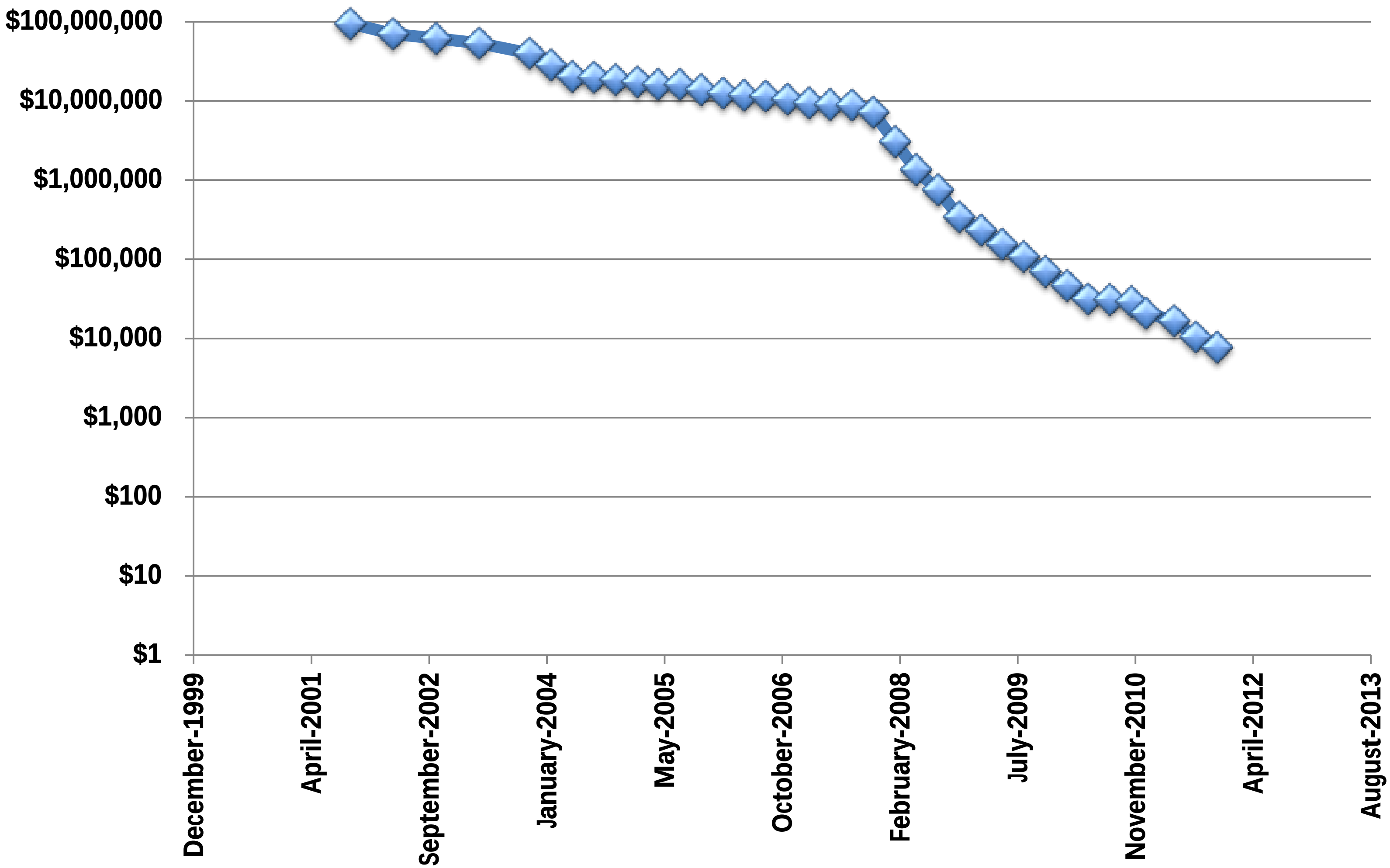
FileRx.com 800-307-7717 RxPads.com

RX_2_GEN_H

VERIFICATION BOX: HOLD BETWEEN THUMB AND FOREFINGER OR BREATHE ON IT. COLOR WILL DISAPPEAR, THEN REAPPEAR

Cost to sequence ***your*** genome

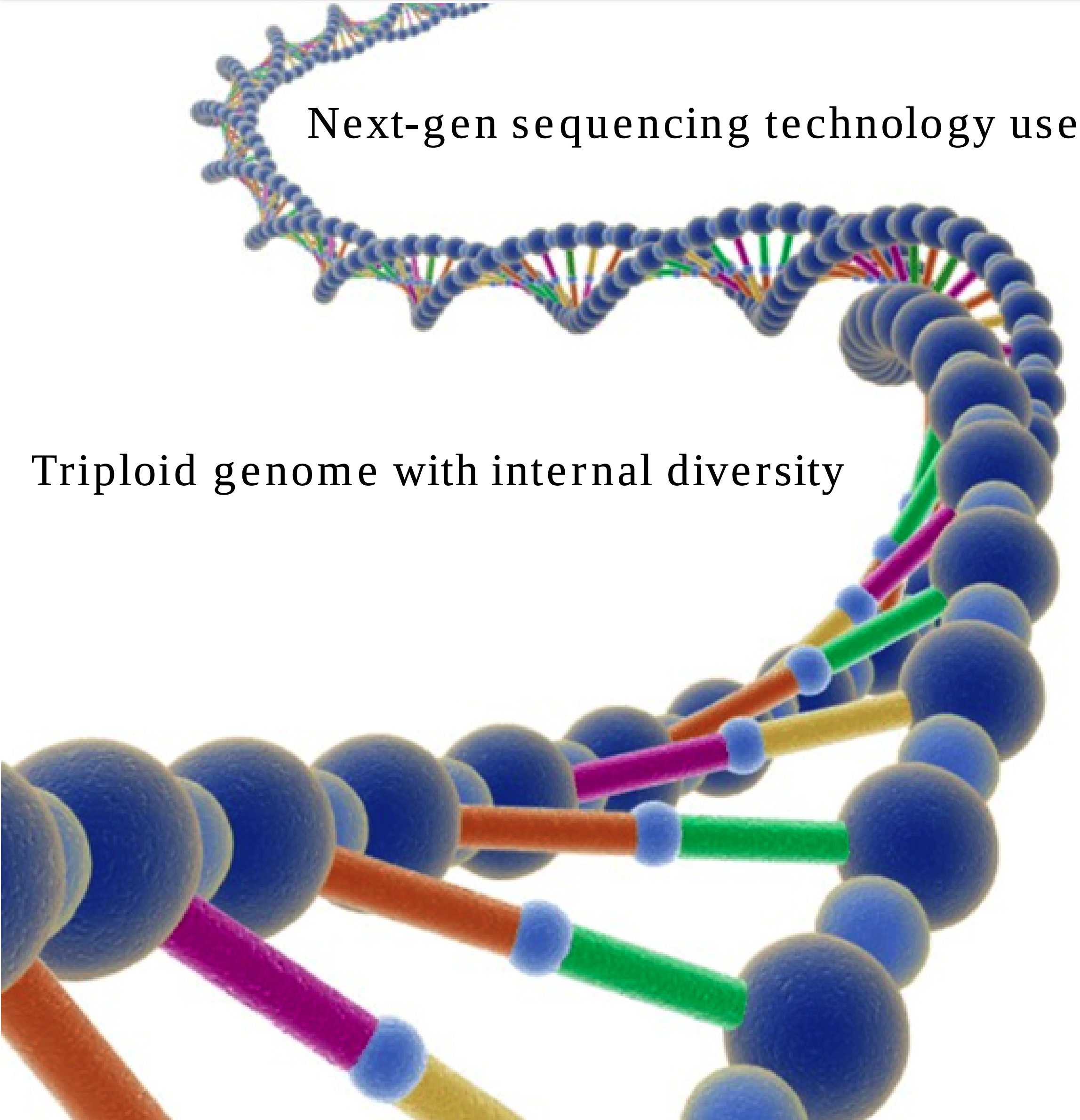
The Australian Wine Research Institute



National Human Genome Research Institute

Future-proofing against ‘Brett’

The Australian Wine Research Institute



Next-gen sequencing technology used to decode the genome of ‘Brett’

Triploid genome with internal diversity

Discovery of key candidate genes:
- ‘Pump’ important in SO_2 tolerance
- Phenolic acid > volatile phenols

Next:
- Potential for ‘super-bug’?
- Diagnostic tests
- Look for ‘chinks in the armour’

VESDA- Developing tools to assist land mangers and industry.

Ricky James- Centre for Expertise in Smoke Taint Research.
DEPI, Rutherglen.

Department of Environment and Primary Industries

State Government Victoria

Overview

Tools for Industry

Tools for land managers.

Smoke taint research relatively new so nothing fit for purpose.

How much smoke????

Determine the relative impacts of controlled burning and wildfire.

- Evaluation of smoke detection monitoring as a tool for measuring smoke intensity and duration of presence and therefore exposure to fruit.

What we know/what we want to know???

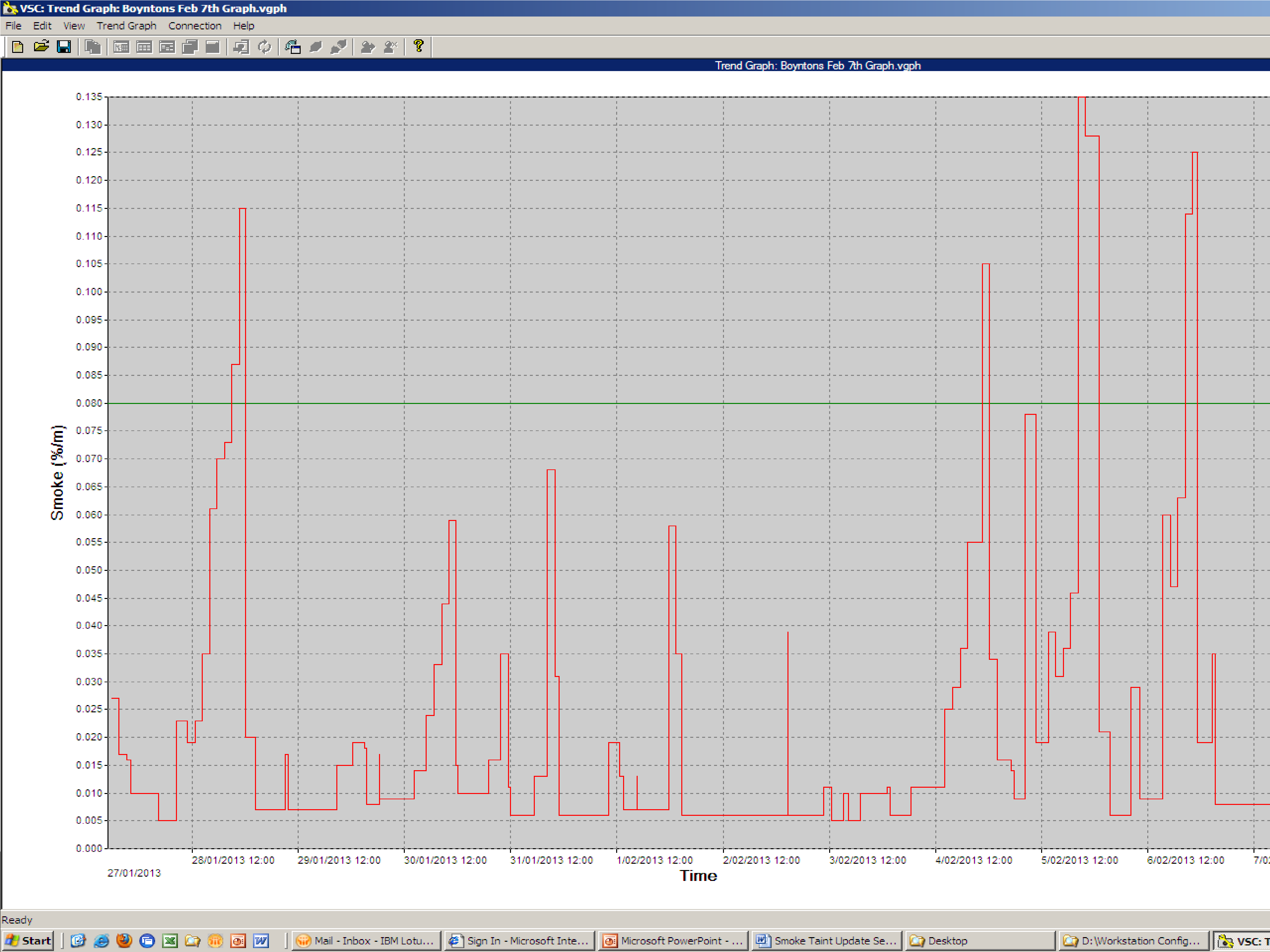
Level of smoke taint is a combination of-

•Intensity of smoke- just like wine, very subjective, need to put a number on it.

•Duration of exposure-how long has the smoke been in the vineyard?

VESDA

- Very Early Warning Aspirating Smoke Detection
- Early warning alarm systems for sealed electrical and telecommunications cabinets.
- Retro fit units to be used in external environment to monitor smoke in vineyards.
- Ability to objectively measure smoke intensity over time and log this data over extended periods and multiple smoke events.


VSC: Trend Graph: Boyntons Feb 7th Graph.vgph
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Trend Graph: Boyntons Feb 7th Graph.vgph
Smoke (%/m)
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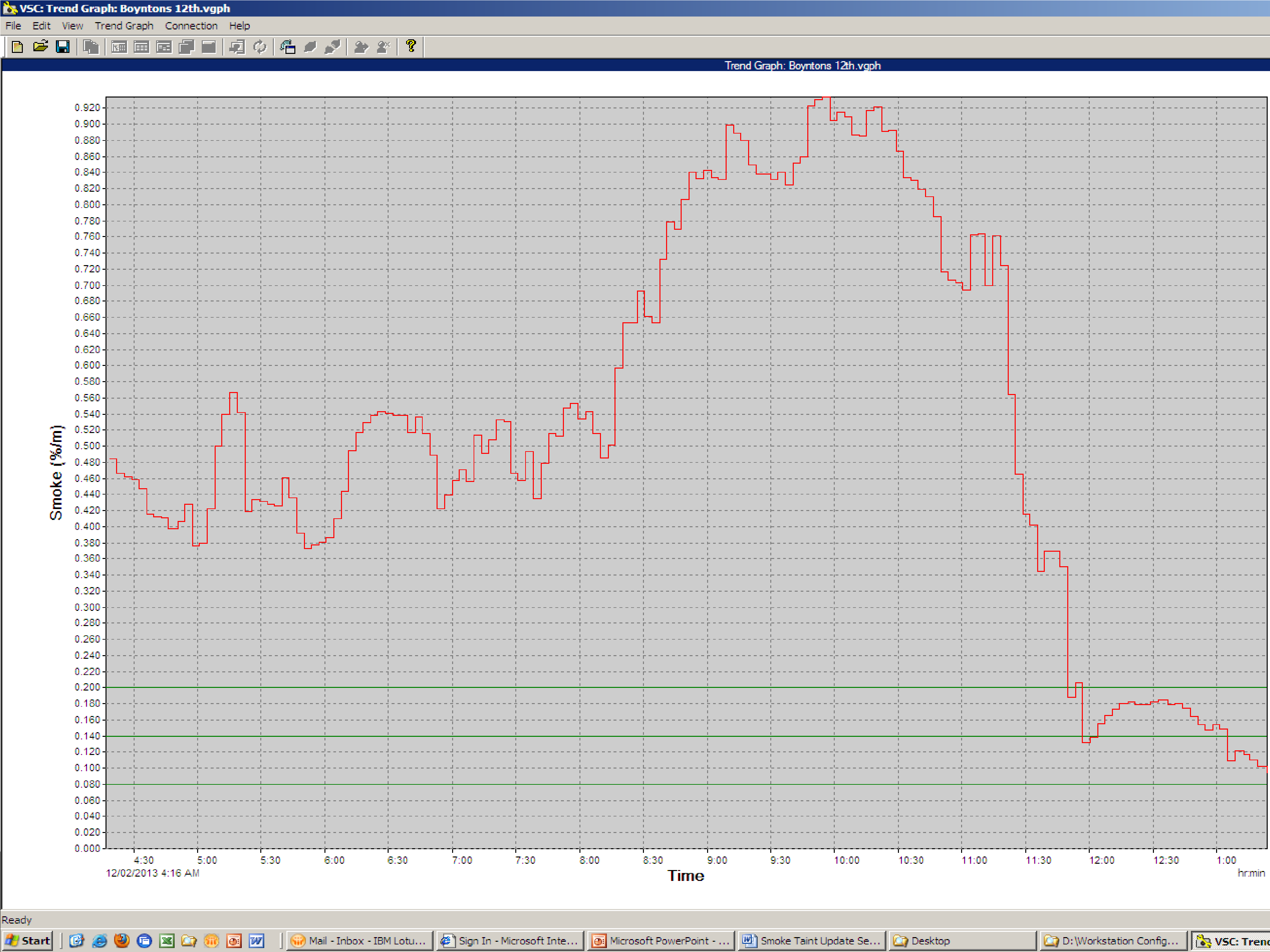
VSC: Trend Graph: Boyntons 12th.vgph
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Trend Graph: Boyntons 12th.vgph
Smoke (%/m)
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0.020
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Sign In - Microsoft Inte...
Microsoft PowerPoint - ...
Smoke Taint Update Se...
Desktop
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VSC: Trend

Positives and Negatives

- + Affordable price for industry- approx $2500
- + Logs intensity and duration
- + Simple installation and data collection
- + Real time data to monitor controlled trials.
- + Local production, knowledge and experience.
- - Not ‘fit for purpose’. External conditions.
- - False positives- dust, moisture, Winnie Blues
- - Very sensitive- logs every change in concentration
- - Correlations with EPA air Quality data.

Next Steps

- Adjust software to enable more suitable and reliable data to be collected.
- Correlation between Obs/m and visual horizon data.
- Ability to create a network across a region when best opportunity arises
- Monitor numerous locations in the one region to compare smoke intensity, duration and affect on fruit.

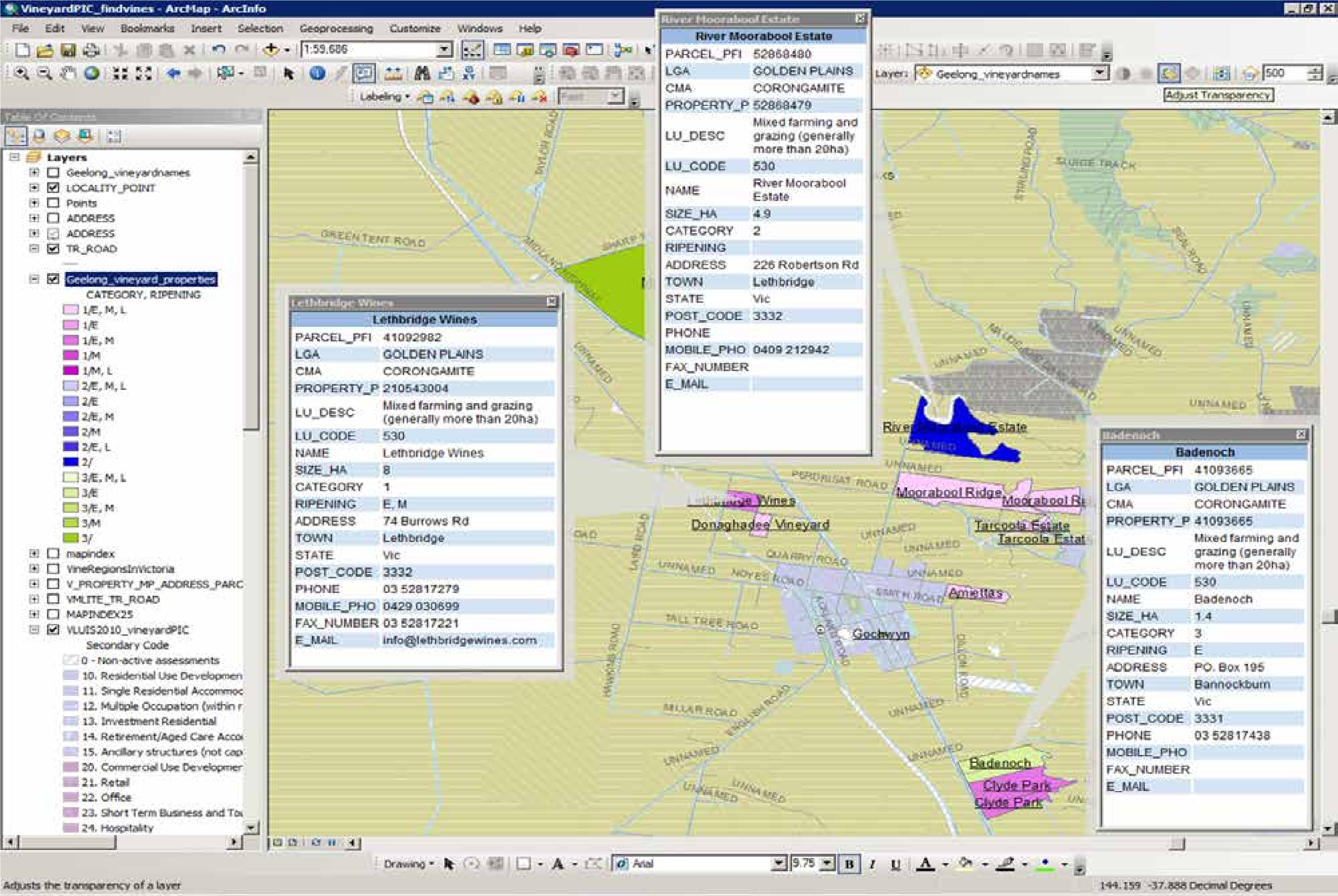
Who, What and Where??

Project Management Plan- Objective 6

Develop and evaluate a risk assessment tool to enable industry and land managers to determine suitable burning periods based on varietal sensitivity and grapevine development.

Fit for purpose mapping for land managers and industry

- VLUIS- Victorian Land Use Information Survey
- Biosecurity Victoria- PIC Codes
- DEPI- Fire Management
- Victorian Wine Industry
- DAFWA- STAR Model



VineyardPIC_findvines - ArcMap - ArcInfo
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ADDRESS
ADDRESS
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Geelong_vineyard_properties
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1/E
1/E, M
1/M
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mapindex
VineRegionsInVictoria
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MAPINDEX25
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Secondary Code
0 - Non-active assessments
10. Residential Use Developmen
11. Single Residential Accommoc
12. Multiple Occupation (within r
13. Investment Residential
14. Retirement/Aged Care Acco
15. Ancillary structures (not cap
20. Commercial Use Developmer
21. Retail
22. Office
23. Short Term Business and To
24. Hospitality
River Moorabool Estate
PARCEL_PFI 52868480
LGA GOLDEN PLAINS
CMA CORONGAMITE
PROPERTY_P 52868479
LU_DESC Mixed farming and grazing (generally more than 20ha)
LU_CODE 530
NAME River Moorabool Estate
SIZE_HA 4.9
CATEGORY 2
RIPENING
ADDRESS 226 Robertson Rd
TOWN Lethbridge
STATE Vic
POST_CODE 3332
PHONE
MOBILE_PHO 0409 212942
FAX_NUMBER
E_MAIL
Lethbridge Wines
PARCEL_PFI 41092982
LGA GOLDEN PLAINS
CMA CORONGAMITE
PROPERTY_P 210543004
LU_DESC Mixed farming and grazing (generally more than 20ha)
LU_CODE 530
NAME Lethbridge Wines
SIZE_HA 8
CATEGORY 1
RIPENING E, M
ADDRESS 74 Burrows Rd
TOWN Lethbridge
STATE Vic
POST_CODE 3332
PHONE 03 52817279
MOBILE_PHO 0429 030699
FAX_NUMBER 03 52817221
E_MAIL info@lethbridgewines.com
Badenoch
PARCEL_PFI 41093665
LGA GOLDEN PLAINS
CMA CORONGAMITE
PROPERTY_P 41093665
LU_DESC Mixed farming and grazing (generally more than 20ha)
LU_CODE 530
NAME Badenoch
SIZE_HA 1.4
CATEGORY 3
RIPENING E
ADDRESS PO. Box 195
TOWN Bannockburn
STATE Vic
POST_CODE 3331
PHONE 03 52817438
MOBILE_PHO
FAX_NUMBER
E_MAIL
GREEN TENT ROAD
Moorabool Ridge
Donaghades Vineyard
Tarcoola Estate
Amietta's
Gochwyn
Badenoch
Clyde Park
QUARRY ROAD
NOYES ROAD
SMITH ROAD
TALL TREE ROAD
MILLAR ROAD
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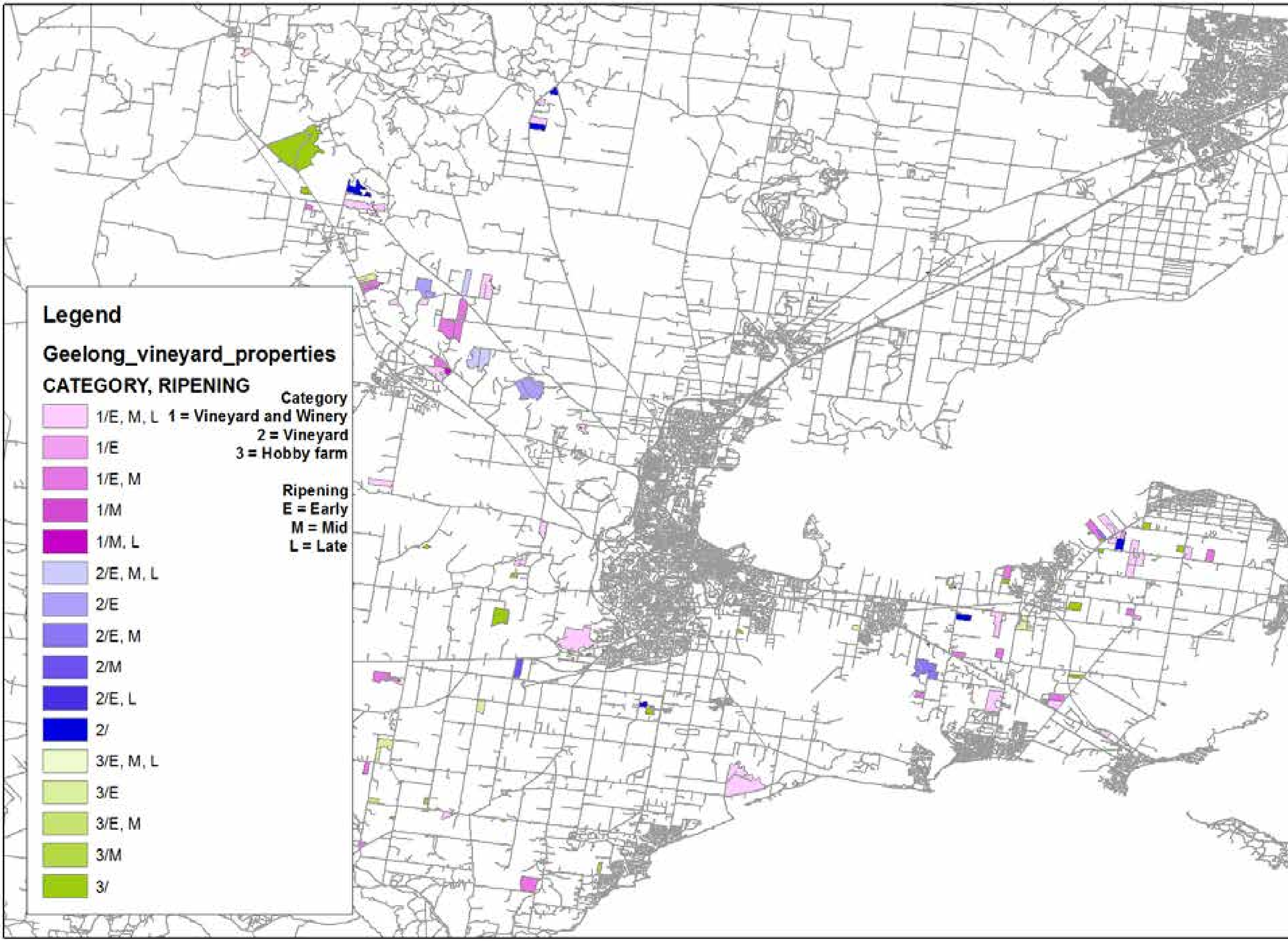
Legend
Geelong_vineyard_properties
CATEGORY, RIPENING
1/E, M, L
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2/E, M
2/M
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2/
3/E, M, L
3/E
3/E, M
3/M
3/
Category
1 = Vineyard and Winery
2 = Vineyard
3 = Hobby farm
Ripening
E = Early
M = Mid
L = Late

Wine Related Research @ DEPI

Smoke Taint- Mark Downey

Tannin measurements in Winegrapes and resulting wines- Rachel Kilmister

Impacts of global warming on grape phenology, vine growth and grape quality- Rachel Kilmister

Soil Health- Ian Porter and Jacky Edwards

Phenology – Veraison
Heated Chamber @ +2 DegC.

23/12/11 29/12/11 4/1/12 20/1/12

Questions???

A W R I

The Australian Wine Research Institute

Strategies for Successful MLF

What can you do to get that MLF through efficiently (and before Christmas!)?

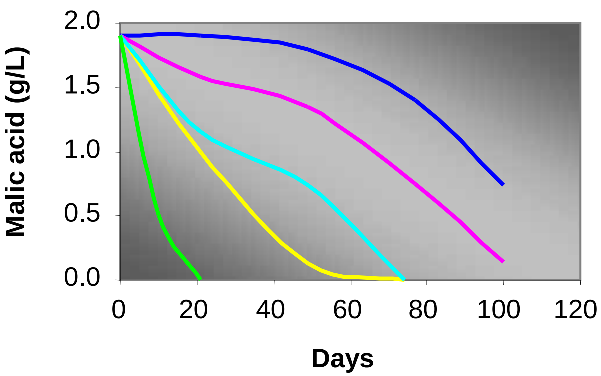
Eveline Bartowsky

Senior Research Microbiologist

What is a successful MLF?

The Australian Wine Research Institute

Different for everyone



- Malic acid metabolism
  - Rapid
  - Complete

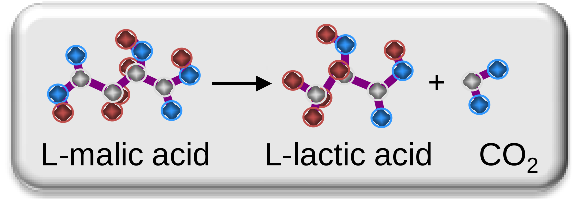
- Sensory changes
  - None
  - Buttery character
  - - fruity & ¯ vegetative characters
  - Improved mouthfeel

Ø *Delayed/failed MLF can increase the risk of wine spoilage, especially Brett and biogenic amines*

As a reminder …

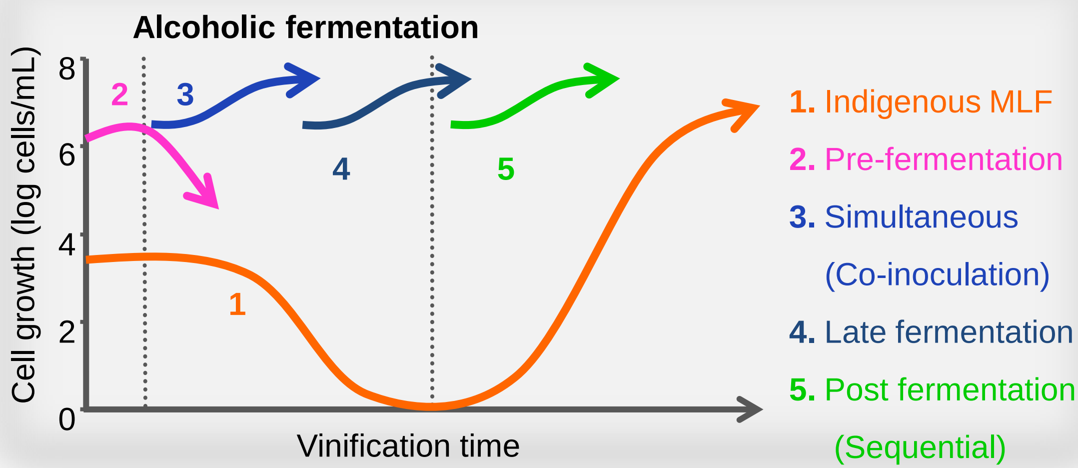
The Australian Wine
Research Institute

- Bacteria mediated



  - Reduce wine acidity
  - Microbial stability
  - Sensory changes

- When can it occur?
  - Spontaneous
  - Inoculated

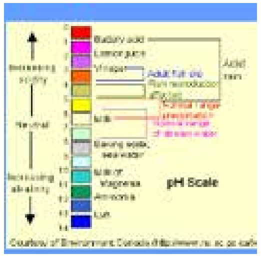


MLF is generally more difficult to manage that AF

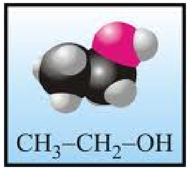
The key to MLF

The Australian Wine
Research Institute

Main factors

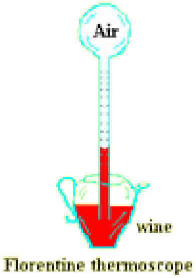


- pH

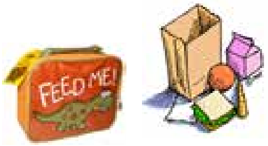


- Alcohol (ethanol)

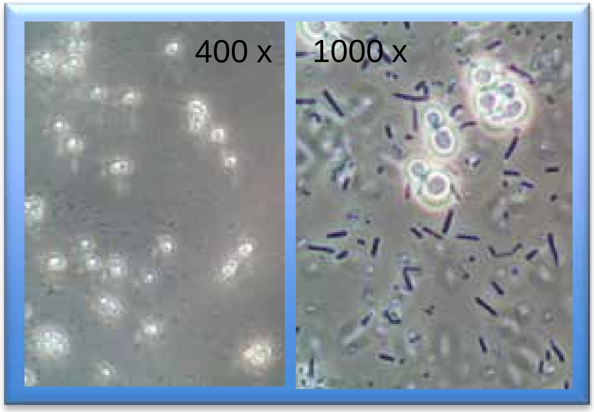
- SO_2



- Temperature
- Bacteria
- Yeast

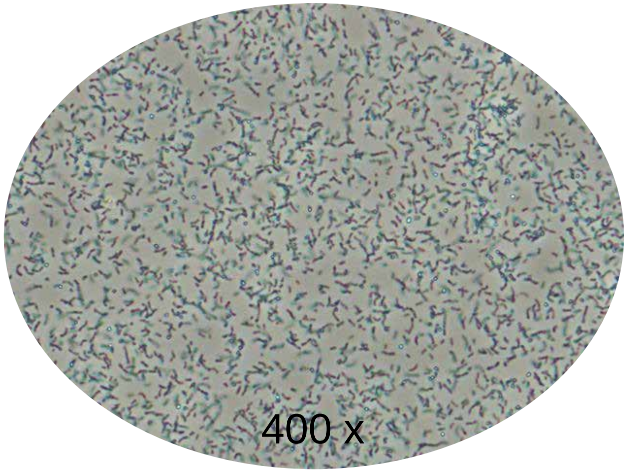


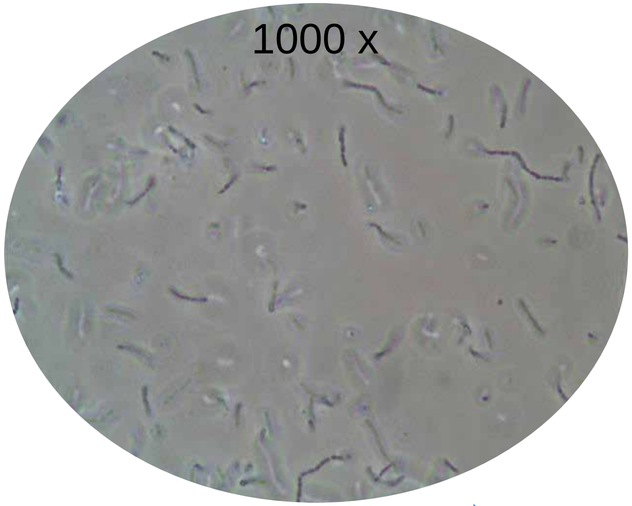
- Nutrients



Under the microscope

The Australian Wine Research Institute





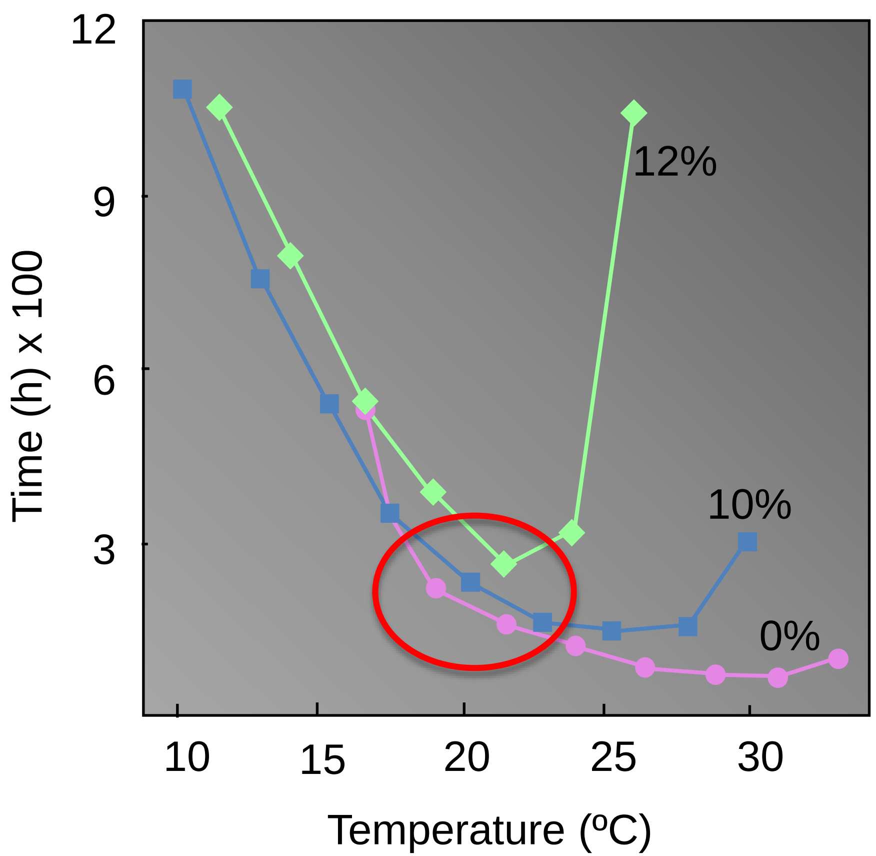
Don't under estimate the power of the microscope

Temperature and ethanol - *O. oeni*

The Australian Wine Research Institute

- LAB generally are more temperature sensitive than yeast
- Most *O. oeni* strains grow slowly < 15˚C
- Ethanol affects cell wall composition

Ø Monitor temperature of wines regularly

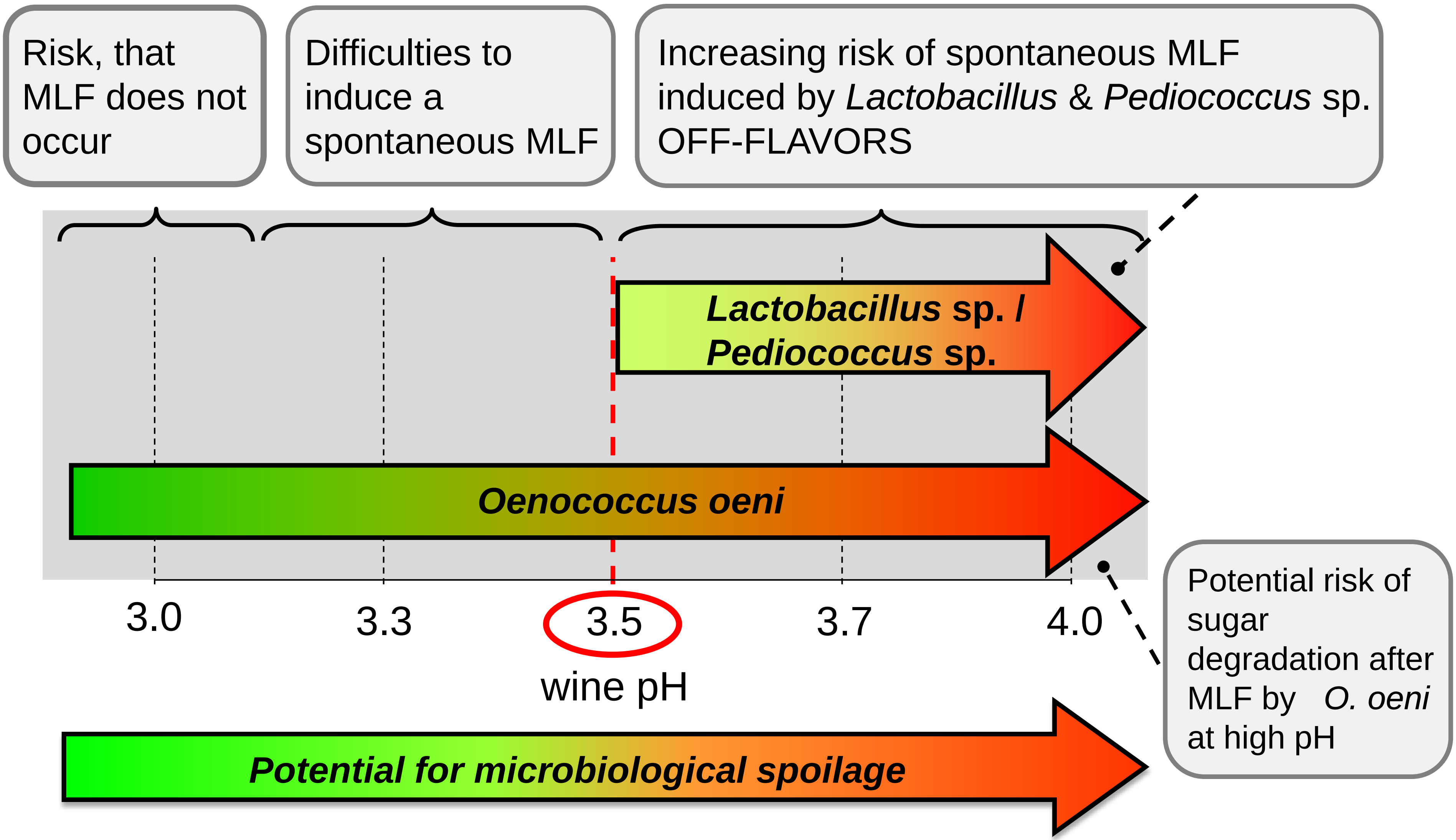


Strain AM20
Asmundson & Kelly (1990)

For harsh wine conditions, adjust temperature within range 18-22˚C

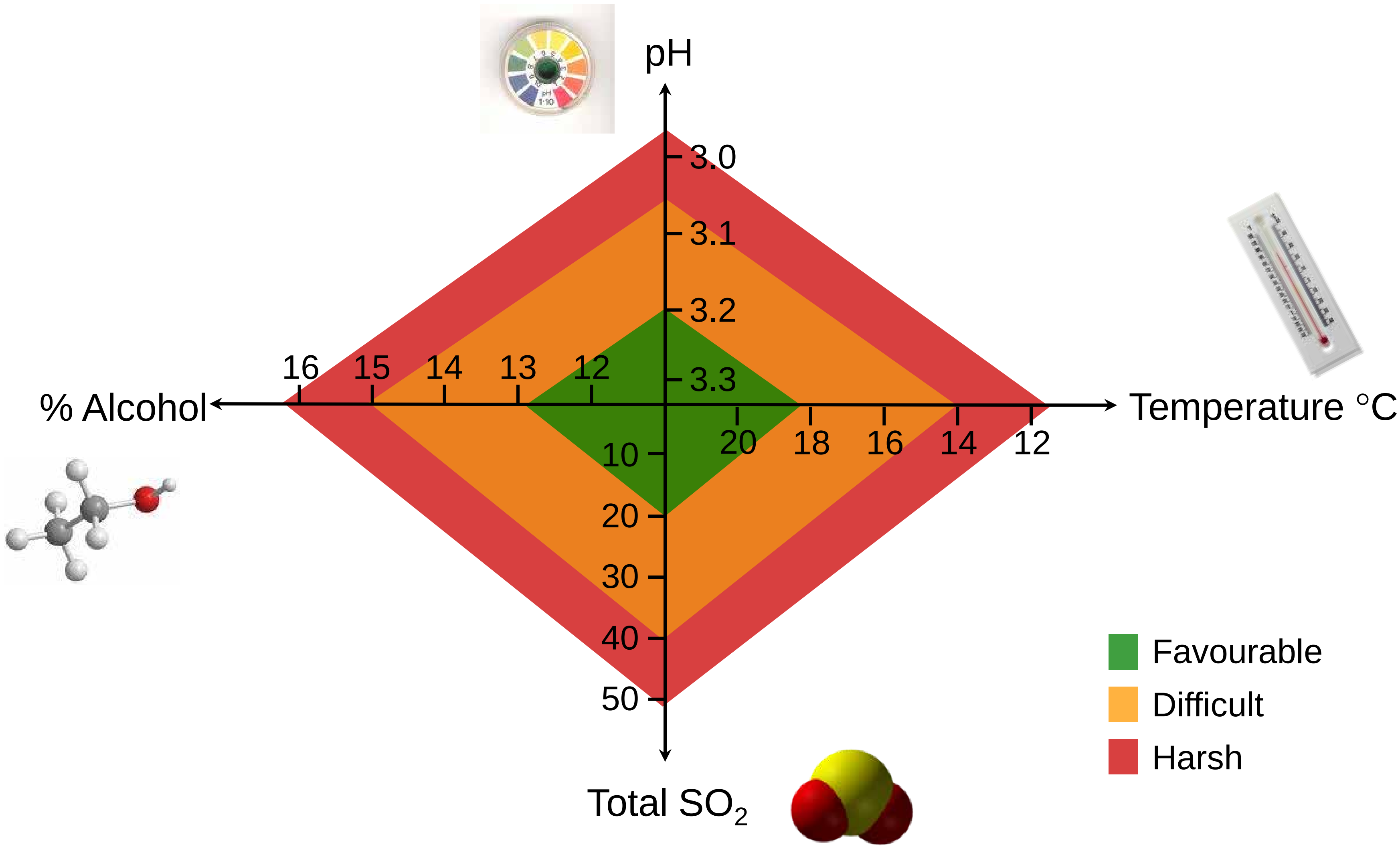
Effect of wine pH and LAB growth

The Australian Wine Research Institute



Additive effect of crucial MLF factors

The Australian Wine Research Institute

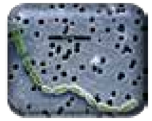


Adapted from Lallemand

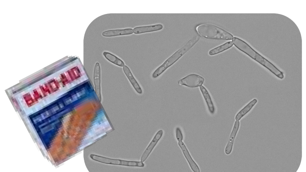
Why inoculate for MLF?

The Australian Wine Research Institute

v Indigenous



- § Diverse population
- § Spontaneous MLF
- Ø Induction can be unpredictable
- Ø Unpredictable quality
- Ø Possible infection by spoilage microorganisms
- Ø Risk of off-flavours, amines, mousy …..
- § Low cost & minimal resources
- § Risk of ‘Brett’ development

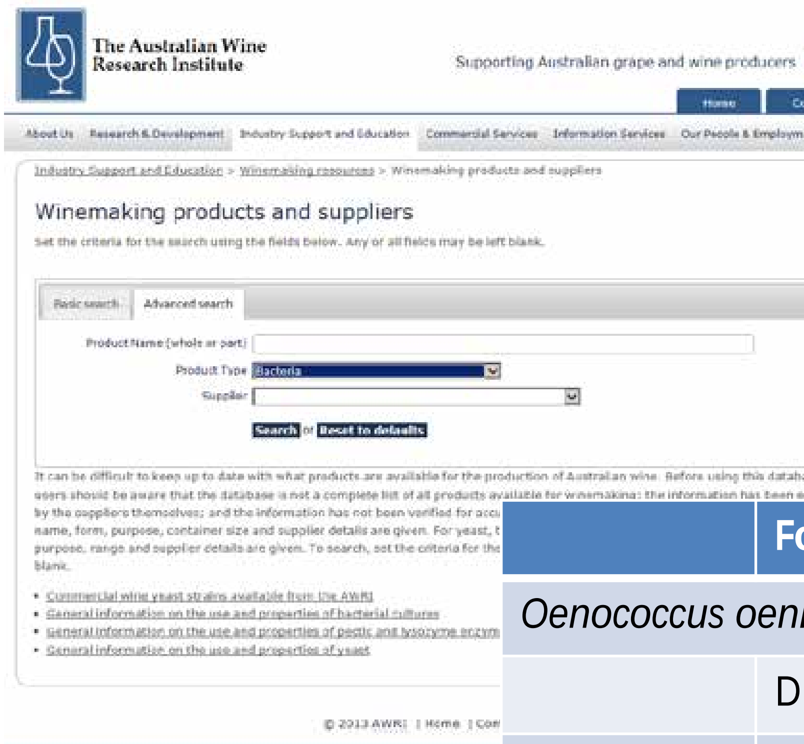


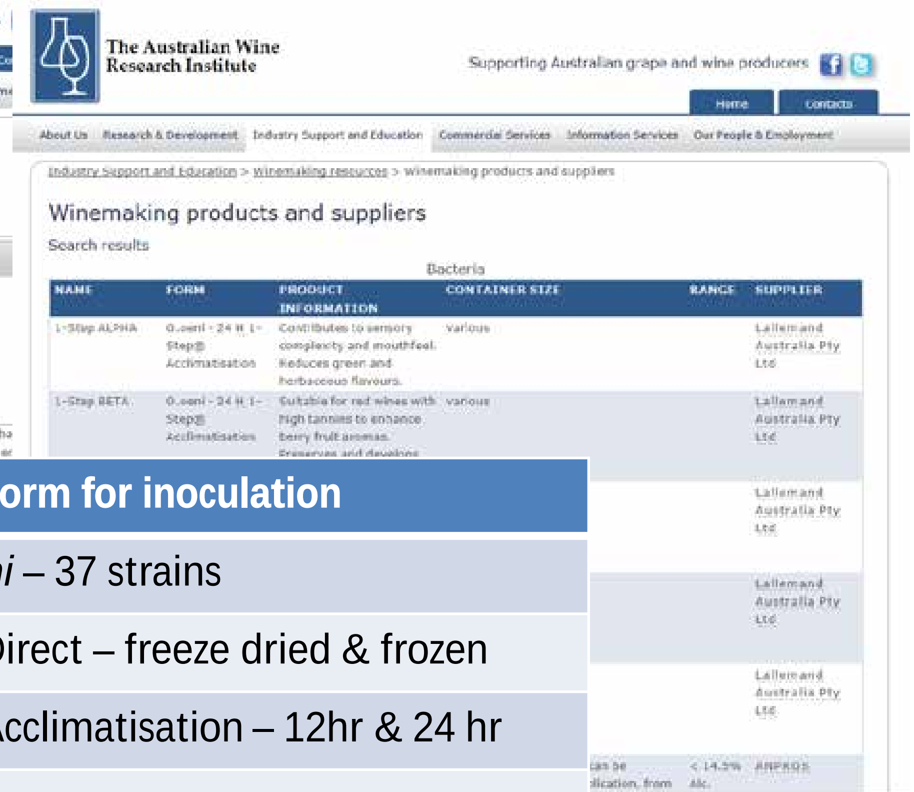
v Selected

- § Unique strain
- § Inoculation - high cfu/mL
- § Induction at a chosen time
- § Predictable kinetics
- § Sensory & quality attributes
- Ø Shorter lag phase
- Ø Better control
- Ø Decreases potential of spoilage LAB (& AAB)
- Ø Strains selected against negative characteristics

Commercial Malolactic Starter Cultures

The Australian Wine Research Institute



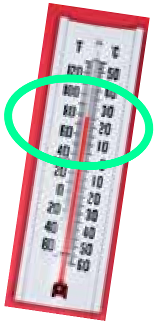


	Form for inoculation
Oenococcus oeni – 37 strains	
	Direct – freeze dried & frozen
	Acclimatisation – 12hr & 24 hr
Lactobacillus plantarum – 1 strain	
	Direct – freeze dried
Updated May 2013	

Management of ML product

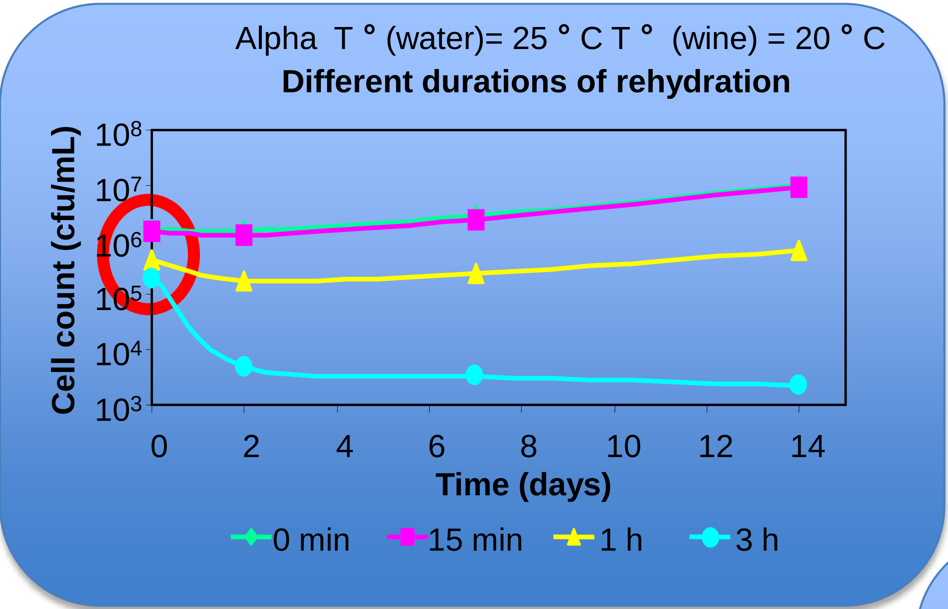
Don't under estimate the importance of correct product management

- Store correctly
  - -18°C
- Use by the 'use by date'
  - Up to ~ 24 months
- Rehydrate according to the instructions
  - Temperature of water or wine
  - Length of rehydration

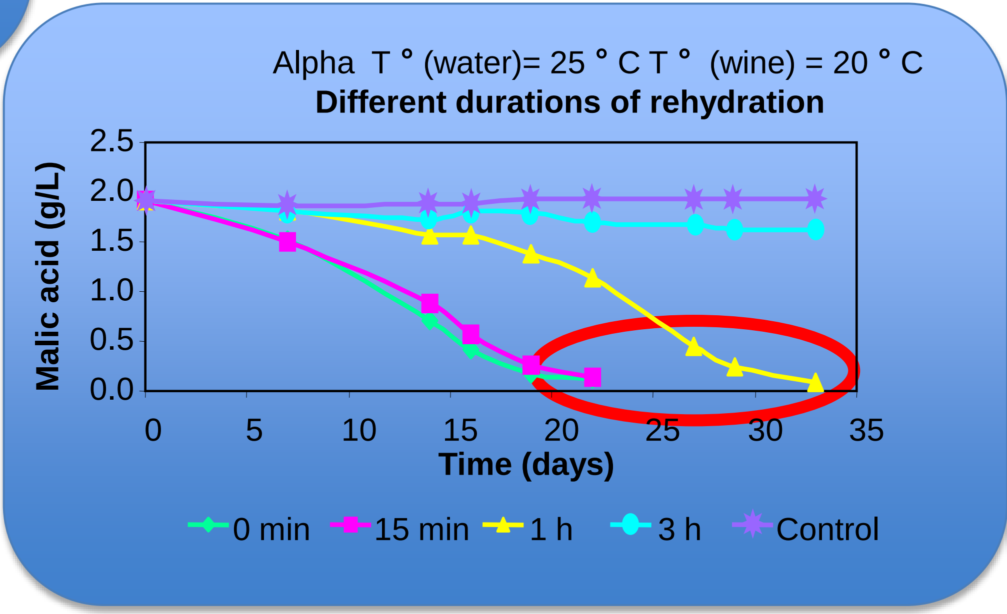


Importance of correct rehydration

The Australian Wine Research Institute



- *0-15 min rehydration – no loss of cell viability*
- *Loss of cell viability if left rehydrating for 1-3 hours*

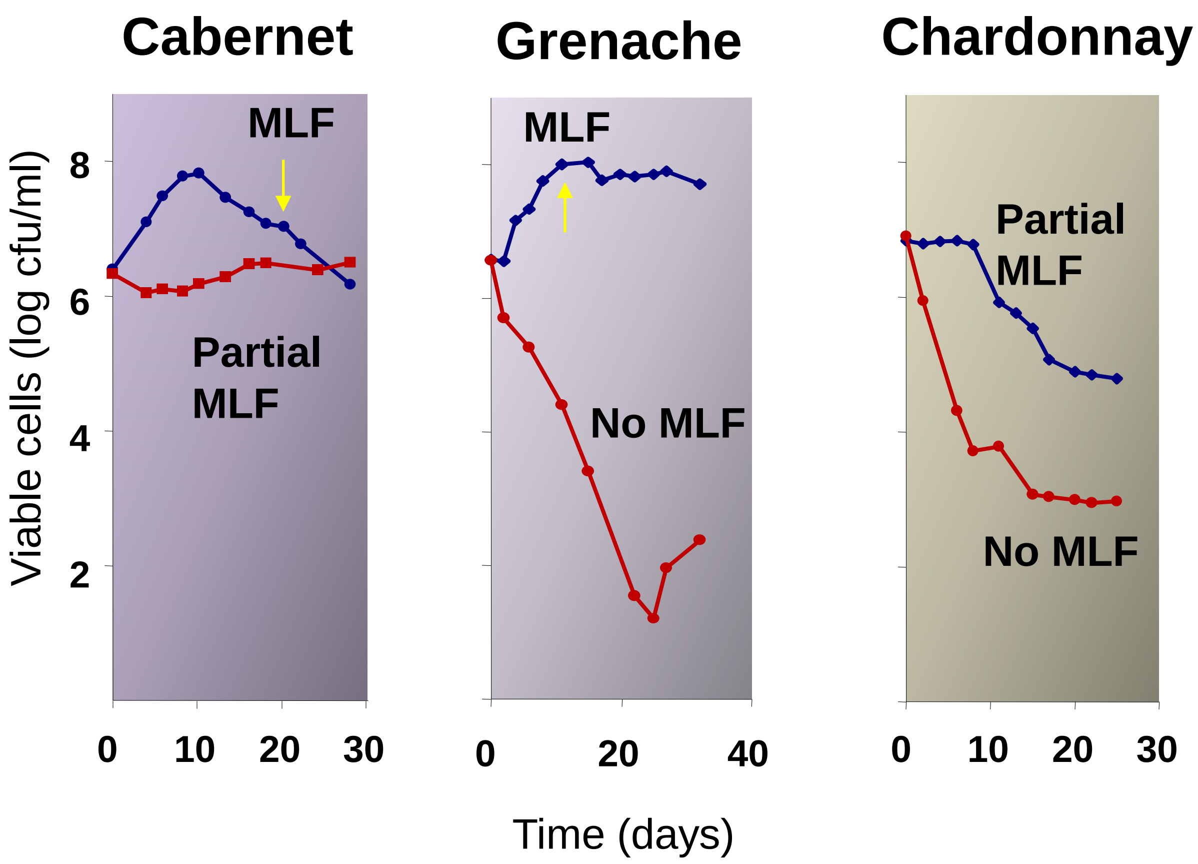


- *0-15 min rehydration – MLF completed sooner*
- *If left rehydrating for 1-3 hours – slower or no MLF*

Magali Déléris-Bou; Lallemand

Growth and MLF in ‘soft’ and ‘harsh’ wines

Viniflora oenos
pH & alcohol



Soft wine
pH 3.5, 12.5% EtOH

Harsh wine
pH 2.9, 14.5% EtOH

Conditions that favour bacteria growth favour MLF

Gockowiak
& Henschke AJGWR (2003)

Why consider Co-inoculation?

The Australian Wine Research Institute

- Enough nutrients
- Ethanol not a factor
- Bypass difficult conditions
- Stabilise faster
- Reduced microbiology risks
- Perceived more fruitier, balanced, integrated

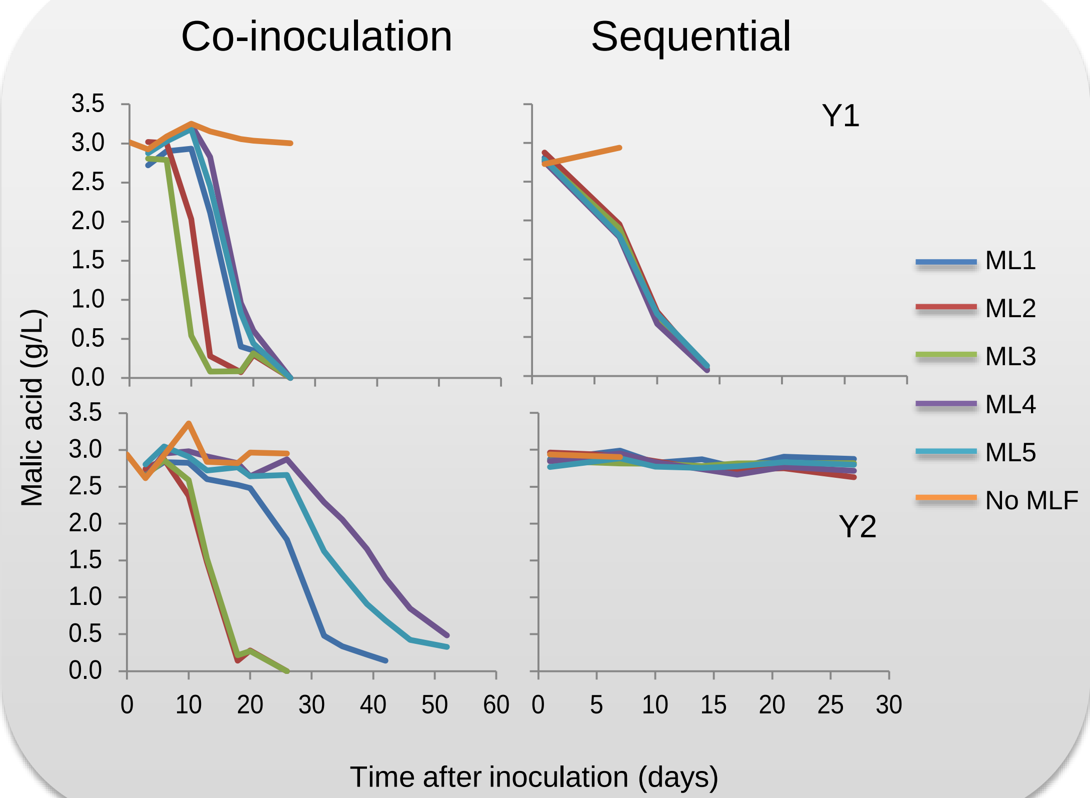
	Pro (Advantages)	Con (Risks)
After AF	Ø No VA ↑ Ø Easy to control	Ø Stuck due to high alcohol Ø Nutrients depleted Ø Production anti-LAB inhibitors
With AF	Ø Ethanol not high Ø Most FSO_2 bound Ø Heat of AF	Ø Potential yeast/bacteria antagonism

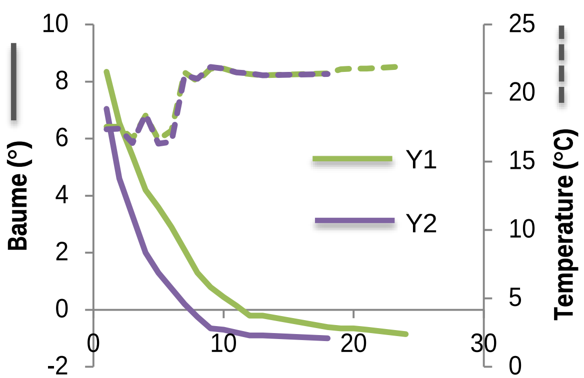
Co-inoculation

The Australian Wine Research Institute

		Juice	Y1	Y2
Brix / Alcohol	° / %	21.6	12.6	12.5
Glu + Fru	g/L		1.1	7.2
pH		3.26	3.4	3.38
TA pH 7.0	g/L	5.2	4.9	5.3
TA pH 8.2	g/L	5.4	5.1	5.6
YAN	mg/L	272	93	85
Ammonia	mg/L	97	8	8
a Amino N	mg/L	192	86	78
SO_2 (free)	mg/L	12	< 4	< 4
SO_2 (total)	mg/L	42.5	50	51
L-malic acid	g/L	3	2.79	2.87

Co-inoculatⁿ			4 wks	6-8 wks
Sequential			4-8 wks	-





Chardonnay (2011)
Barossa Valley
17°C

Co-inoculation can help bacteria adapt to 'harsh' wine composition

Yeast and bacteria ?

The Australian Wine Research Institute

- Yeast may
  - Deplete complex nutrients & growth factors required by bacteria
    - Essential amino acids
  - Release of bioactive metabolites
    - SO_2
    - Fatty acids (C6, C8, C10)

Inhibition

➢ *Conditions that favour yeast growth (AF) tend not to favour MLF*

  - Release of metabolites
    - Amino acids, vitamins, peptides
    - Mannoproteins

Stimulation

Grape must with low nutrients (higher DAP usage)	>>	May cause yeast to produce increased levels of SO_2	>>	May inhibit MLF

Yeast and bacteria ?

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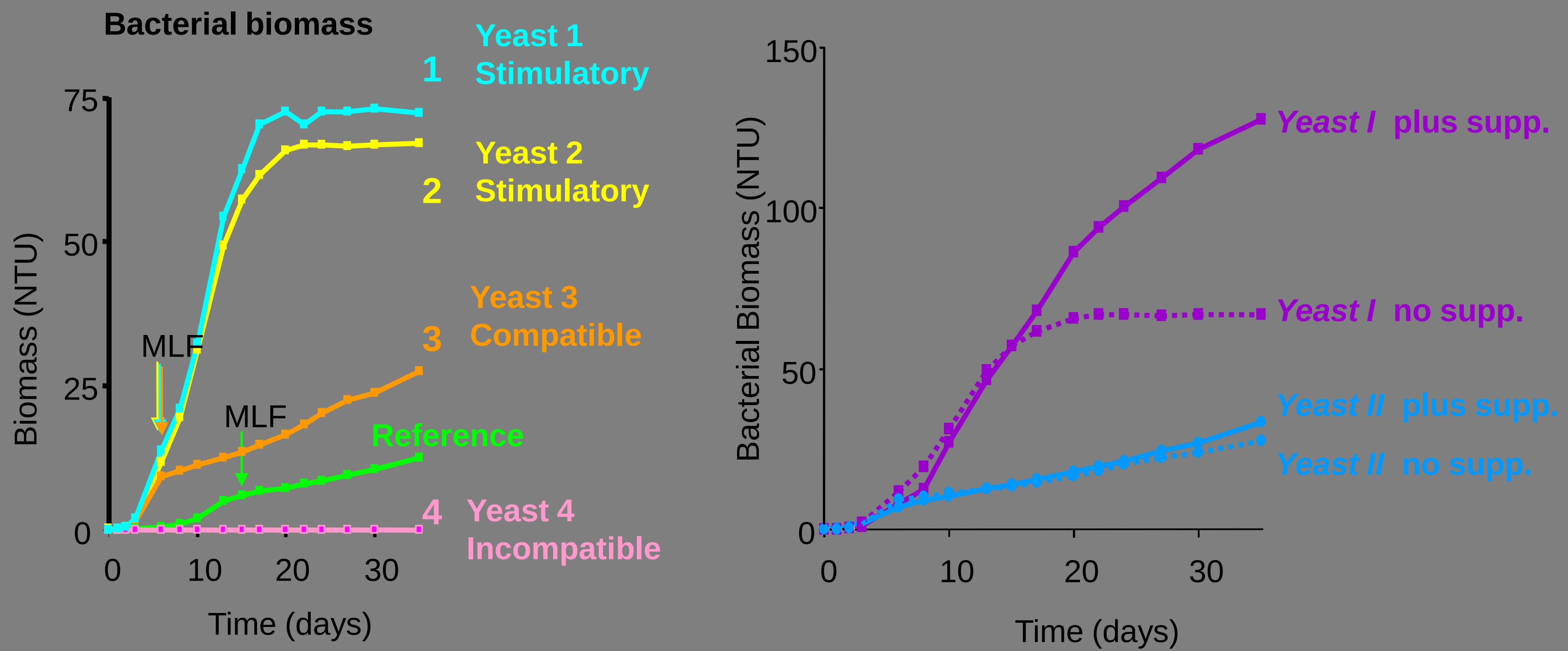
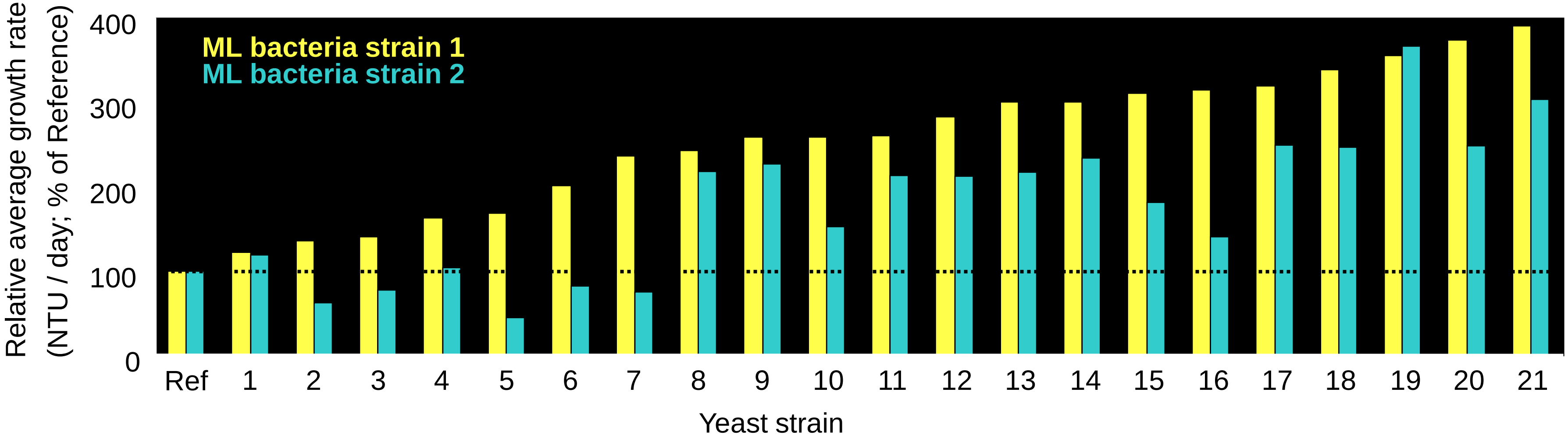


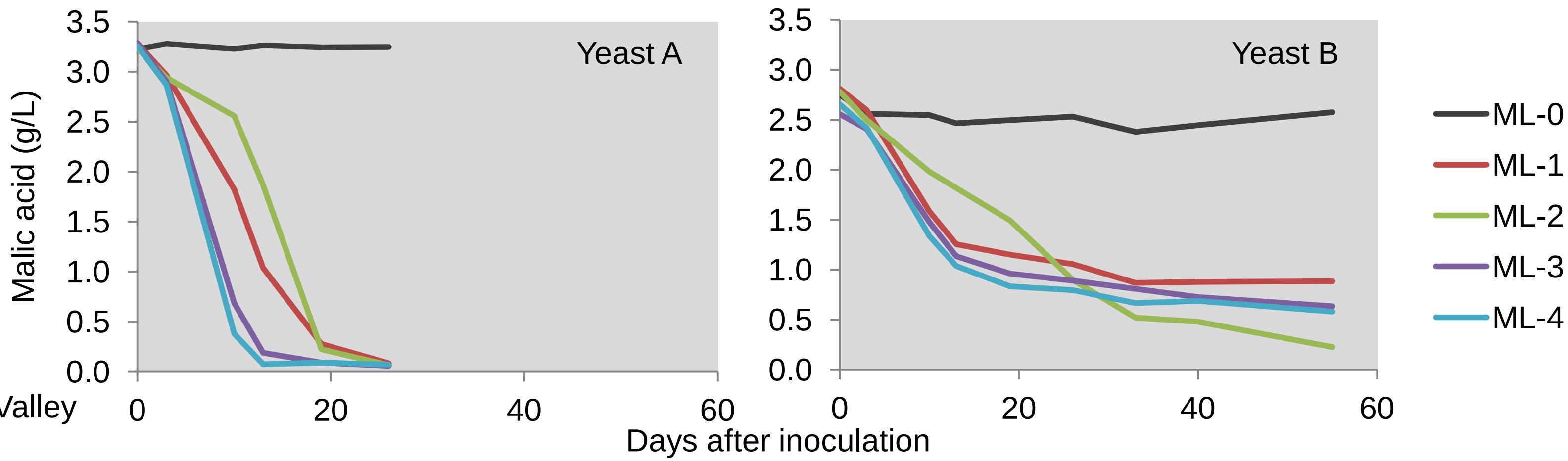
Intrinsic incompatibility by Yeast II for MLF
Addition of nutrients may not solve incompatibility

Yeast and bacteria ?

The Australian Wine Research Institute



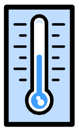
ML bacteria strain 1 more tolerant to incompatible yeast strains



Shiraz
Clare Valley
2008

Wine conditions – *a general guide*

The Australian Wine Research Institute




Condition	Wine type	Limiting	Optimal
Temperature (˚C)	White	<16, >24	18 - 22
	Red	<15, >25	18 – 22
pH	White	< 3.1	> 3.2
	Red	< 3.0	> 3.1
Alcohol (% v/v)	White	13.5	< 12.5
	Red	14	<13
Total SO_2 (ppm)	White	> 30	< 15
	Red	> 30	< 15
Microbial			
Bacteria	Follow manufacturer's instructions		
Yeast	Check compatibility with ML bacteria		

Confirm wine parameters by measurement/analysis

What to do to ensure a successful MLF

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- Check your wine parameters
  - pH, alcohol, SO_2, temperature
- Select a ML strain to suit your purposes
  - Tolerance to wine pH, alcohol & SO_2
  - Sensory
- Co-inoculation
- Yeast strain
  - High nutrient demand?
  - Produces 'toxic' compounds?
  - Compatibility with the ML strain?
- Take time to prepare your ML culture
  - Rehydration
  - Consider pre-adapting the bacteria to your wine conditions
- Microscope
  - don't be afraid to use it
- *Further information*
  - AWRI website for products
  - Consult ML producer & website
  - AWRI

Acknowledgments

The Australian Wine Research Institute

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- Caroline Abrahamse
- Jane McCarthy

- Paul Henschke
- Holger Gockowiak (former member)

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gwrdc GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

AWRI

The Australian Wine Research Institute

Causes and Management of Slow and Stuck Fermentations

Paul Henschke

Peter Godden

and AWRI Industry Development & Support team

- Con Simos
- Adrian Coulter
- Geoff Cowey
- Matt Holdstock

The problem of sub-optimal fermentation

- A common seasonal problem, but exacerbated by hot weather
- Affects most wineries at some stage, both in Australia and overseas
- White, red & sparkling wines, in tanks & barrels
- Multifactorial problem, including yeast, nutrients, toxic substances and fermentation conditions/management
- Most (all ?) yeast types are affected, including benchmark EC1118/PDM/Prise de Mousse
- Expensive in resources (time, energy, yeast, tank space) and loss of quality

>>> This talk contains practical information on how to reduce the risk

Sub-optimal fermentation profile

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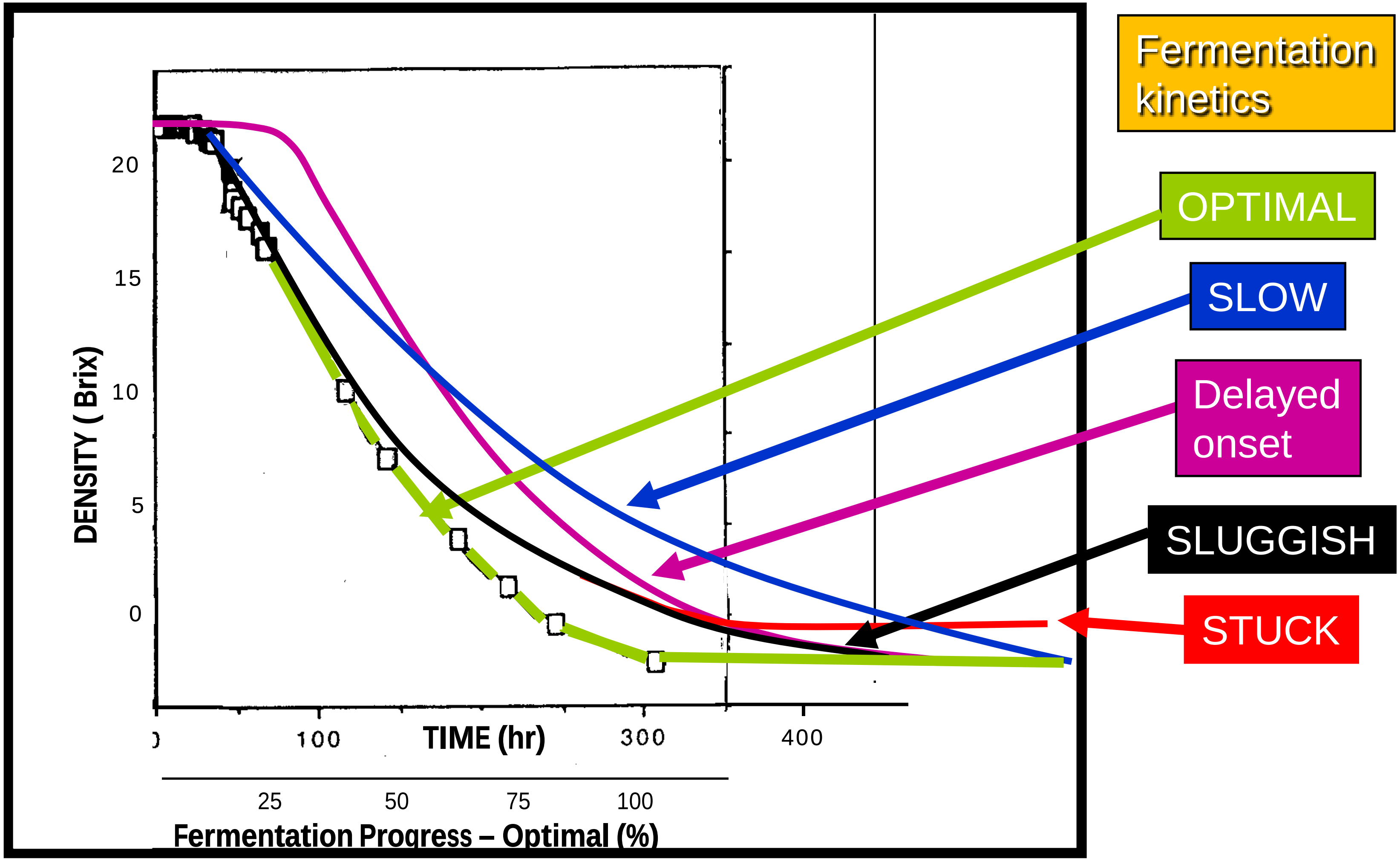

Environmental changes during fermentation major stresses to which yeast must adapt

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Factor	Grape juice	Wine
Sugar (g/L)	180 – 260	0 – 4
Alcohol (% v/v)	0	10 – 16
Nutrients:		
YAN (mg N/L)	50 – 300	<50
Oxygen (ppm)	0 – 9	0
Conditions	***Nutrient rich***	***Nutrient poor*** ***High conc. toxic products***

Failure to adapt results in sub-optimal fermentation

Causes of sub-optimal fermentation

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Delayed onset of fermentation

Causes:

- Poor quality starter culture
  - Low viability or low cell count/inoculation rate
  - Poor physiological condition (low metabolic rate)
- High SO_2, resulting in growth inhibition until level of free SO_2 has decreased below a critical point

Diagnosis:

- Perform a microscopic cell count before & after treating the sample with vital stain, eg methylene blue (see Iland et al. 2007)
- Viability <75% indicates poor yeast culture or must toxicity, eg SO_2
- Measure must/juice SO_2; should be <10-15 mg/L free SO_2

Methylene blue staining of yeast culture assessing culture viability

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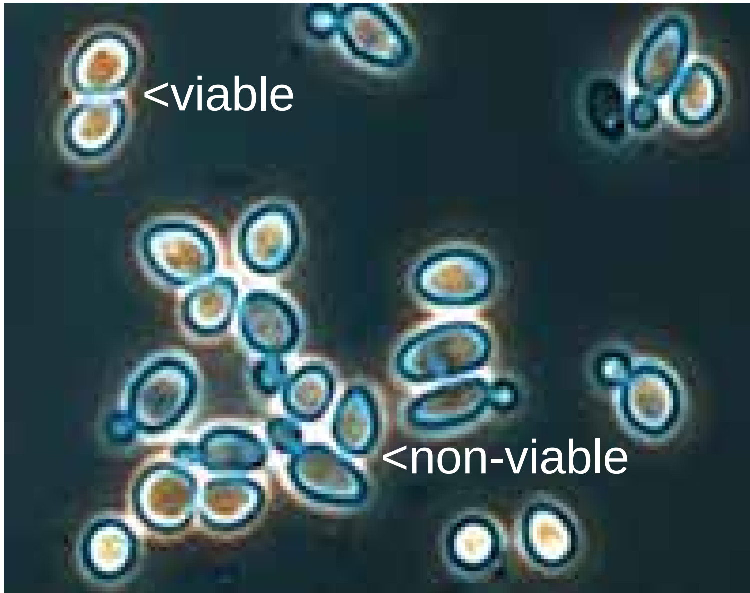
- Methylene blue is a dye that is used to differentiate live and dead yeast cells in a culture.
- Methylene blue is a redox sensitive dye, such that metabolically active cells reduce it to the colourless form; viable cells are highly reductive.
- Dead cells (non-metabolically active) stain blue, ie the oxidised form.
- Population viability is a strong indicator of culture health:
  - Healthy culture typically contains >95-98% viable cells
  - <75% viability indicates toxicity, which can lead to stalled fermentation



Consult Iland et al (2007): Microbiological analysis of grapes and wine: techniques and concepts

Causes of sub-optimal fermentation

Slow (continuously) fermentation

Causes:

- Low yeast biomass or cell number
- Low budding index
- Low level of key nutrient, typically YAN, O_2 or lipids

Diagnosis:

- Confirm by microscopic cell count:
  - 0% FP (Fermentation Progress) count should be >1-5x10^6 cells/mL;
  - 35% FP should exceed 50x10^6 cells/mL
- Measure juice/must YAN, should exceed 100-150 mg N/L
- Failure of aeration or grape solids addition to stimulate fermentation suggests deficiency of a key nutrient, eg YAN

Causes of sub-optimal fermentation

v Sluggish & Stuck fermentation

Causes:

§ Multifactorial problem

Interaction between:

1. yeast strain
2. juice/must (nutrients, toxic substances) and
3. fermentation conditions/management (under control of winemaker)

§ Most yeast types are affected, including the industry benchmark strains EC1118 / PDM / Prise de Mousse

Diagnosis: complex & the subject of this talk

Sub-optimal fermentation kinetics
Risk Factors

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Yeast-related factors

- incorrect choice (alcohol stress tolerance)
- poor quality starter culture
  - rehydration / reactivation
  - viability / vitality
- unsuccessful inoculation
- indigenous microflora
- degree of must clarification
- temperature stress
- vigour and sedimentation

Nutrient deficiency

- yeast assimilable nitrogen (YAN)
- oxygen / lipids
- vitamins
- minerals

Inhibitors

- high concentration of sugar (high °Be)
- high ethanol
- fatty acids (acetic acid & mid chain length FAs)
- SO_2
- toxic (killer) proteins/other organisms
- residues (pesticides, cleaning agents)

Adapted from Henschke (1997)
ASVO Seminar Procs pp. 30-38,41

A list of Alcohol Tolerances of Fermentation Yeast*

choice guide only – data most relevant to ‘cellar bright’ juice ferments†

Sugar Conc (g/L)	Degree Baume	Degree Brix	Maximum alcohol produc’d (% v/v)	Strain – Commercial name
200	11	20	12	Uvaferm CEG, CM UCD 522-Montrachet CH158-Siha 4
218	12	21.5	13	Hefix 1000 VRB
235	12.7	23	14	Fermivin Simi white Lalvin Actiflore, Assmannhausen, B, ICV D-47, ICV K1, CSM, M1107, M2, QA23, T306 Maurivin AWRI 796 Zymaflore VL1, VL3a
>250	>13.5	>24.5	> 15	**Fermivin PDM**, Fermichamp Lalvin L-2056, **L-2226**, L-2323**, L-43**, V1116, BDX, BM45, CY3079, D254, DV10, **EC1118**, M1, RC212, S6U, Syrah, O 16, Agglo, Enoferm R2, **Uvaferm 43** **IOC 18-2007, Prise de Mousse**, **Maurivin PDM**, AWRI 350, AWRI R2 WET 136-Siha 3 Uvaferm PM
			unspecified	Siha 5

Source: Cunier, ITV Manual (1994) ; Bold, recommended for restarting fermentation

***Measured as the maximum [EtOH] produced by standardised fermentation test, with surplus sugar.**

†Presence of grape solids (phytolipids) or oxygen/YAN can increase yeast tolerance to alcohol

Yeast-related risk factors

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Active Dried Yeast - rehydration/reactivation (1)

- Follow manufacturers instructions precisely
- Choice of rehydration medium
  - Mineral water is preferable to rain water
  - If using tap water, remove Cl_2 by boiling/sparging when necessary
  - Water with grape sugar concentrate (10% sugar)
  - Diluted preservative-free (SO_2) grape juice (sterile)
- Temperature of medium should be 38-40 °C
  - measure temperature with a thermometer

  (optimum for reformation of yeast lipid membranes)
- For high risk juices: high sugar (>13 Be), bright (low solids), low YAN (<150 mg/L), to be fermented cold (<15C) consider proprietary 'inactivated yeast' nutrients rich in sterols

Yeast-related risk factors

The Australian Wine Research Institute

Active Dried Yeast - rehydration/reactivation (2)

- Add yeast slowly to container with large surface area. Avoid clumping - clumping produces non-wetted, and hence, non-rehydrated yeast
- Avoid vigorous (mechanical) stirring during re-hydration step, which reduces viability
- Leave yeast for 15 min before mixing/aerating
- Use yeast after 20-30 min from start of rehydration

  – do not use yeast after this time unless grape sugar or juice has been added, because reactivated yeast rapidly loses activity in water

Hydration temperature is very important

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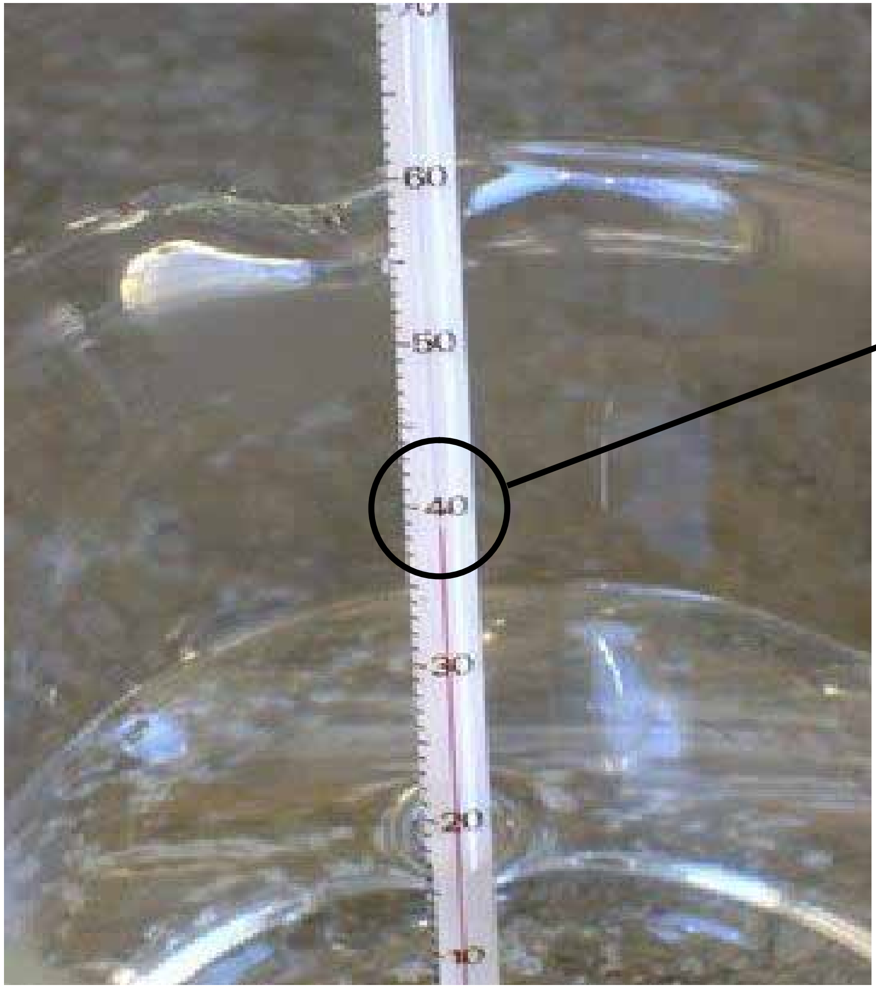

Critical
38 – 40 °C

Hydration step – prevent 'dry lumps' of yeast

The Australian Wine Research Institute

Correct

Incorrect

Hydration final step – temperature amelioration

Culture amelioration before inoculation of difficult to ferment juices/musts

Step-wise cooling by adding juice to the culture can be beneficial for cold juices or musts (post cold maceration) and/or of high Be/Brix

Yeast-related risk factors

Active Dried Yeast - rehydration/reactivation (3)

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- Add rehydrated yeast to pre-warmed juice (ie after cold settling or cold soak, preferably >15ºC
- For cold (<15 ° C), highly clarified, high sugar juice ferments, step-wise cool reactivated yeast in 5-10 ° C steps at 5 min intervals by adding appropriate volumes of the juice to be inoculated
- Ensure sufficient time has elapsed after SO_2 addition to must to avoid damaging yeast (<10 ppm SO_2 @ pH3.5) – consider adding a 'sacrificial culture' of about 15–20% of aerated active yeast (containing aldehyde) to the juice in order to bind SO_2 and other potentially inhibiting substances, about 30 min before inoculation
- Do not use old yeast stocks for high risk juices

Yeast-related fermentation factors

Fermentation management

- Add yeast hulls for high risk ferments (detoxification role)
- Allow ~10% of sugar to ferment before cooling
  - It is critical to build-up cell number (growing yeast - v. stress sensitive)
  - Do not cool in greater than 2-4 ° C increments
- Monitor fermentation progress & temperature daily
  - Spreadsheets provide an efficient record of fermentation data, comparison with similar ferments and early identification of problems
- Look for a steady fermentation rate; compare with previous data of similar ferments and/or previous years data to identify problems
- Cell numbers should reach 70 x 10^6 cells per ml for cellar bright juice ferments (determine with microscope and haemocytometer)
  - Monitor budding % as an indication of yeast growth or problems
  - Expect high % budding during first third stage of fermentation
  - Vital staining (eg methylene blue) is also a useful diagnostic for dead yeast cell estimation – check when fermentation rate becomes slow
  - Also look for presence of (lactic acid) bacteria, which can adversely affect yeast activity and lead to fermentation arrest

Yeast-related fermentation factors

Factors affecting yeast implantation

- Pure culture inoculation strategy
  - Maximising the benefits of selected yeast strains

- Minimise indigenous yeast population of must (<10^5 cfu/ml)

  - Minimise must exposure to moderate-hot temperature, during harvest, transport, juice preparation (enzyme treatment, clarification, etc) which otherwise promotes indigenous yeast & bacteria growth

  - Add sufficient SO_2 (50-100 ppm, depending on fruit condition) during machine harvest to limit indigenous microbial growth

  - Clarification procedures can lower indigenous microbial growth

  - High indigenous yeast count can indicate nutrient depletion – add nutrs.

- Optimise yeast starter culture population

  - Yeast propagator should exceed 10^8 cfu/ml, but be capable of producing maximum population of 3-4 x 10^8 /ml

  - ADWY viable cell population should exceed $2x10^{10}$ cfu/g

- Recommended Inoculation rates

  - whites: 5 x 10^6 cells/ml(typically 250 g ADWY per kL juice);

  - reds: 4 x 10^6 cells/ml ; lower rates can compromise yeast implantation

Yeast-related factors

Factors affecting yeast implantation

Control of indigenous yeasts and nutrient loss

- **Grape condition**
  - **- Damaged grapes (rain/bird damage/mouldy/heat wave) have higher wild microbial load, including wild yeast, acetic and lactic acid bacteria**
- **Method of harvest and transport**
  - **- Mechanical harvest gives higher microbial load due to poor harvester/transport bins hygiene – eg adjust sulfite: time, temperature**
- **Must processing**
  - **- Time (minimise) / Temperature (as low as practical)**
  - **- Chemical antimicrobials (effective levels of sulfite (measure pH)**
  - **- Physical removal of microbes (ie enzyme / cold settling, filtration, centrifugation)**
  - **- Hygiene (clean/sanitise harvester and transport bins regularly)**
  - **- Minimise must dO_2 & contact with O_2 (stimulates oxidative microbes)**
- **Fermentation conditions**
  - **- Temperature (18-27 ° C favours *S. cerevisiae (Sc)* over non-*Sacch. sp*)**
  - **- pH (<3.5 favours *S. cerevisiae*)**
  - **- SO_2 (favours *S. cerevisiae,* generally tolerant to 10 ppm free)**
  - **- O_2 at 30-40% Ferm Progress prolongs survival of *Sc* yeast**

Nutritional deficiency risk factors

The Australian Wine Research Institute

- Yeast Assimilable Nitrogen (YAN)
  - A variable proportion of Australian juices/musts have inadequate YAN
  - Measure YAN on a grape maturity sample or juice sample
  - Low YAN of <150 mg N/L for whites or < ~ 100 mg N/L for reds increases risk of slow/stuck fermentation
  - Maximum growth achieved at approx. 400 mg N/L (NB high heat productn)
- Lipid deficiency (phytosterols and unsaturated fatty acids (UFA))
  - Over clarification removes lipids necessary for yeast growth
  - i.e. when <0.1-0.5 v/v juice solids (ie ‘cellar bright’) or <5 NTU
  - Addition of “fine” settled grape solids highly stimulatory to yeast growth
  - Avoid “hard” settled grape solids, which can impart phenolic coarseness, hotness, bitterness to wine
  - Rehydrate yeast with inactivated yeast product rich in sterols
- Dissolved Oxygen (dO_2)
  - dO_2 is highly variable in juice/must – ranging 0 – 8 ppm (air-saturated)
  - Aerating fermentations at least once, at the stage when they are most active (after 35-50% sugar has been fermented) is highly beneficial
  - Aerate to give ~5 ppm oxygen (sparge, pump over, rack-return, etc)
  - Oxygen alleviates yeast REDOX imbalance & stimulates sterol formation

Nutritional deficiency risk factors

The Australian Wine Research Institute

Vitamins

- Vitamin status of Australian musts/juices is unknown
- **Thiamine** - essential for ethanol production by yeast
  - losses caused by high SO_2 use and wild yeast growth
- Vitamins (thiamine, niacin, biotin, pantothenate, pyridoxine, inositol) can be added to starter cultures under ANZFA Wine Regulations
- Some proprietary yeast foods provide a useful source of vitamins

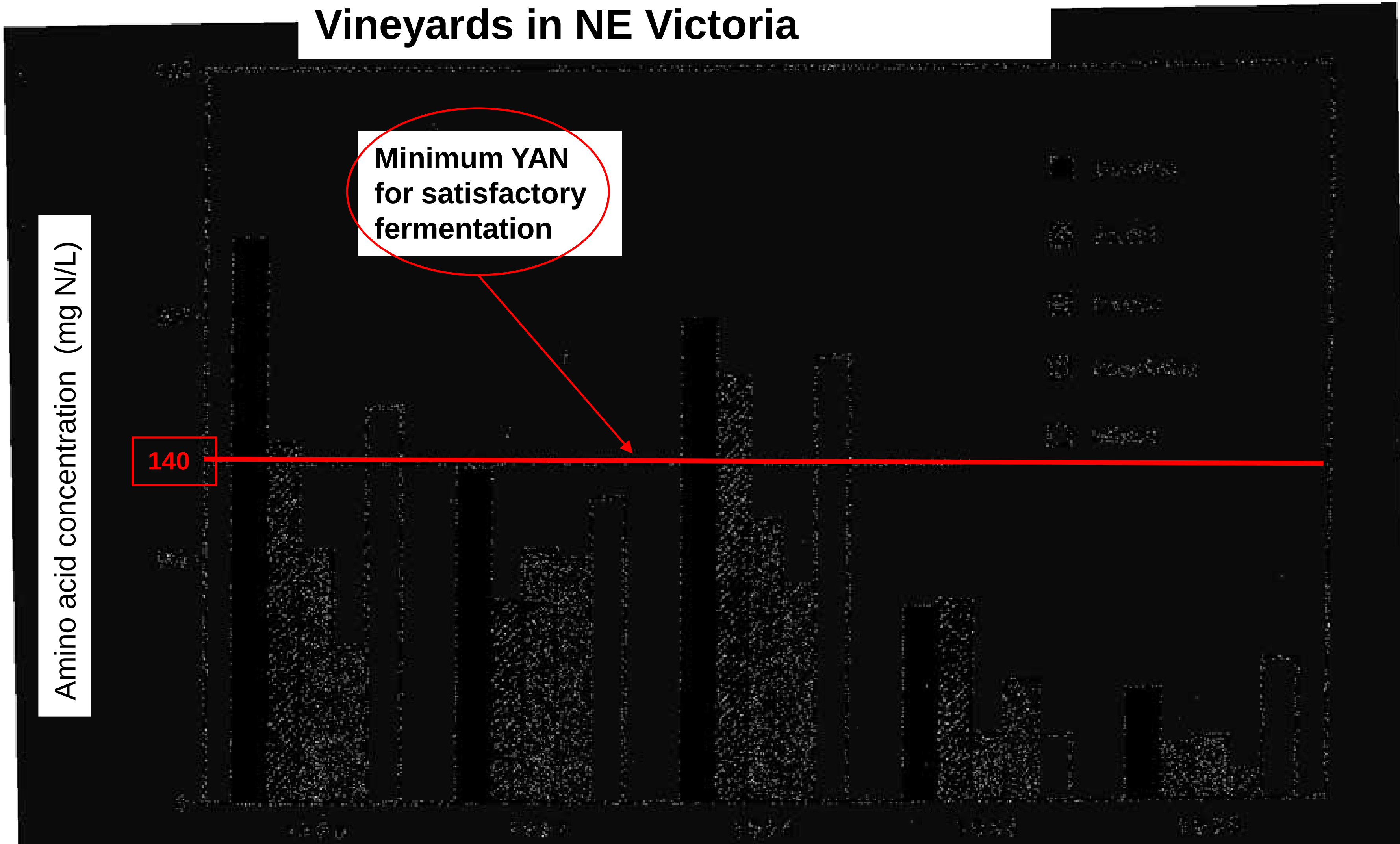
Minerals

- Mineral status of Australian musts/juices is unknown
- **Phosphate – normally considered adequate; can be added with DAP**
- **Low K^+/Low pH** can lead to stuck ferms with some yeast strains (sparkling/tirage)
- **Magnesium, zinc, manganese, which are enzyme co-factors are thought to be sub-optimal** (these cannot be added under ANZFA Wine Regulations)
- Some proprietary yeast foods provide a limited source of minerals

Low YAN juices/musts

- Low YAN musts are typically also low in other nutrients
- **Useful to add proprietary inactivated yeast nutrients to yeast starter cultures when deficiencies are suspected**

Vineyard & Year effect on juice YAN

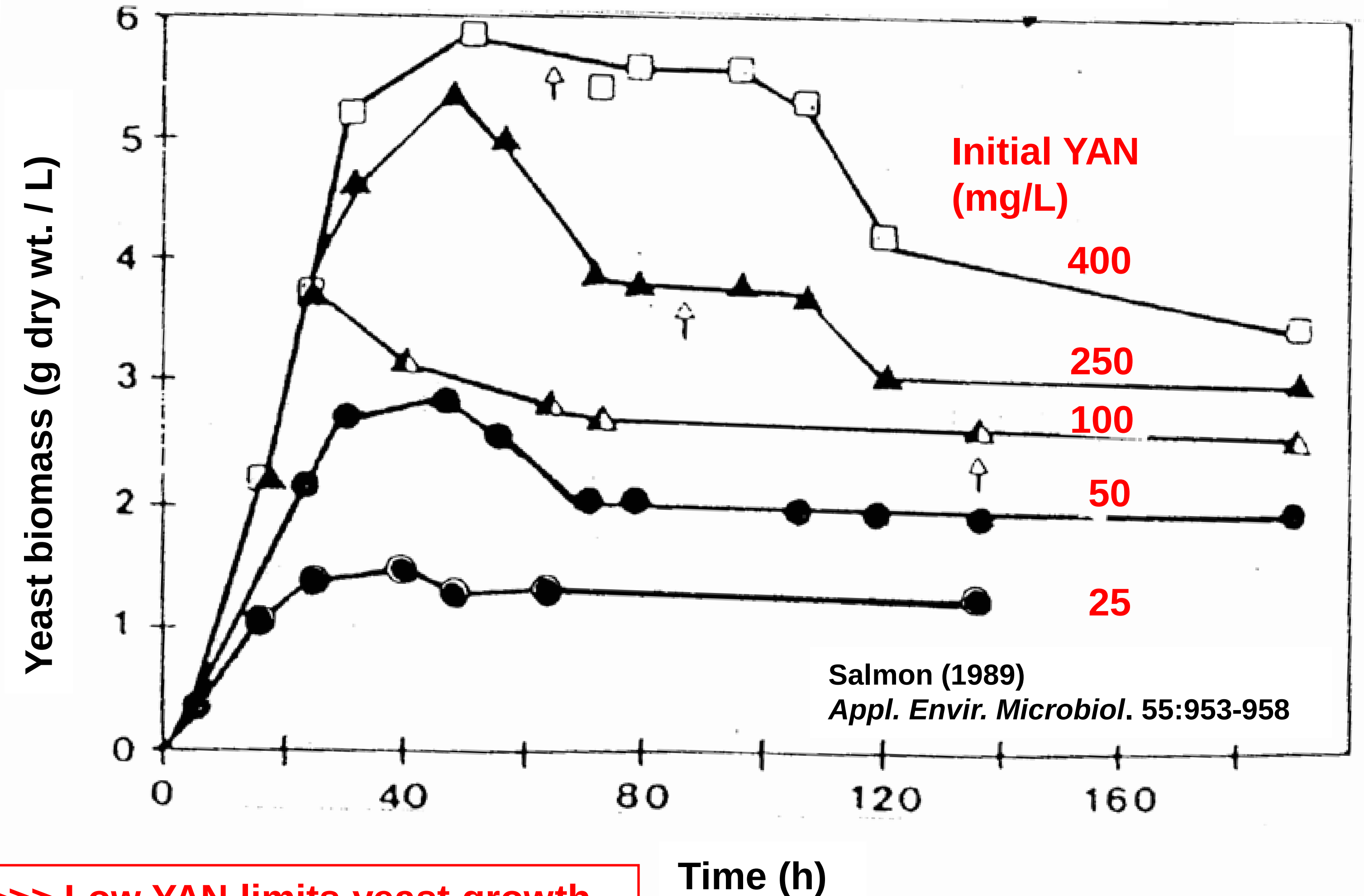


>>> Note large variation in YAN by vineyard as well as by year

Gockowiak & Henschke (1992) *Aust. Grapegrower Winemaker*

Yeast growth response to YAN

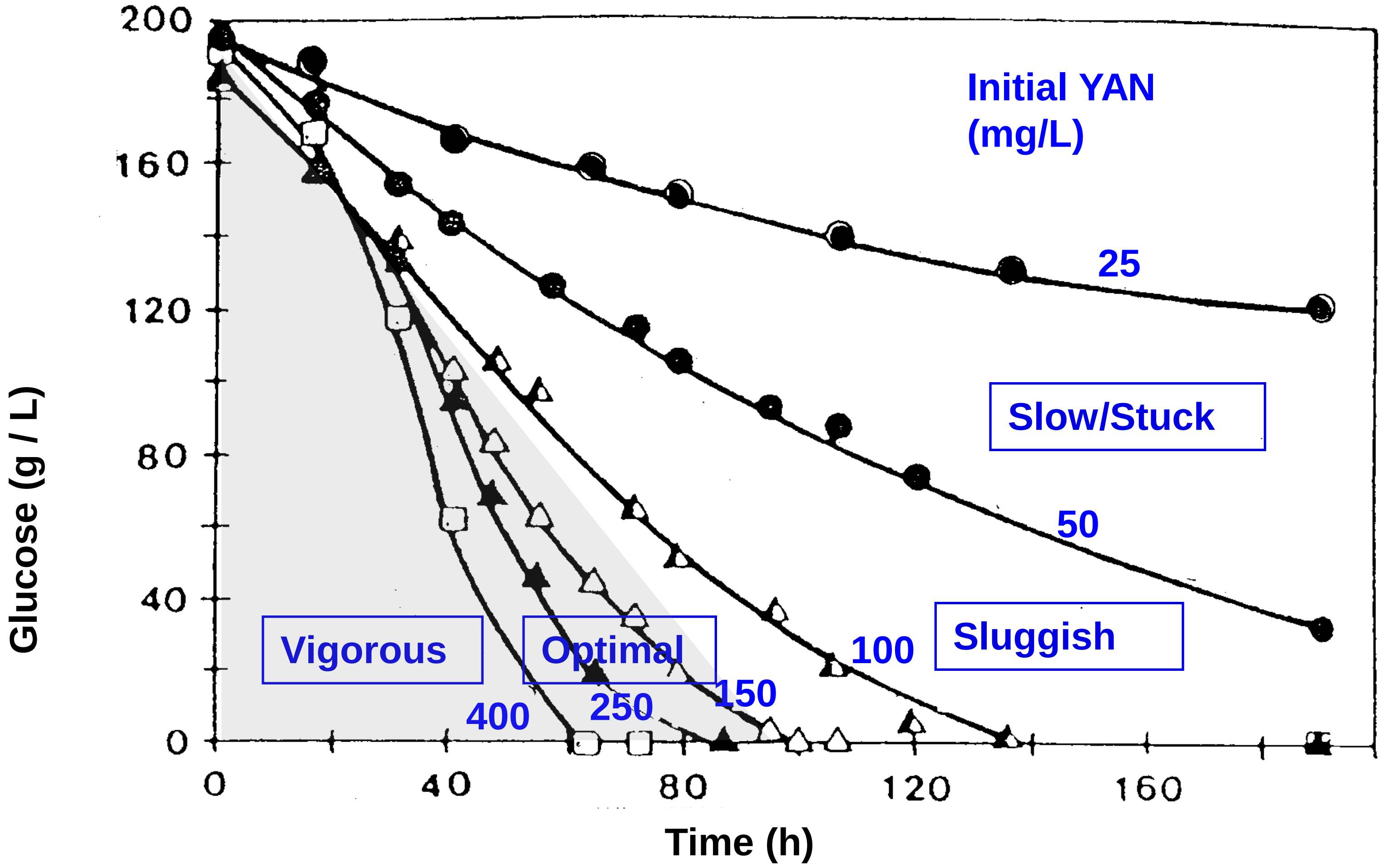
Synthetic juice ≡ 'cellar bright' juice
(all other nutrients are adequate)



>>> Low YAN limits yeast growth
Excessive YAN has no benefit

Fermentation response to YAN

Synthetic juice ≡ ‘cellar bright’ juice
All other nutrients are adequate, representing Nitrogen-limited growth



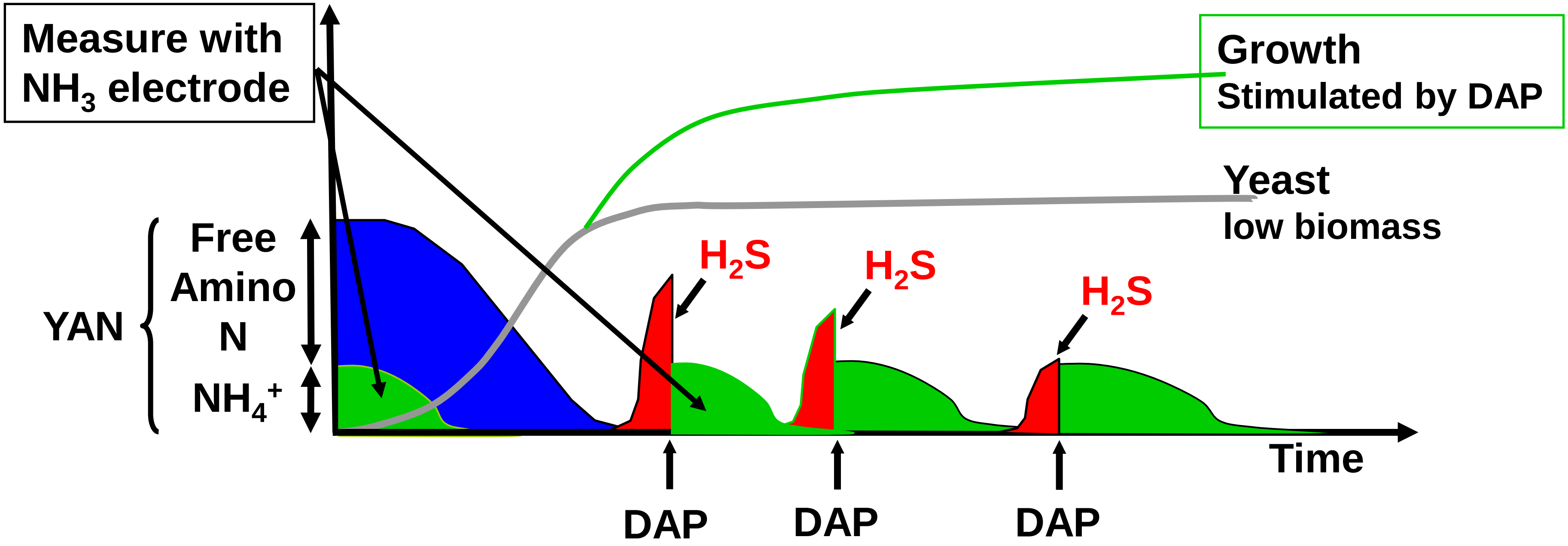
>>> Low YAN - slow/stuck fermentation
Excessive YAN – too vigorous

Sourced from Salmon (1989)
Appl. Envir. Microbiol. 55:953-958

Nitrogen utilisation during fermentation

The Australian Wine Research Institute

Low Nitrogen (<200 mg N/L)
Low biomass increases risk of slow/stuck fermentation and H_2S production



- Inverse relationship between Initial YAN and H_2S production
- Initial YAN should exceed 250 mg N/L YAN to prevent H_2S but H_2S profile depends on yeast strain X juice/must interactn
- Not all Yeast H_2S responds to DAP; could be a vitamin deficiency?

YAN Requirements of Yeast

(white juice, low solids fermentation conditions)

The Australian Wine Research Institute

1. **Maximum N demand:**
   Mean = 400 mg N/L
   Range = 330 – 470 mg N/L
2. **Minimum YAN requirement**
   Whites – approx. 150 mg/L
   Reds – approx. 100 mg/L
3. **Minimum YAN to prevent H_2S**
   approx. 250 – 350 mg/L (yeast x must dependent)
4. **Optimum flavour formation**
   Whites (strong style effects – complex thr' to fruity)
   – Chardonnay – fruity: 250–350 mg/L; <200: complex
   – Sauvignon Blanc – ? mg/L
   – Reds – fruity: 250–350 mg/L ; <200: complex

N-demand of fermentation yeast

examples from Lallemand range

The Australian Wine Research Institute

N demand	Yeast	Type
Low	71B/Actiflore	R/(W) Estery
(1-1.5 mg N/g CO2)	DV10	W/R/S Neutral
	QA-23	W/S EVC
	D47	W EVC
	M1107 / Uvaferm CM	R EVC
	Lalvin EC1118	W/S Neutral
Medium	V1116	W Neutral
	D254	R/W EVC
	L2056	W/R Estery
	Uvaferm CEG/Epernay 2	W/S (barrel)
	R2	W Estery
(1.5-2 mg N/g CO2)	RC212	R EVC
	S6U	W EVC
	BDX	R EVC
	CSM	R EVC
	CY-3079	W (barrel) EVC
	L2226	R Neutral
	L2323	R EVC
High	BM 45	R (barrel) EVC
	K1M	
(>2 mg N/g CO2)	VL1 / VL3	W EVC
	Sb 1176 / 1375	R/W EVC

R, red; W, white; S, sparkling; EVC, enhances varietal character

Defined as Nitrogen needed to maintain constant fermentation rate in synthetic medium with initial YAN = 100 mg/L; sugar = 200 g/L

Adapted from Lallemand Product Catalogue (2000) & Julien, Roustan, Dulau & Sablayrolles (2000) AJEV

Yeast growth response to O_2 added at start of fermentation

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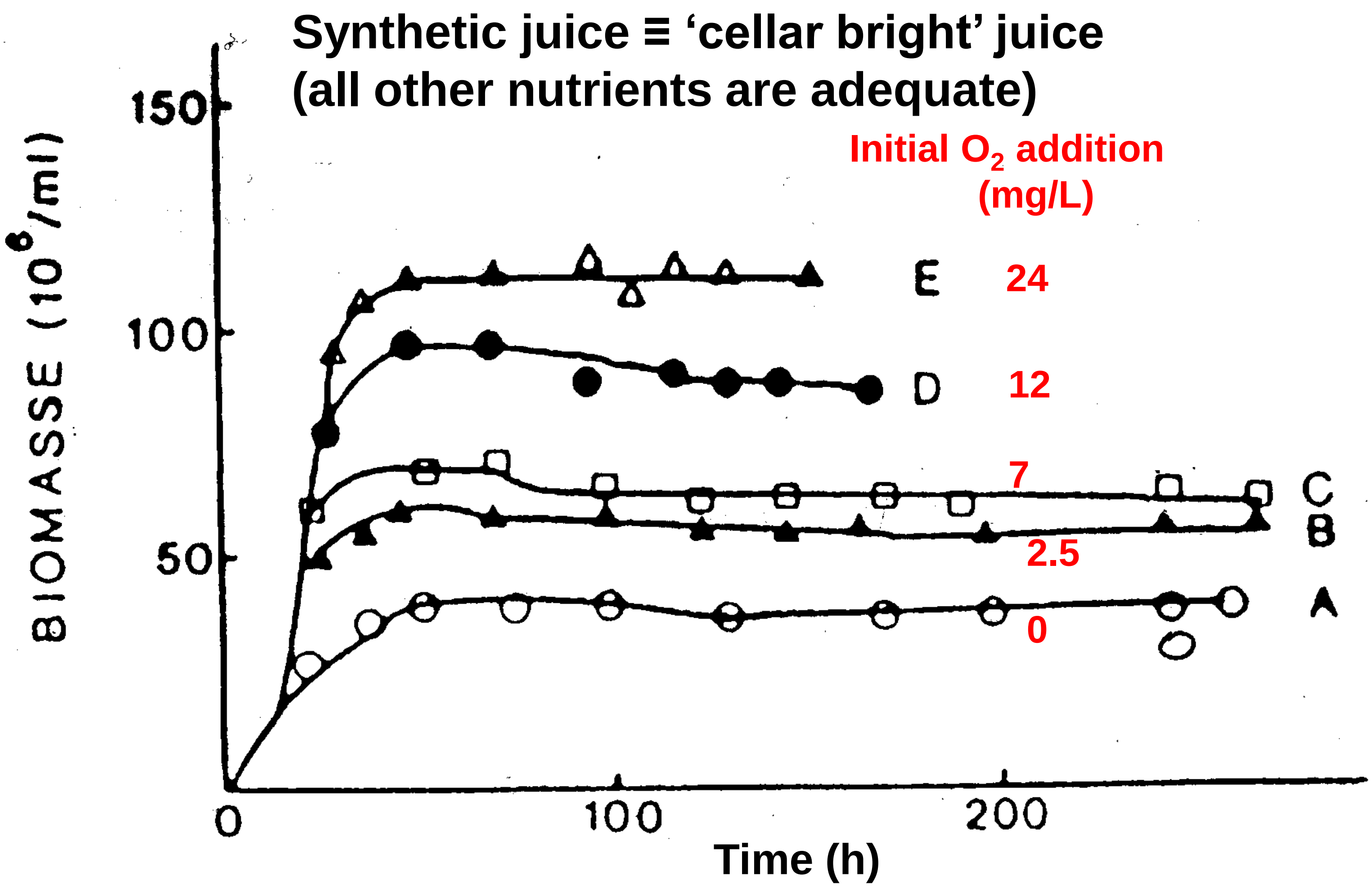
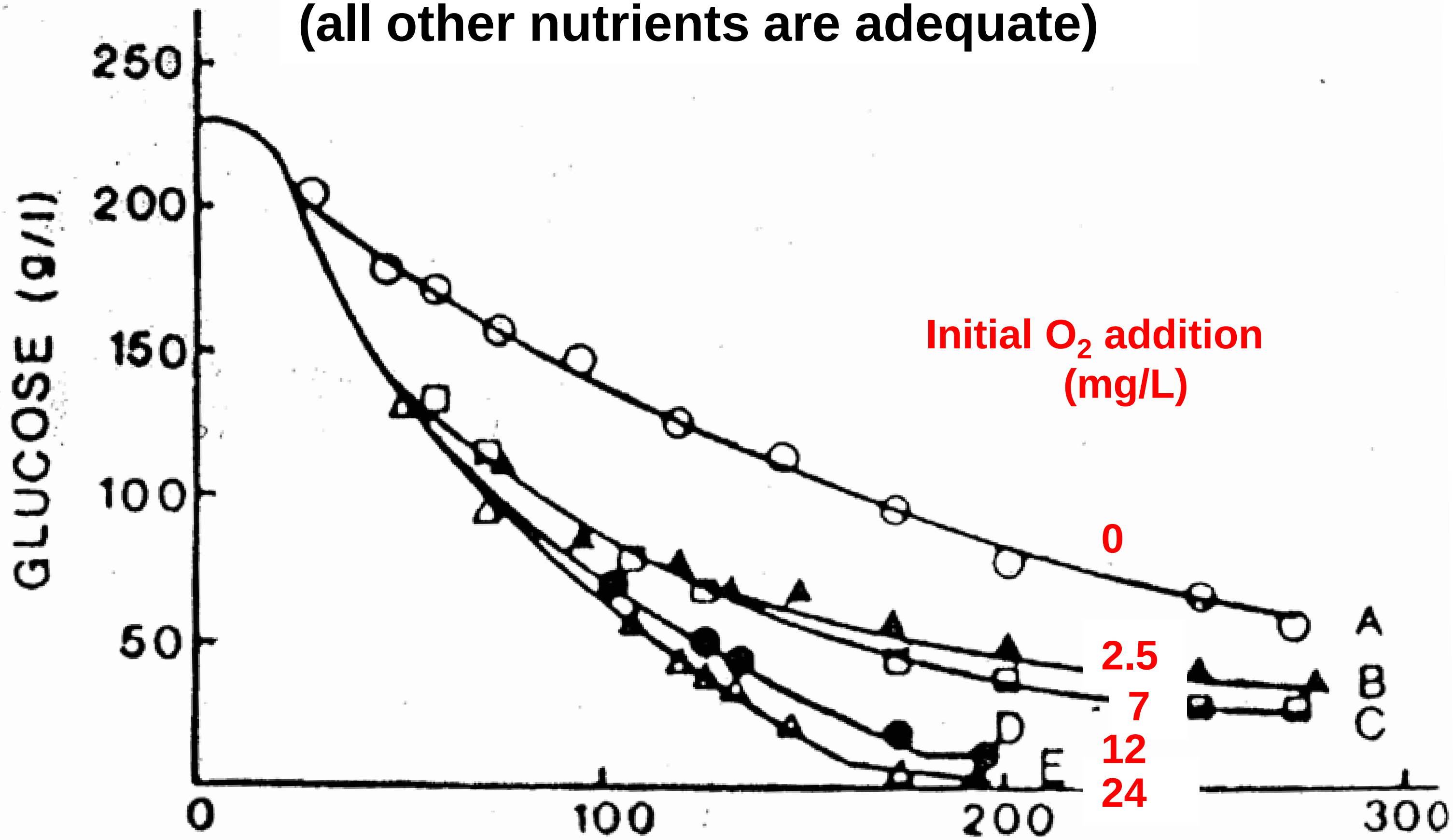


>>> Oxygen stimulates yeast growth

Sablayrolles & Barre (1986)
Sciences des Aliments 6:177-185

Fermentation response to O_2

Synthetic juice ≡ 'cellar bright' juice (all other nutrients are adequate)



>>> Oxygen stimulates fermentation rate

Sablayrolles & Barre (1986) Sciences des Aliments 6:177-185

Effect of O_2 on fermentation rate

The Australian Wine Research Institute

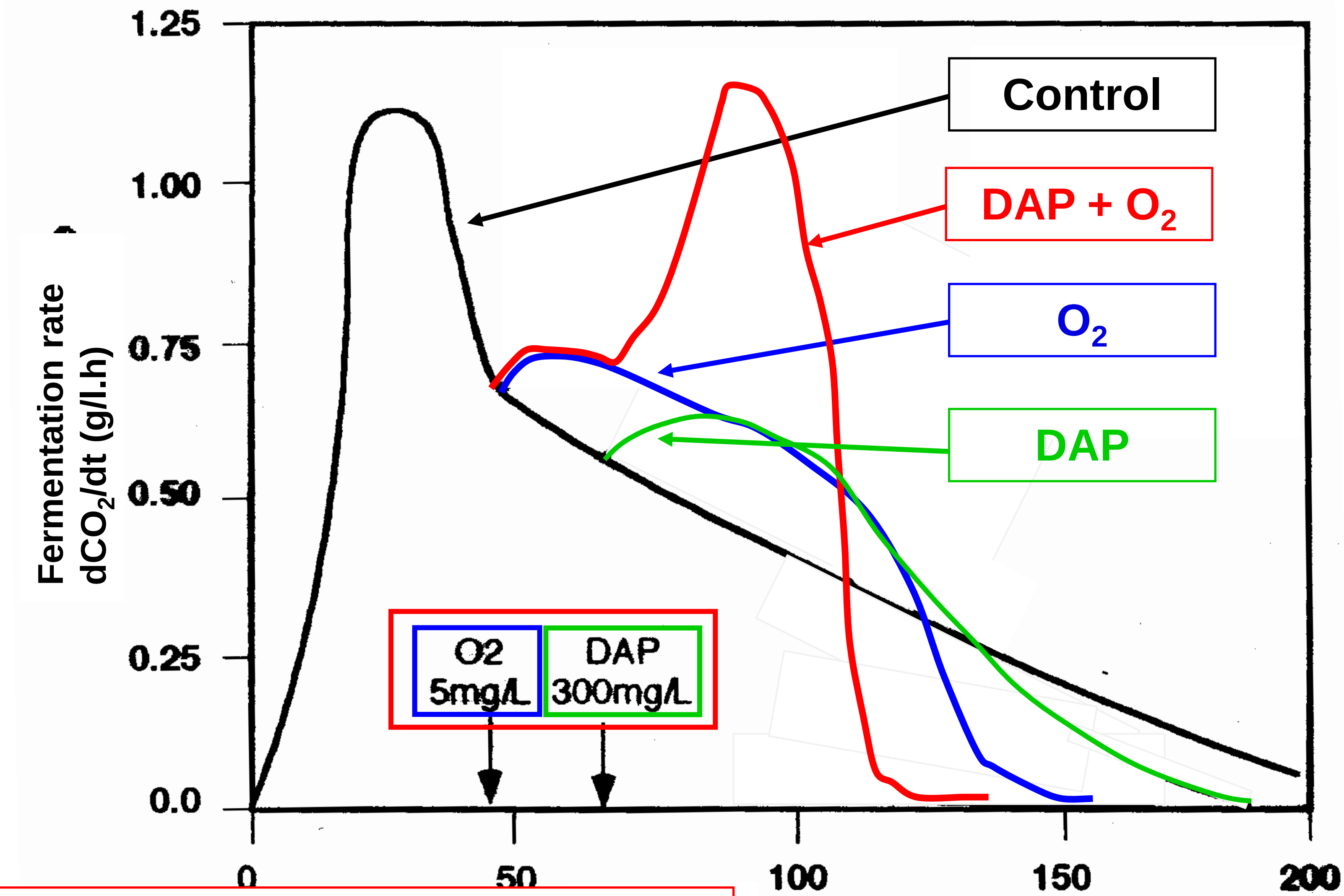
Rate Anaerobic (Max rate of CO_2 production)	**Yeast**	**%Rate gain** with O_2 added at 1/3 fermentation progress	**Yeast**
Low	S6U	**Low**	CSM
<0.5	CEG	(16%)	K1M
	EC1118		V1116
	DV10		CY3079
	QA23		R2
Medium	K1M		71B/Actiflore
0.6-0.7	VL3		S6U
	L2323		VL1
	L2226	**Medium**	D47
	VL1	(30%)	DV10
	71B		QA23
	R2		
High	D47	**High**	EC1118
>0.7	CSM	(45%)	IOC182007
	V1116		
	CY3079		

Adapted from Lallemand Product Catalogue (2000) & Julien, Roustan, Dulau & Sablayrolles (2000) AJEV 51:215-222

Combined effect of DAP + O_2 on fermentation

Nutrient strategy for stimulating fermentation

The Australian Wine Institute



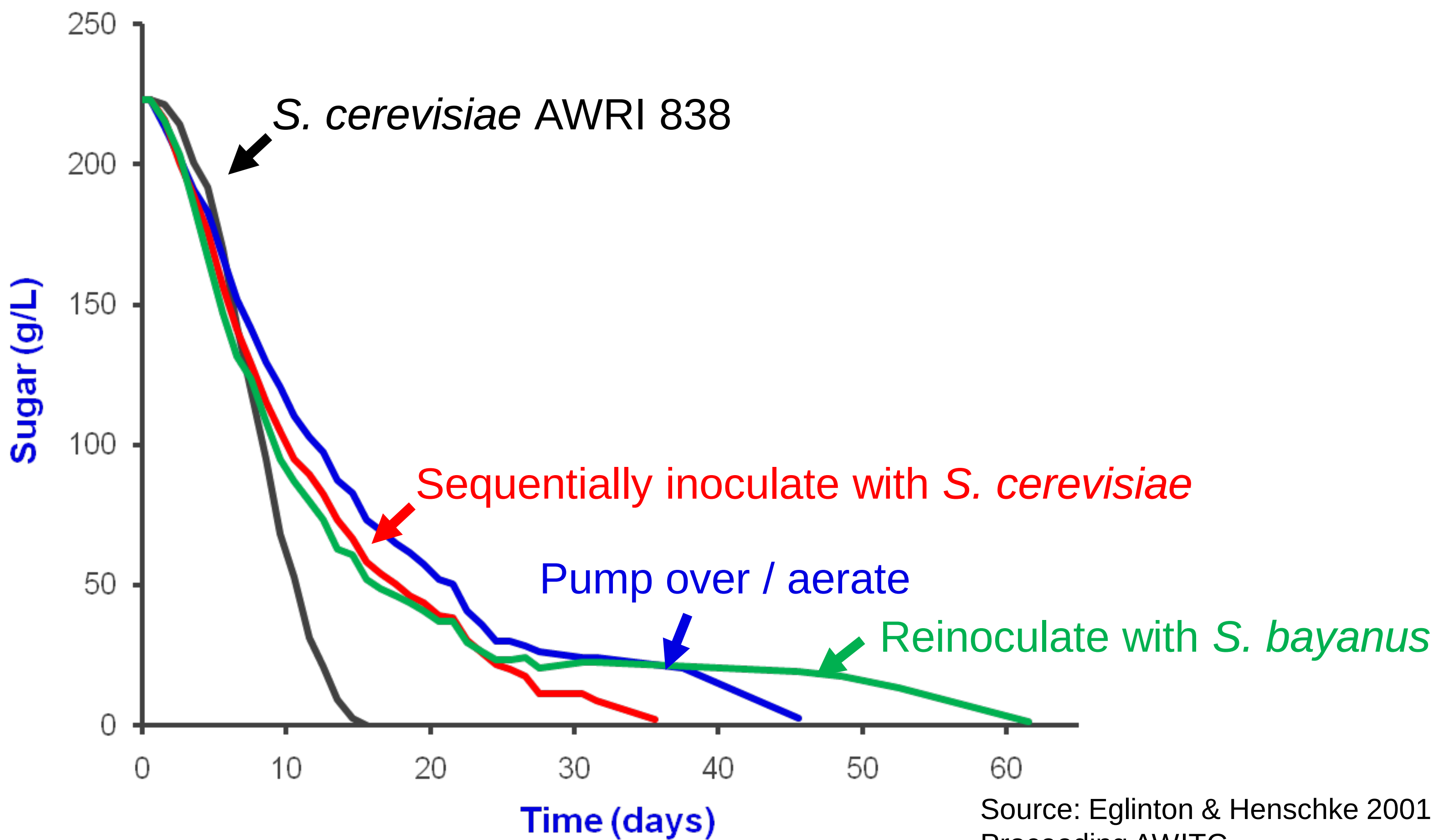
>>> Combined O2 and DAP gives greatest stimulation of fermentation rate

Sablayrolles, Dubois, Manginot & Barre (1986) *J. Ferment. Bioeng*. 82:377-381

Practical strategies for ensuring a complete fermentation with low vigour yeasts

eg S. bayanus AWRI 1375

The Australian Wine Research Institute



Source: Eglinton & Henschke 2001 Proceeding AWITC

All treatments tested promoted refermentation

N.B. Rescue cultures were prepared by AWRI step-wise acclimatisation procedure

Juice Clarification affects Fermentation Rate and Wine Residual Sugar

The Australian Wine Research Institute

Ferment rate	Wine residual sugar	turbidity	Clarification treatment
Highest	Lowest		Cold settled
			Bentonite treated and settled
			Enzyme treated and settled
			Centrifugation, 10 min at 1500g
			Coarse filtration
			Centrifugation, 20 min at 10000g
Lowest	Highest		Fine filtration (eg Sietz EK)

High clarity increases fermentation risk but enhances varietal character
Therefore, turbidity is adjusted to balance yeast performance and flavour

Inhibitory substances – risk factors

The Australian Wine Research Institute

- Ethanol – probably largest cause of stuck ferments
  - strain dependent: growth at 8-12%, fermentation >12 %
  - **determined by grape maturity at harvest**
- SO_2
  - strain dependent, typ. >10 mg/L free SO_2 at pH 3.5
  - cell death at 45 mg SO_2/L, pH 3.5 (0.8 mg/L mol. SO_2)
- Fatty acids (good hygiene / aerate ferments)
  - acetic acid: yeast growth at >1.5 g/L at 8% EtOH
    fermentation inhibited at 3-4 g/L
  - aliphatics (C6, C8, C10): ca. >3 mg/L at 10% EtOH
- Toxins (low risk except for lactic acid bacteria infection)
  - yeast toxins most active in low solids (bright) ferments
  - some wine yeast are tolerant
  - some Lactobacillus toxins can inhibit ferm. (high/low solids)
- Agrochemical residues (very uncommon)
  - copper oxychloride 10-15 mg/L
  - captan, fenarimol (eg Rubigan) / triadimenol (Bayfidan)
- Residues of winery sanitisers (uncommon)
- Yeast hulls can be used as a broad spectrum detoxification additive

From Henschke (1997)
ASVO Seminar Procs pp. 30-38,41

Role of acetic acid in stuck fermentation

Origin of acetic acid

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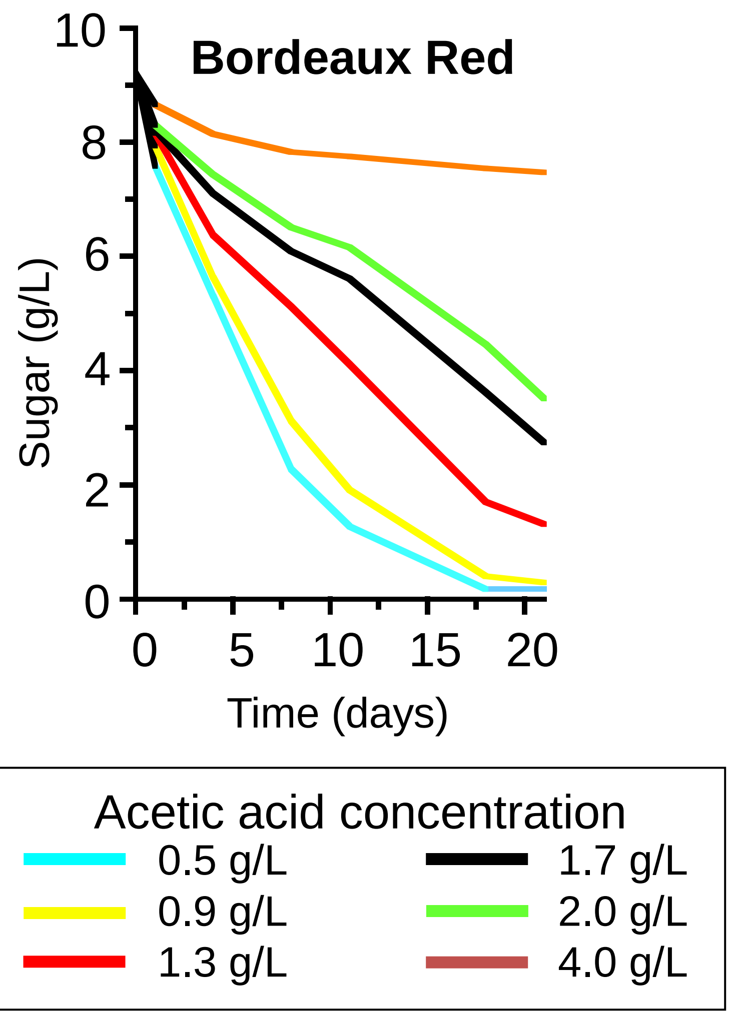
- inoculated yeast
  - Ø (excessive) nicotinic acid can stimulate production
- wild yeast
  - Ø apiculate yeasts (*Kloeckera/Hanseniaspora*)
- lactic acid bacteria – most important
  - Ø principally from citric acid
- acetic acid bacteria
  - Ø requires significant O_2

Effect of acetic acid on refermentation

Fermentation rate

The Australian Wine Research Institute

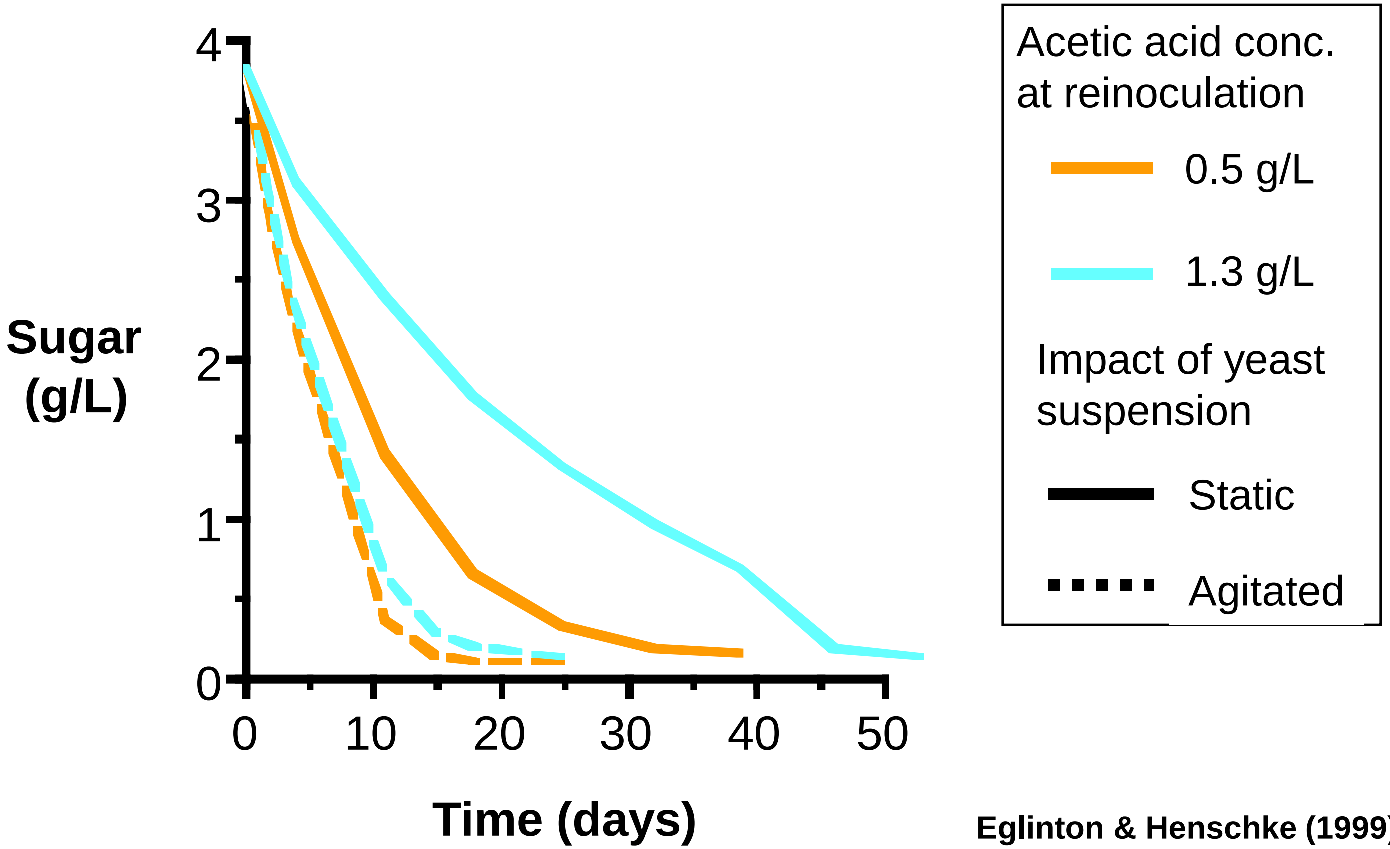
Stuck ferments containing different conc. acetic acid were inoculated with rescue yeast previously acclimatised to the stuck wine



Eglinton & Henschke (1999)
Aust. J. Grape Wine Res. **5:71-78**

Agitation aids refermentation

The Australian Wine Research Institute



Eglinton & Henschke (1999)
Aust. J. Grape Wine Res. **5:71-78**

>>> When restarting fermentation, important to keep yeast in suspension by physical means until CO_2 production commences, which then maintains yeast in suspension

Conclusions

- Yeast starter cultures are more effective rescue cultures when prepared by step-wise acclimatisation to the stuck ferment wine.
- Acclimatised rescue cultures can effectively restart incomplete ferments which contain up to 2 g/L acetic acid.
- Yeast strains however vary in their ability to act as a rescue culture
- Use of an acclimatised rescue culture largely negates the need to remove acetic acid by RO before rescue unless a very high concentration of acetic acid is present.
- Since additional acetic acid can be formed during the rescue procedure it is best to remove the acetic acid following refermentation of the wine.

Fermentation management – Risk factors

- Temperature stress

  Do not commence cooling until 10% sugar fermented
  Excessive temperature (32-35 ºC depend on [EtOH])
  Over-cooling for particular yeast (non-cryogenic) / may need to use methods to maintain yeast in suspension if <13-15 ºC
  Excess heating or cooling (transition exceeding 5 ºC)
  Cooling preferably should be <3 ºC per day

- Vigour and sedimentation (flocculation)

  Yeast sediments in low vigour ferments (CO_2 bubbles keep yeast in suspension and assists ferment circulation)
  Physical stirring can help prevent sedimentation
  Avoid flocculating strains in cool, cellar bright, anaerobic, high sugar ferments

- Grape solids

  Beneficial to wine style but deprives yeast of key nutrients
  Lipids increase yeast tolerance to ethanol stress

- Nutrients

  If known or suspected lack of nutrients (especially YAN and O2) recommend aeration (ca. 5 ppm O_2) and adding 300 mg/l DAP at 30-50% fermentation progress

Henschke (1997) ASVO Seminar pp. 30-38,41

Problem fermentations

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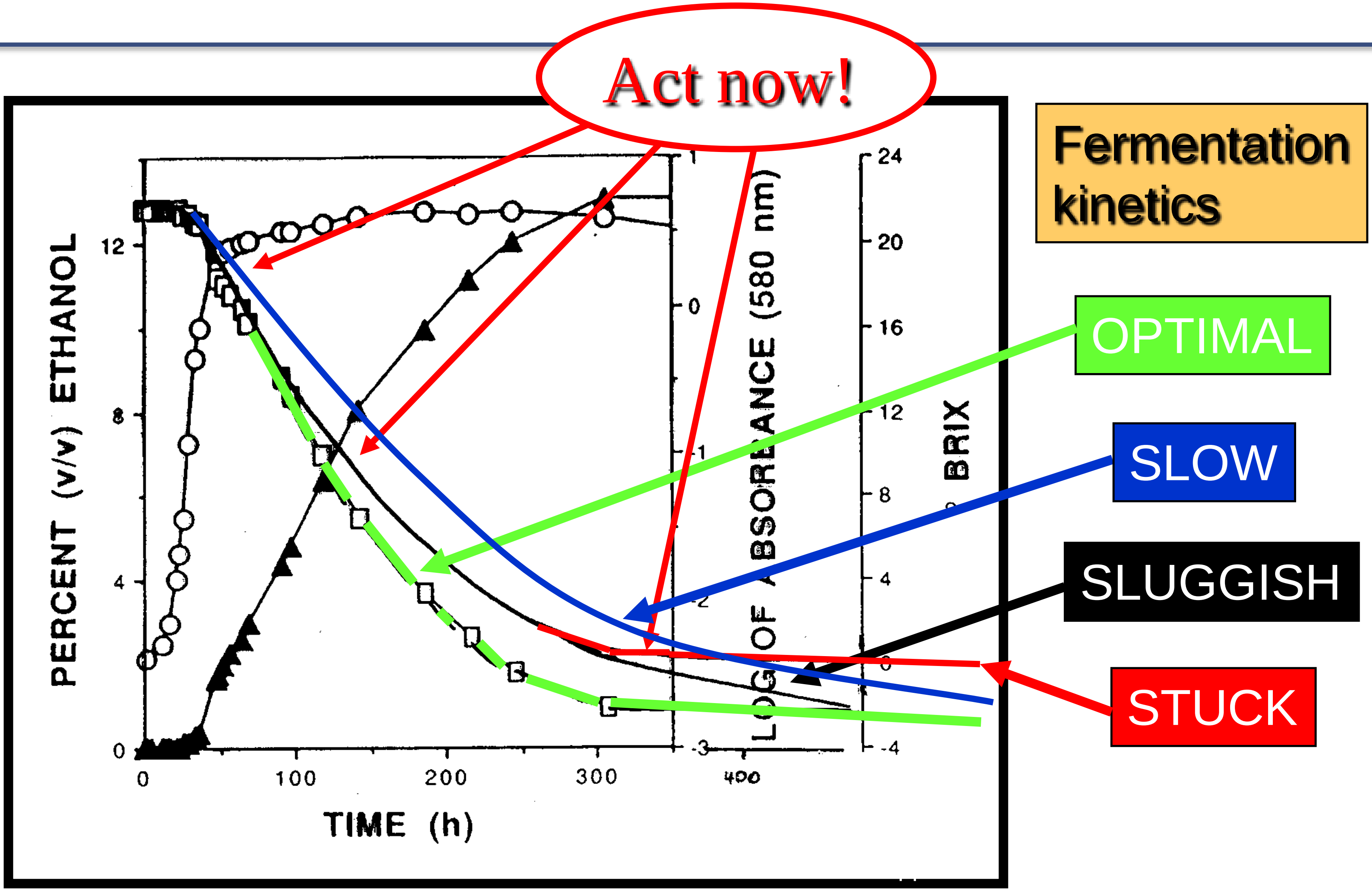

Take corrective action early

Re-yeasting: AWRI Rescue procedure

The Australian Wine Research Institute

- Add 500 mg/L EC1118, PDM, Uvaferm 43, etc
- Rehydrate with sterol-rich reactivation nutrient
- don't let culture run dry – go onto next stage when 50% of sugar has gone
- Avoid temperature shock
- Add wine to culture, rather than culture to wine
- Add SO_2 if bacteria present in stuck wine
- Rack or centrifuge stuck wine (remove dead yeast)
- Add DAP and aerate once active
- Yeast hulls often beneficial
- Keep yeast in suspension
- Keep good records

Choice of rescue procedure

If ferment stops with <10 g/L residual sugar and the alcohol content is <12 % v/v:

- Then recommend preparing a starter culture in grape juice with a recommended yeast. This procedure is relatively quick and will produce moderate tolerance to alcohol
- **Otherwise** use a rescue culture prepared by stepwise acclimatisation of a recommended rescue yeast. This procedure builds tolerance to the toxic substances present in the problem ferment

Yeast acclimatisation procedure for restarting difficult and stuck ferments

(See AWRI Website for details)

The Australian Wine Research Institute

Procedure for 1000 L of ferment

Stage	Function		Cumulative volume
1	Preparation of rescue culture		20 L
2	Acclimatisation		
	Step	Proportion of ferment	
	1	50%	40 L
	2	75%	80 L
	3	88%	160 L
	4	94%	320 L
3	Inoculate problem ferment		1020 L

Adapted from Henschke (1997)
ASVO Seminar Procs pp. 30-38,41

Important principles of rescue procedure

- Stepwise acclimatisation of yeast to toxic substances of the problem ferment – if possible, incrementally add the ferment to the culture rather than the culture to the ferment
- No sugar depletion stress
- No nitrogen depletion stress
- Continuous aeration
- Agitation prevents nutrient starvation stress

For more information

The Australian Wine Research Institute

- **AWRI website – wealth of practical information**
- **ASVO seminar 1996**

  papers by: Henschke, Monk & Four industry practitioners
- **Industry Services Group ; AWRI Technical Note 05**
- **Contact Industry Development & Support team: Con Simos, Adrian Coulter, Geoff Cowey, Matthew Holdstock for technical advice**

Acknowledgments

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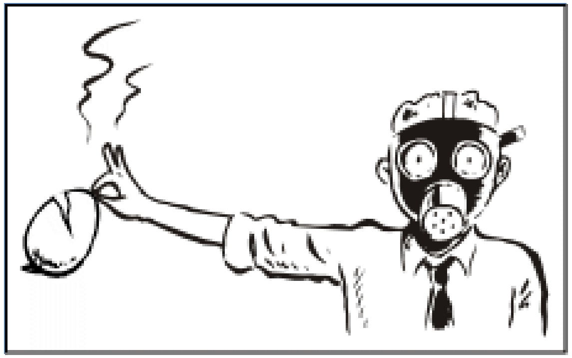
Rotten egg, cabbage and rubber: compounds responsible for reductive off-flavours in wines

Leigh Francis



Compounds found in wine

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Low MW Sulfur Compound		Odour Descriptor	Aroma Threshold (µg/L)	Detected (µg/L)	
				Literature Review	AWRI
Hydrogen Sulfide	H_2S	rotten egg, sewage like	1	nd - 370	nd - 56
Methanethiol	MeSH	rotten cabbage, burnt rubber, putrefacation	1.5	nd - 16	nd - 11
Ethanethiol	EtSH	onion, rubbery, burnt match, sulfidy, earthy	1.5	nd - 50	nd - 3
Dimethyl sulfide	DMS	*blackcurrant* , cooked cabbage, asparagus, canned corn, molasses	25	nd - 474	nd - 980
Carbon disulfide	CS_2	*sweet, ethereal, slight green* , rubber, sulfidy, chokingly repulsive	5	nd - 18	nd - 140
Diethyl sulfide	DES	garlic, rubbery	1	nd - 10	nd
Methyl thioacetate	MeSAc	sulfurous, cheesy, egg	40	nd - 115	nd - 53
Dimethyl disulfide	DMDS	vegetal, cabbage, intense onion-like (at high levels)	10	nd - 22	nd - 2
Ethyl thioacetate	EtSAc	sulfurous, garlic, onion	70	nd -180	nd - 32
Diethyl disulfide	DEDS	bad smelling, onion	4	nd - 85	nd - 1.5

Hydrogen sulfide detection threshold

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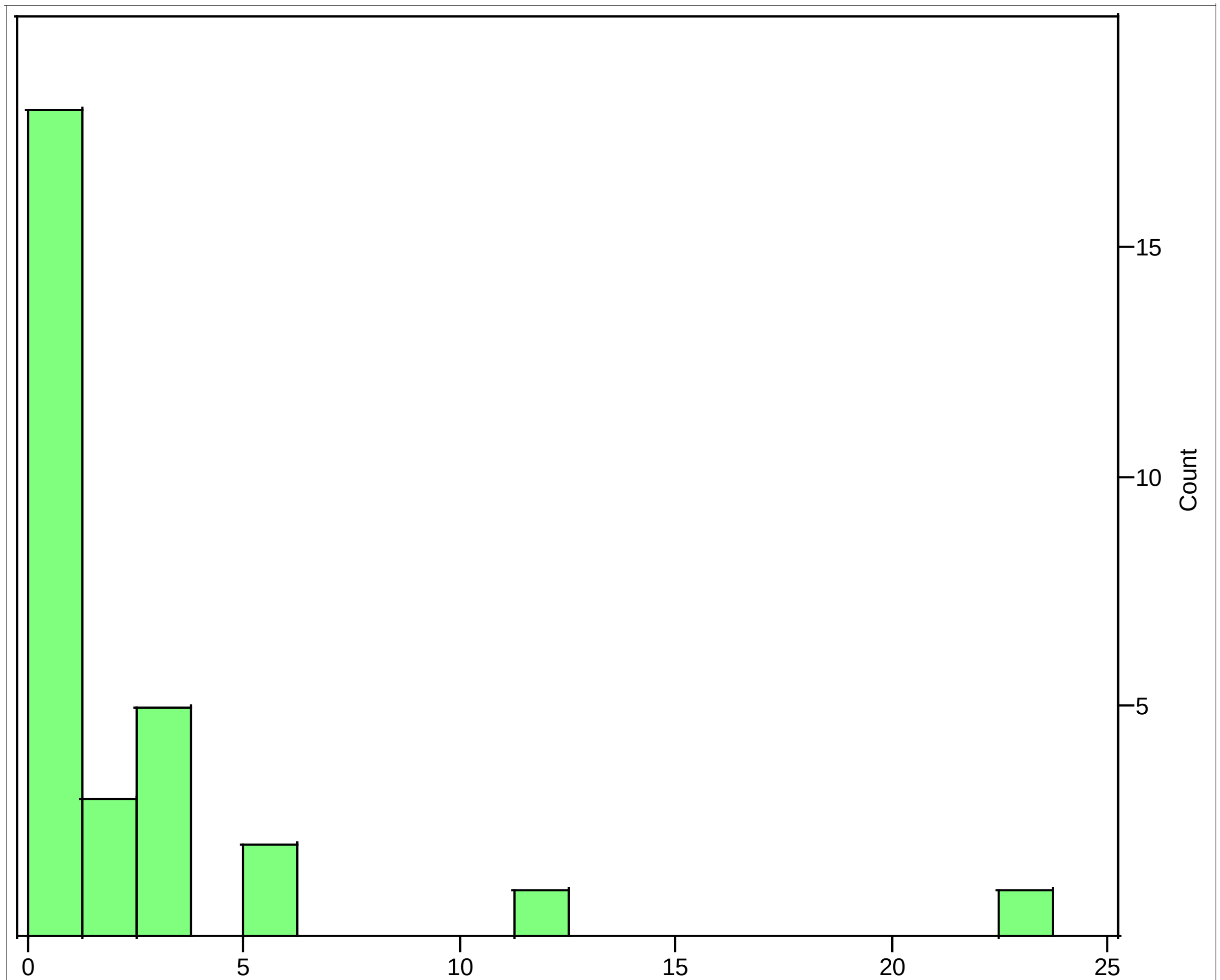

Individual BET values (µg/L)

H_2S and methanethiol act additively, benzenemethanethiol not so stinky

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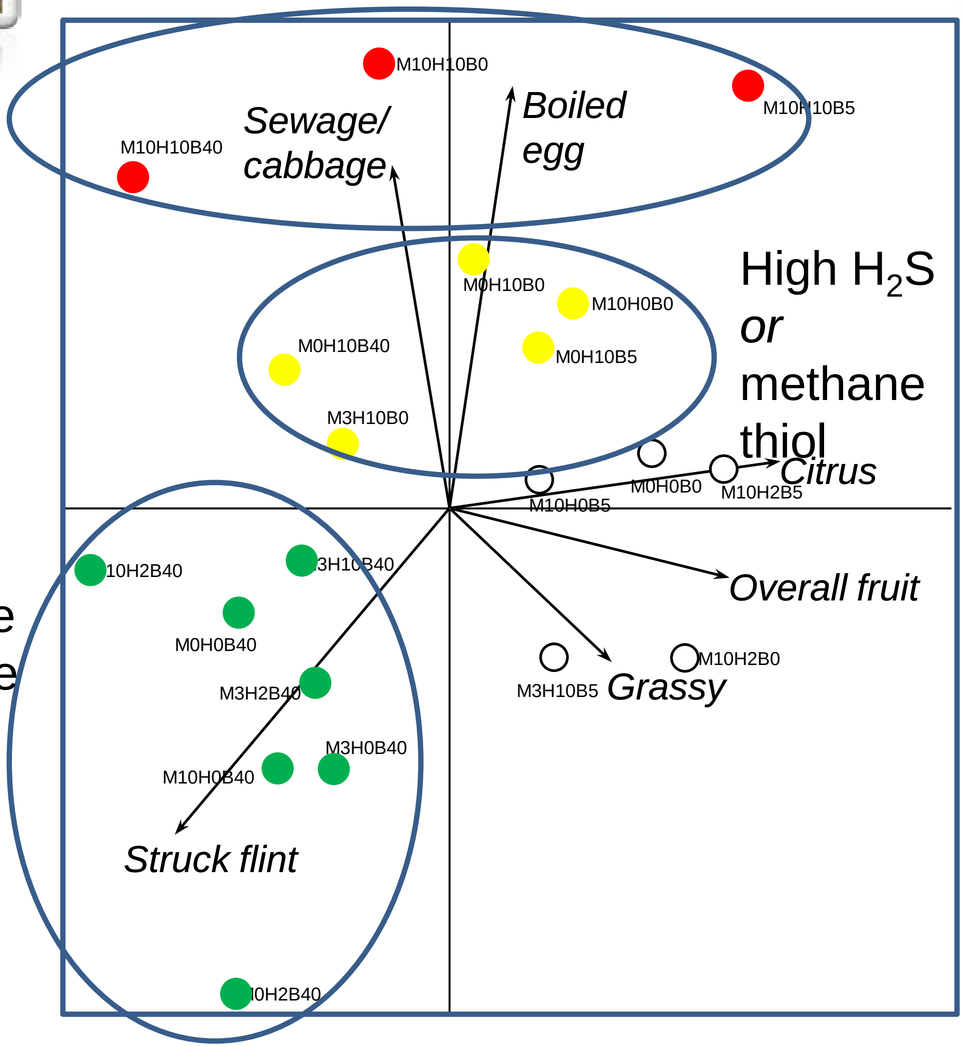
High H_2S *and* methane thiol



High H_2S *or* methane thiol

High benzene methane thiol

Reductive flavour most commonly relates to H_2S and methane thiol

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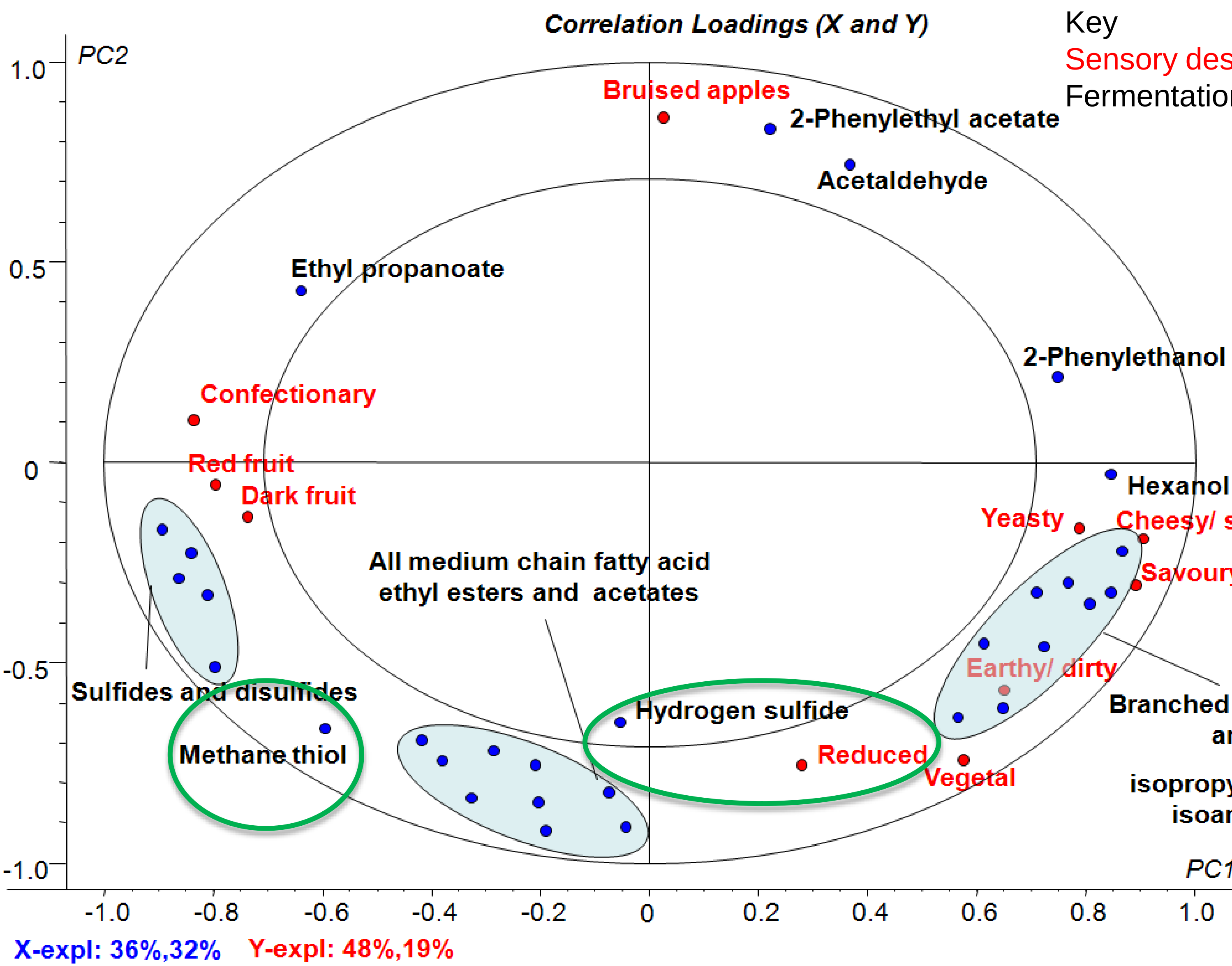

Key
Sensory descriptors in red
Fermentation aromas in black

Ugliano et al.
Food Chem. (2010)

Formation and degradation of H2S

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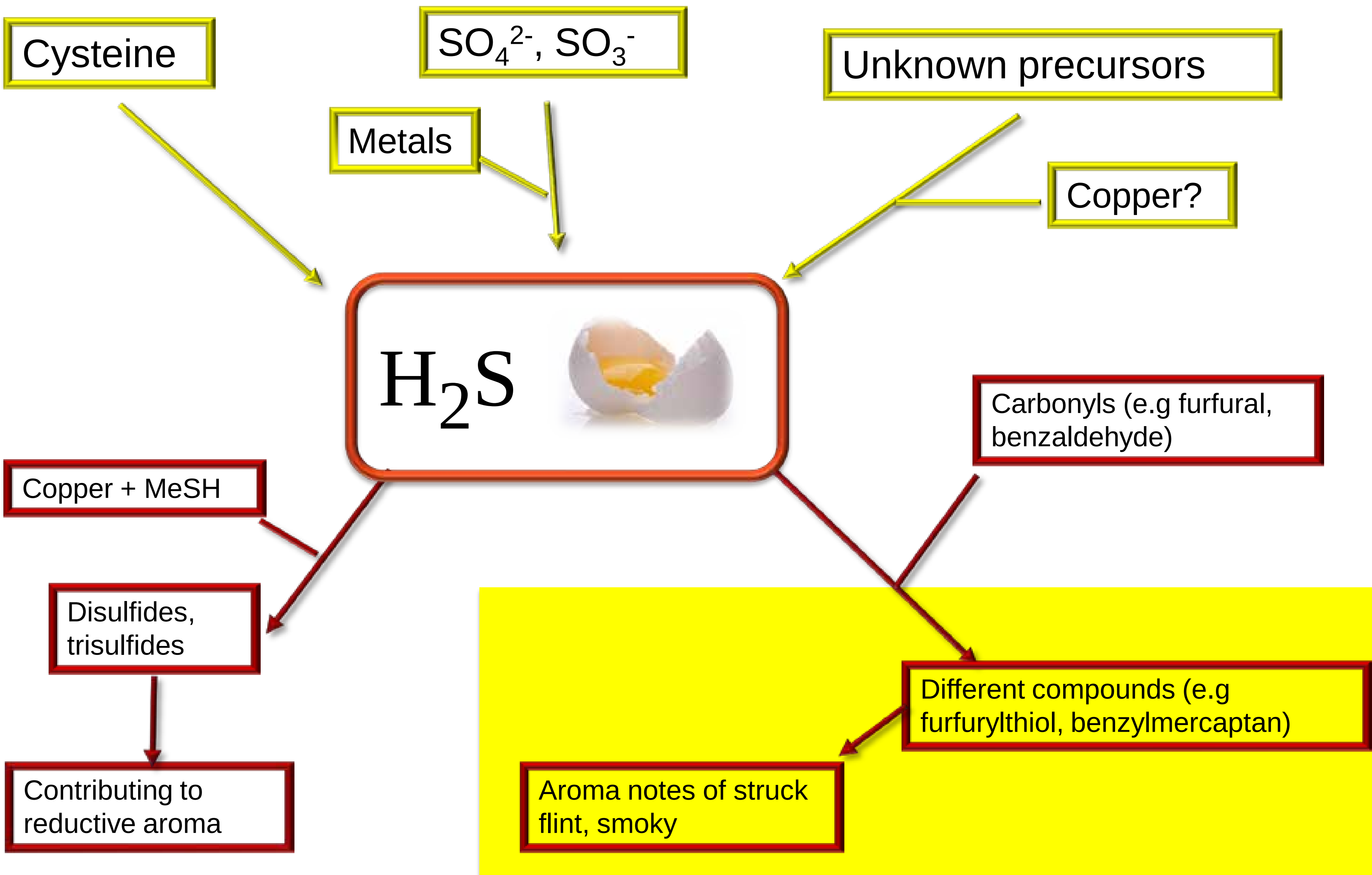

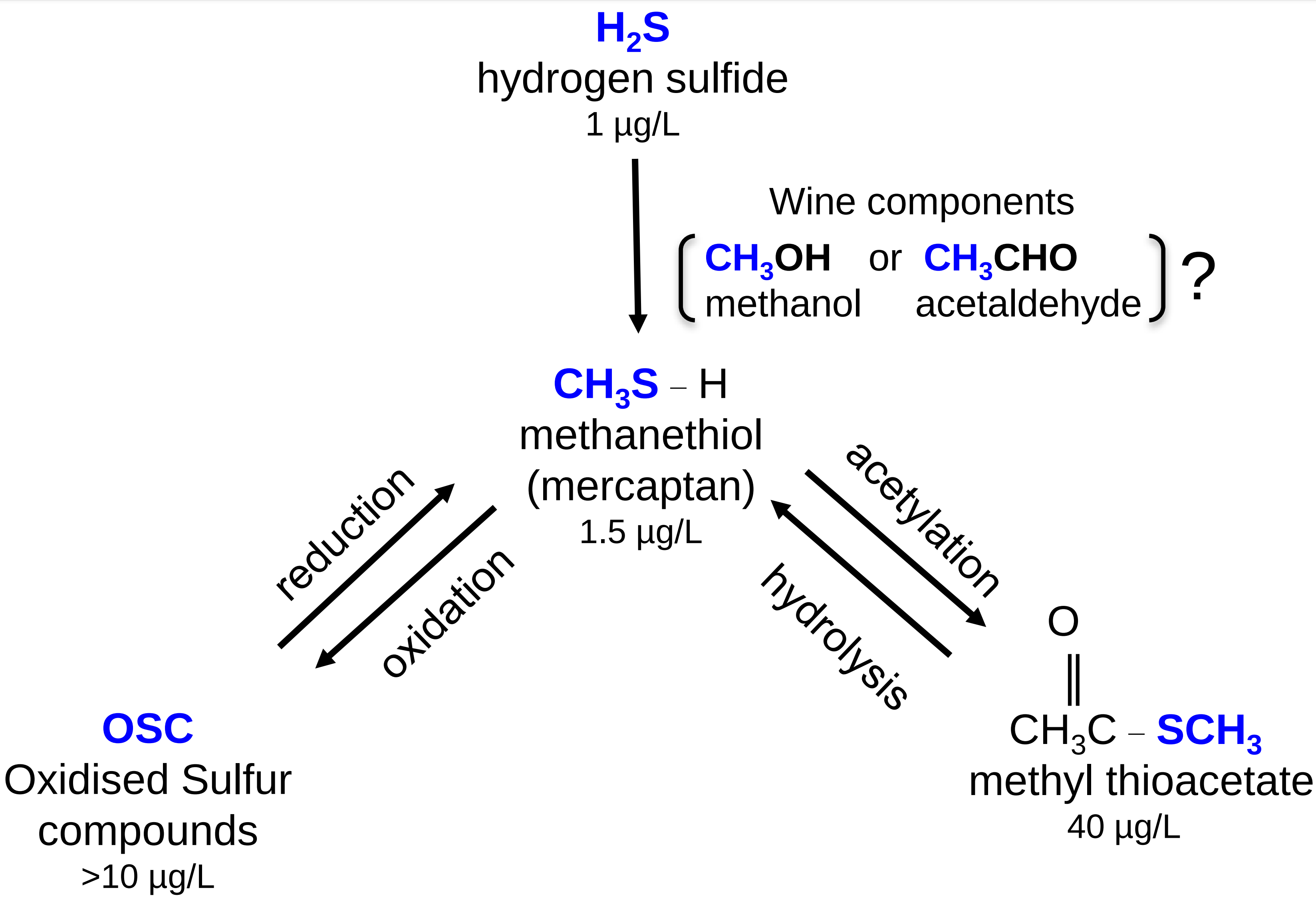
H2S
hydrogen sulfide
1 µg/L
Wine components
CH3OH or CH3CHO
methanol acetaldehyde
?
CH3S – H
methanethiol
(mercaptan)
1.5 µg/L
reduction
oxidation
acetylation
hydrolysis
OSC
Oxidised Sulfur compounds
>10 µg/L
O
CH3C – SCH3
methyl thioacetate
40 µg/L

Copper additions can be very effective at removing sulfur compounds

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H_2S $H_2S + Cu^{2+}$ ® CuS ¯

mercaptan $CH_3CH_2SH + Cu^{2+}$ ® $Cu(CH_3CH_2S)_2$ ¯

reduction ¯ _ oxidation

DMDS $CH_3S\text{-}SCH_3 + Cu^{2+}$ ® unreactive

DMS $CH_3SCH_3 + Cu^{2+}$ ® unreactive

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Downsides of residual copper

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- Hazes
- Increased loss of 3-MH and 3-MHA
- More rapid loss of SO_2
- Increases in sulfides

Hazes and protein instability

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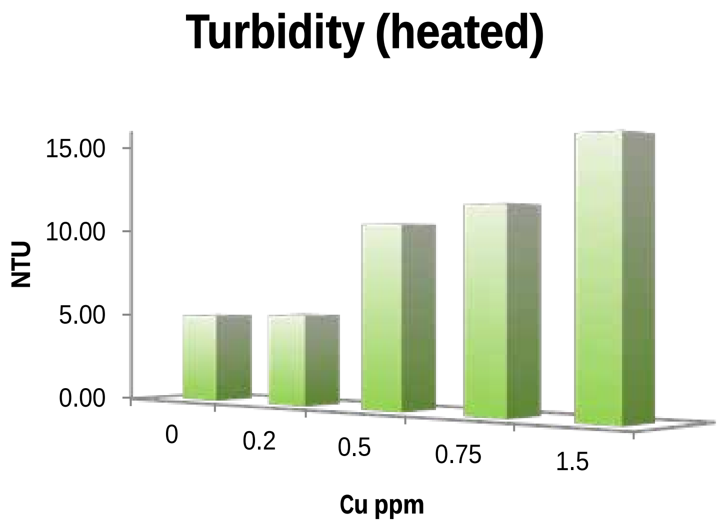

- Increased copper levels in bottled wine are well known to increase protein instability
- Generally recommended to keep levels below 0.5 ppm, but limit depends on the wine

Increased loss of 3-MH and 3-MHA

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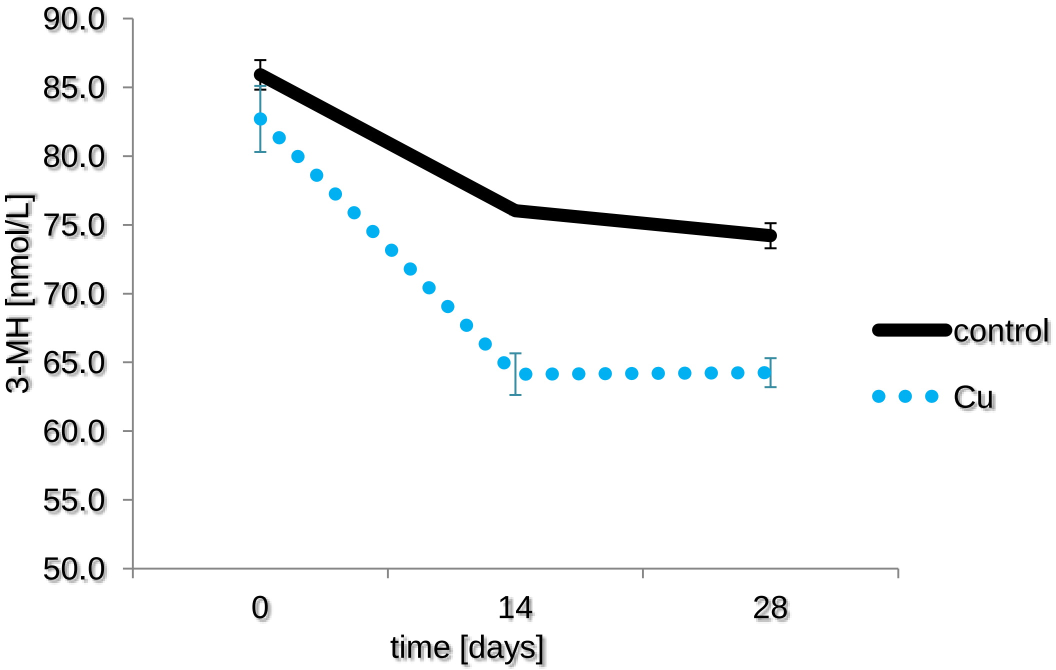
3-MH (3-Mercaptohexan-1-ol)

3-MHA (3-Mercaptohexan-1-ol acetate)



Dr. Mandy Herbst-Johnstone
School of Chemical Sciences
The University of Auckland

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More rapid loss of SO_2

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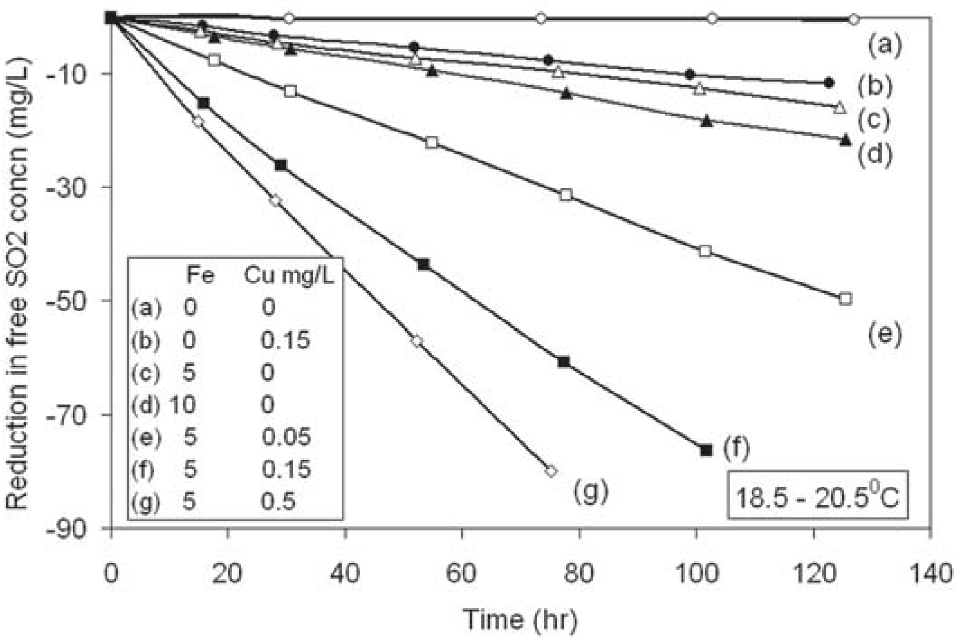
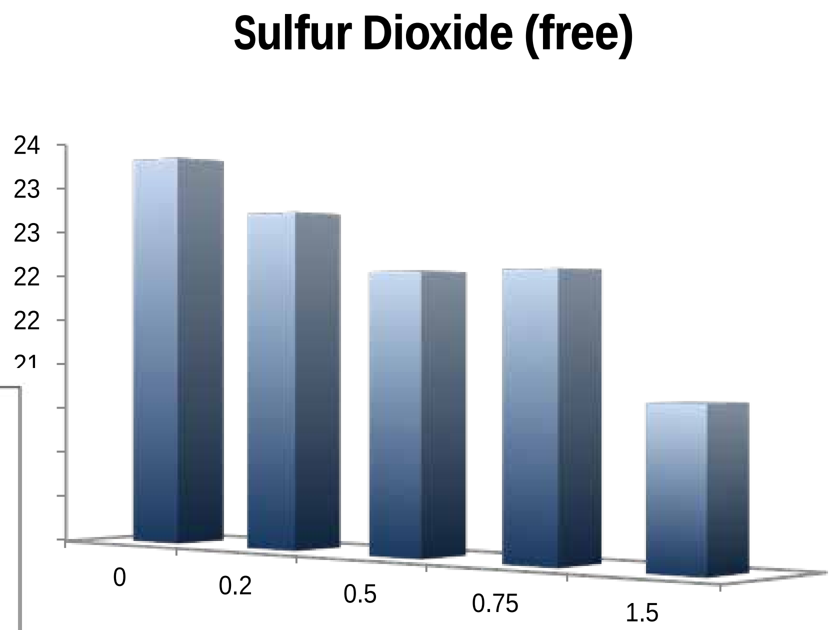
SO_2 cannot interact with O_2 directly
It requires the presence of metals such as copper and iron.





After just two months in bottle!

Danilewicz, J. (2007). Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *American journal of enology and ….*

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Increases in sulfides

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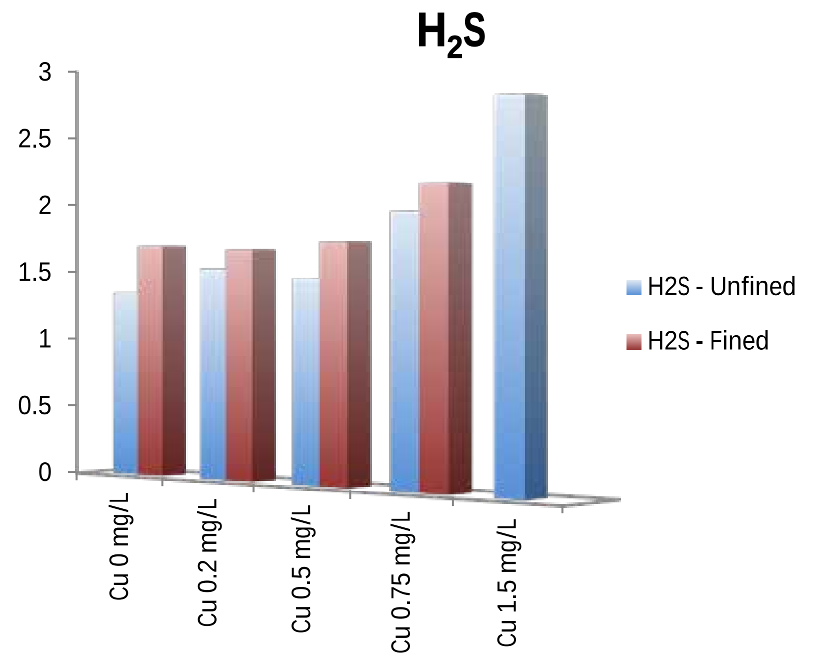
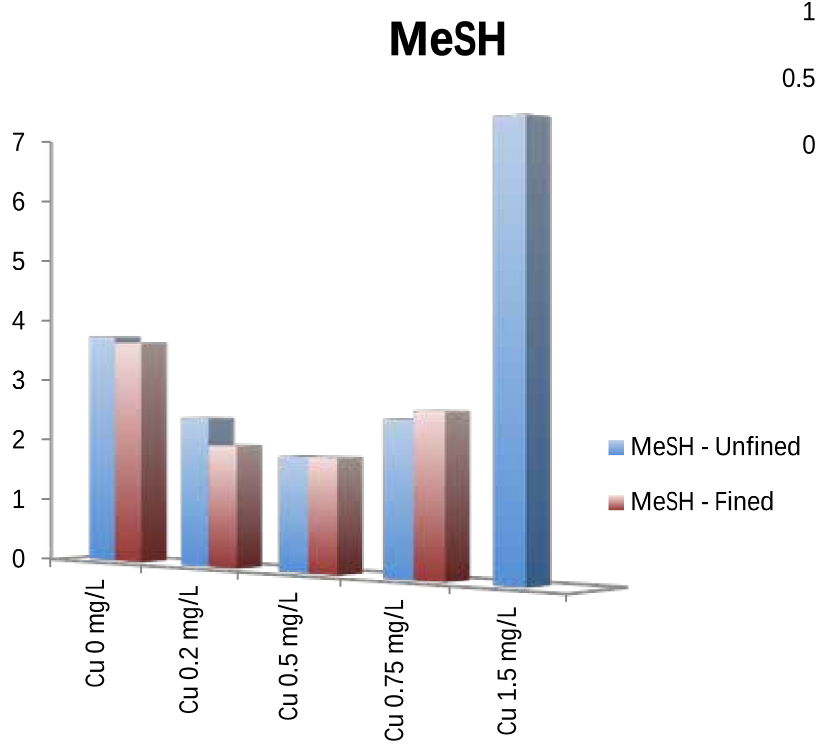
After just two months this Chardonnay was already showing the impact of increased copper





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When is it ok to use copper?

- Best time to add is at the end of fermentation
  - Eliminate the potential precursors as early as possible
  - Use the solids to remove as much of the excess copper as possible

- If you have to do it later
  - Know what sulfur compounds you are treating (copper/cadmium test)
  - Add the minimum amount of copper
  - Give it time to stabilize before bottling
  - Test the copper levels before and after addition

Never add on the day of bottling

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Principle of Cu/Cd test

The principle of this test is that different reductive volatiles react with different fining agents, including copper and cadmium salts (Note that cadmium sulfate is not an allowable fining agent and is only to be used in this diagnostic test). A fining trial can be conducted using these agents to determination of the type of reductive fault present in a wine to determine the appropriate course of action to remove the fault.

Reagents

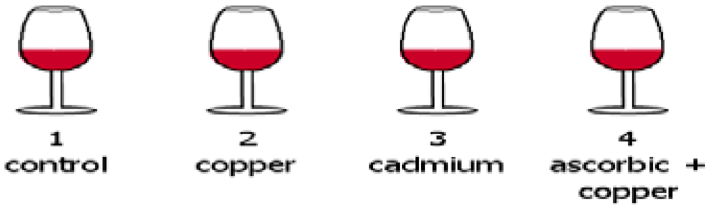
1) 1% w/v Copper (II) Sulfate solution (1 g of CuSO4.5H2O in 10% Ethanol)
2) 1% w/v Cadmium (II) Sulfate solution (1 g of CdSO4.8 H2O in 10% Ethanol)

Caution:
Cadmium is TOXIC
Do not taste samples to which cadmium has been added. Assess by aroma only.
Cadmium sulfate is not an allowable wine additive or processing aid and is only to be used in this diagnostic test

3) 10% w/v Ascorbic acid (10 g ascorbic acid in 100 ml of 10% Ethanol)

Procedure

1. Place 50 mL of wine into four separate glasses.
2. Label the four glasses: (1) control, (2) copper, (3) cadmium, and (4) ascorbic acid +copper
3. Add 1 mL of reagent 1, the copper sulfate solution to glass 2 ('copper' glass).
4. Add 1 mL of reagent 2, the cadmium sulfate solution to glass 3 ('cadmium' glass).
5. Add 0.5 mL of reagent 3, the ascorbic acid solution to glass 4 ('ascorbic + Cu' glass), wait 2 minutes, then add 1 mL of reagent 1, the copper sulfate solution to the same glass 4.



- Copper can be very effective in preventing the development of sulfur off-flavours
- However if excess is left in the wine it can lead to
  - the development of the same undesirable characters
  - hazes
  - degradation of SO_2 levels and desirable sulfur compounds
- Copper is best added early in the wine's life when fermentation solids can help to remove it. Later additions can lead to a build up of available copper
- Not all copper is stripped from wine post addition as sulfides
- Careful trials can lead to successful management of copper levels

Blackcurrant or canned corn??

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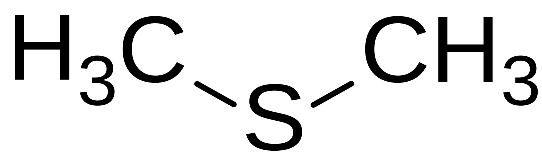
Dimethylsulfide (DMS)



25 µg/L

Acknowledgements

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The Australian Wine Research Institute

Automating Juice and Wine Analysis

Dr Eric Wilkes

Group Manager Commercial Services

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SO MUCH MORE THAN A GREAT LAB

We have all seen CSI/NCIS

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It all tends to be a trade off!

Cost v speed

Accuracy v convenience

Cost v accuracy

Speed v Maintenance

Only one thing for sure, no perfect one size fits all solution.

Questions to ask yourself?

How quickly do I really need that result?

How much is it worth to me?

If I replace the people am I losing other skills?

Would lean/organization solve the problem?

Is a better LIMS going to make a difference?

Before you do anything…….

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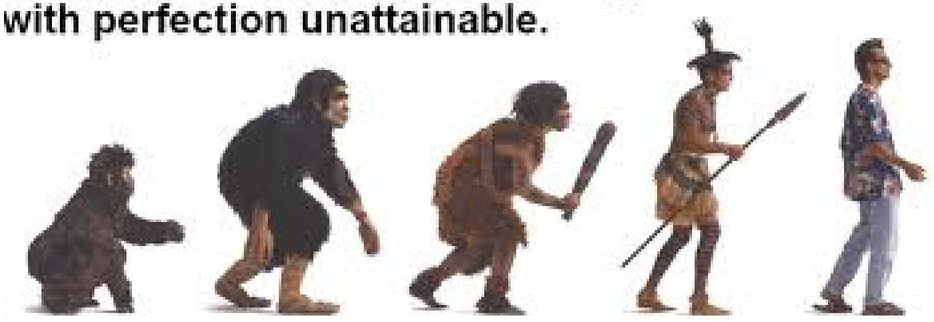
GET LEAN

It's a journey—of continuous improvement with perfection unattainable.



Digital refractometers

Brix / Baume

$1.5K~$20K

✔Quick
✔Accurate
✔Easy to use
✖bubble cause issues
✖Temperature corrected, sort of
✖expensive

Density meters

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Brix, SG, alcohol

$3K~$20K

- ✔ Quick
- ✔ Accurate
- ✔ Easy to use
- ✖ Need careful cleaning
- ✖ Sensitive to degassing
- ✖ Don’t bounce

NIR Alcohol

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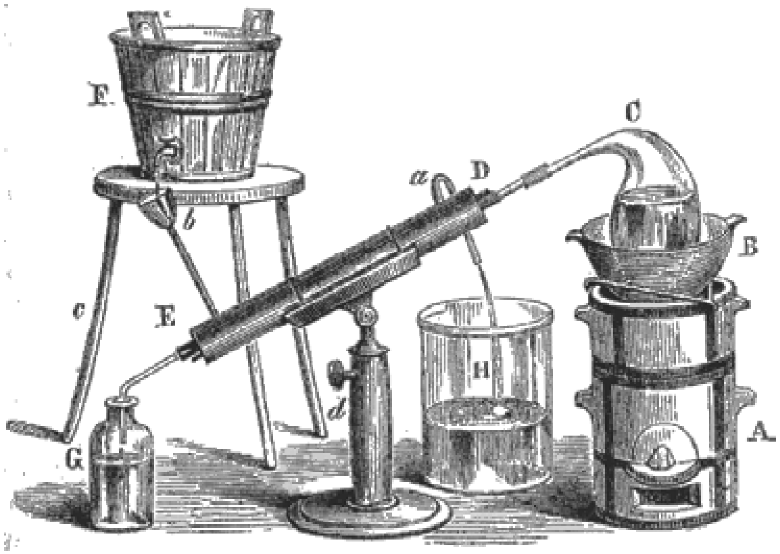

Alcohol

$25K

✔Quick

✔Accurate

✔Easy to calibrate

✖Need careful cleaning

✖Sensitive to degassing

✖Can get unexplained outliers

Ebulliometers

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Research Institute

Autotitrators

The Australian Wine Research Institute

pH/TA, SO_2

$3K~$50K

✔Quickish

✔no less accurate

✔can be left unattended

✔Can have auto-degassing

✖Need careful maintenance and cleaning

✖difficult to troubleshoot

Flow Injection Analysis

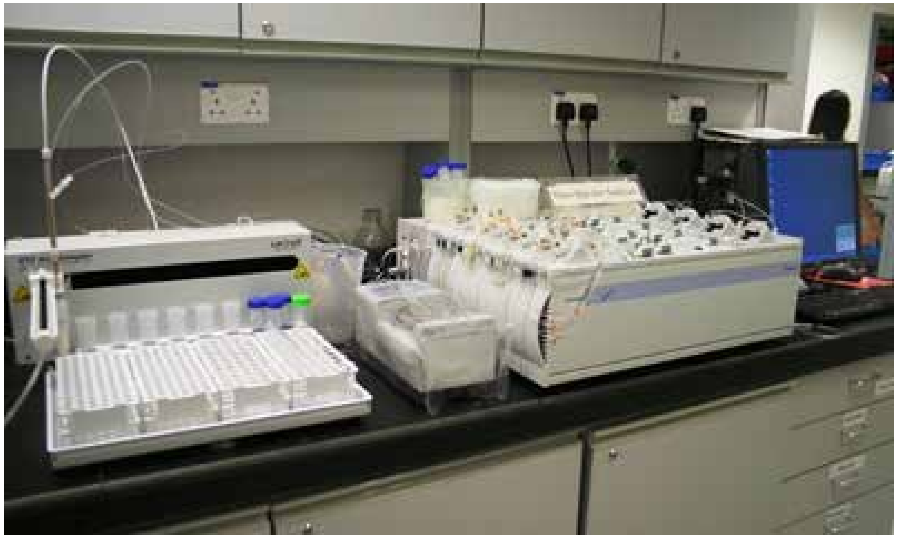
SO_2, VA ~$40K

✔ Huge throughput

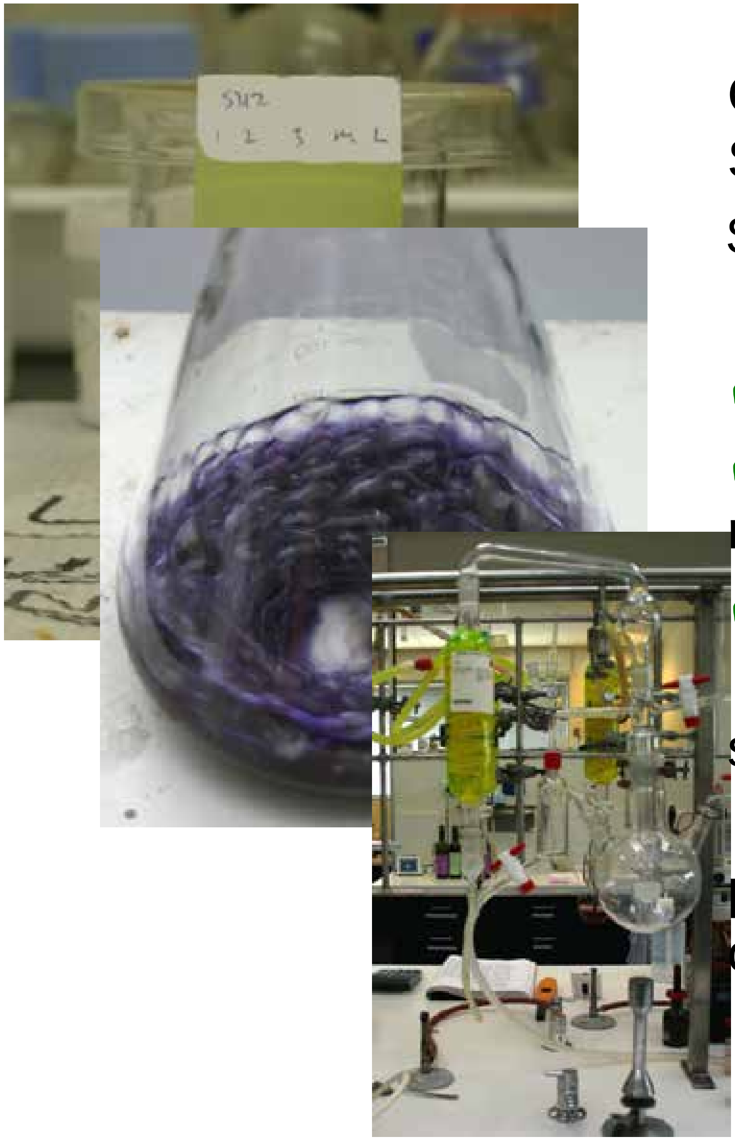
✖ Need careful cleaning

✖ High Maintenance

✖ some dodgy reagents



Enzymatic Analysis



G/F, malic, YAN, VA, SO_2

$1K~$30K

- ✔Quickish
- ✔accurate and reliable
- ✔less interferants
- ✖Need good tech skills
- ✖expensive and perishable consumables

Automated enzymatic analysis

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G/F, malic, YAN, VA, SO_2

$20K~$100K

✔Quickish

✔High throughput

✔accurate and reliable

✔less interferants

✖Need good tech skills

✖Maintenance

✖expensive and perishable consumables

FTIR

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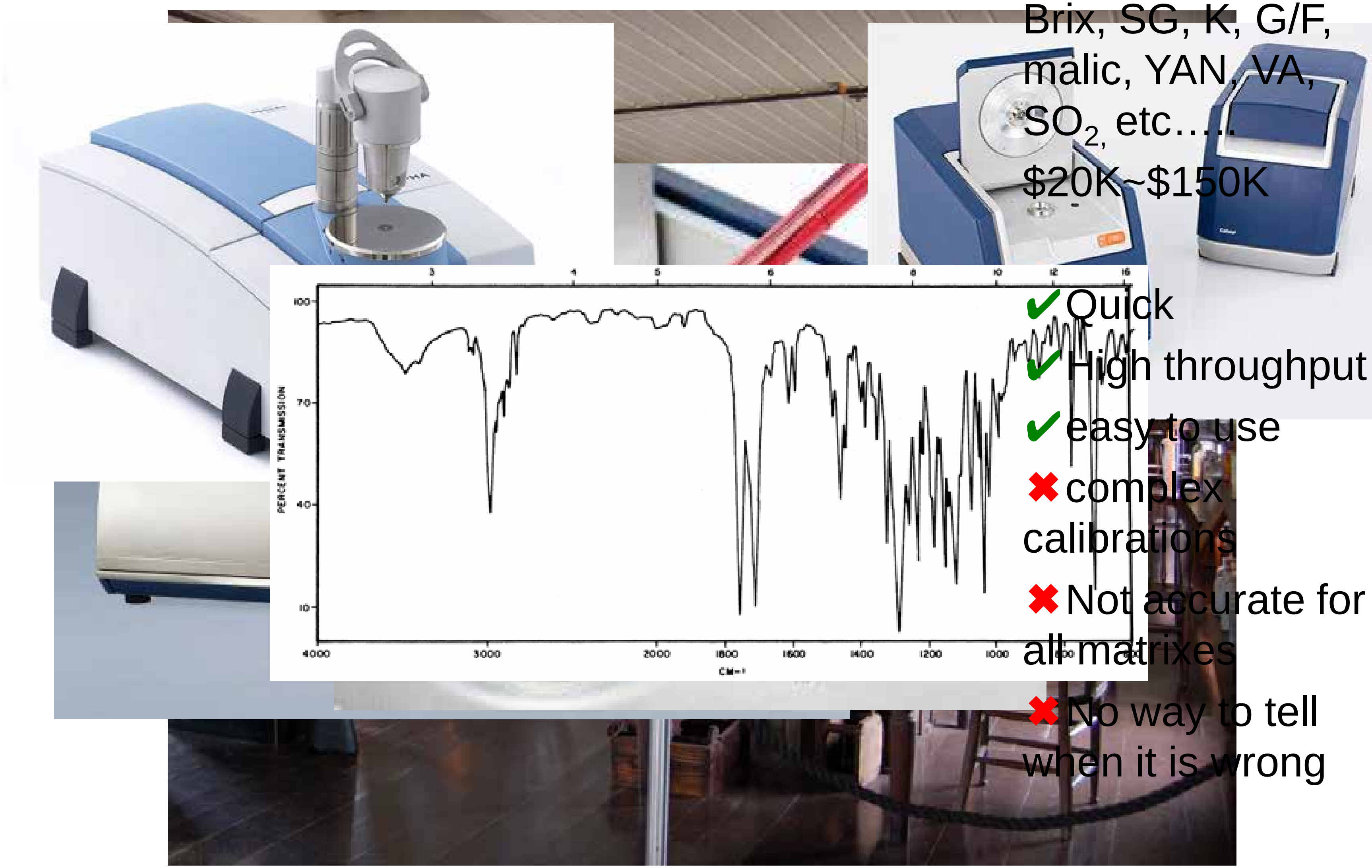

Brix, SG, K, G/F, malic, YAN, VA, SO_2, etc…..

$20K~$150K

- ✔ Quick
- ✔ High throughput
- ✔ easy to use
- ✖ complex calibrations
- ✖ Not accurate for all matrixes
- ✖ No way to tell when it is wrong

Questions?

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AWRI

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Using MLF to accentuate wine aroma and flavour

Eveline Bartowsky

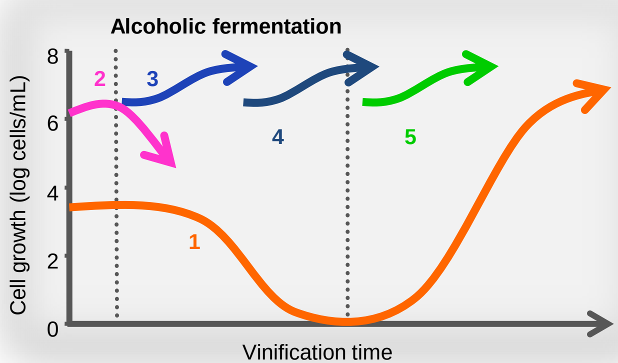
Senior Research Microbiologist

Malolactic fermentation

The Australian Wine Research Institute

- MLF ...
  - Reduce wine acidity
  - Microbial stability
  - Sensory changes

- When can it occur?
  - Spontaneous
  - Inoculated



- Sensory impact
  - Buttery character
  - - fruity & ¯ vegetative characters
  - Improved mouthfeel

- Delayed/failed MLF
  - Can increase the risk of wine spoilage, especially Brett & biogenic amines

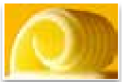
MLF is generally more difficult to manage that AF

It’s all about aroma & flavour

The Australian Wine Research Institute

- Talk will concentrate on sensory aspect of MLF

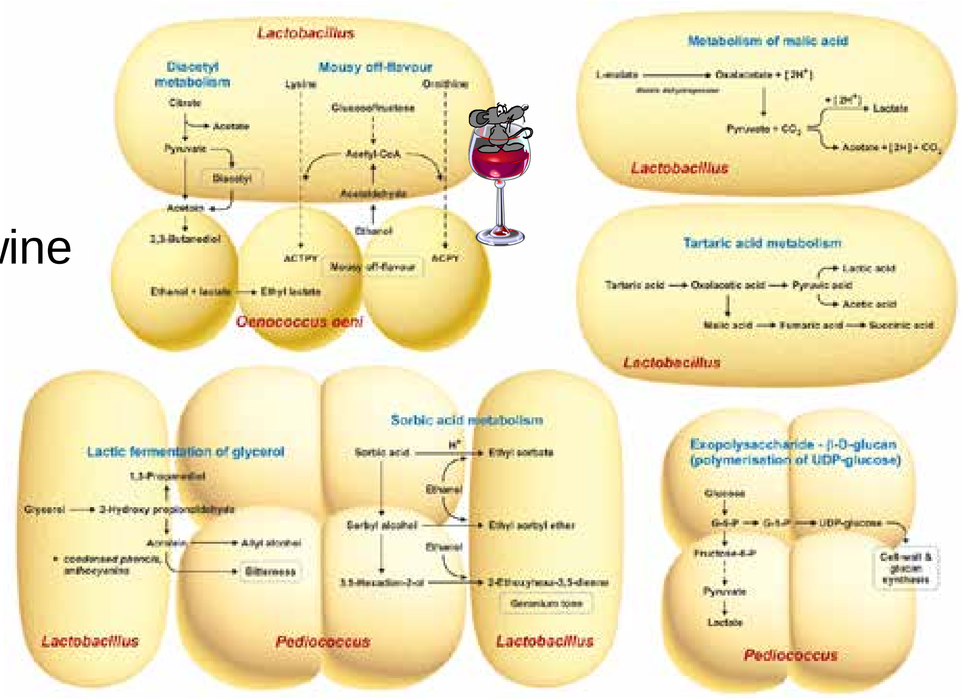
- Sensory



Diacetyl

Berry-fruit characters in red wine

  - Polysaccharides (mouthfeel)



Buttery aroma - Diacetyl

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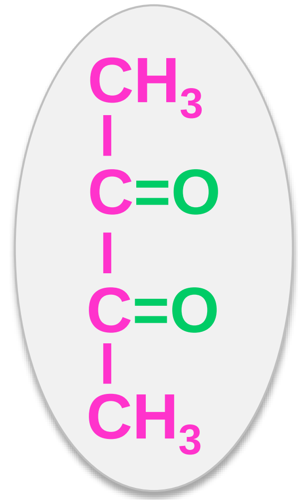
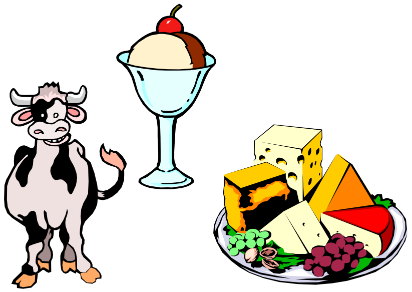
- *O. oeni* during MLF
- Derived from citric acid metabolism
- Aroma
  - buttery, nutty, butterscotch
  - 1 - 4 mg/L = enhance flavour complexity
  - > 5 - 7 mg/L = undesirable buttery aroma
  - Taste threshold
    - Chardonnay 0.2 mg/L
    - Pinot Noir 0.9 mg/L
    - Cabernet Sauvignon 2.8 mg/L





2 important considerations for diacetyl producn

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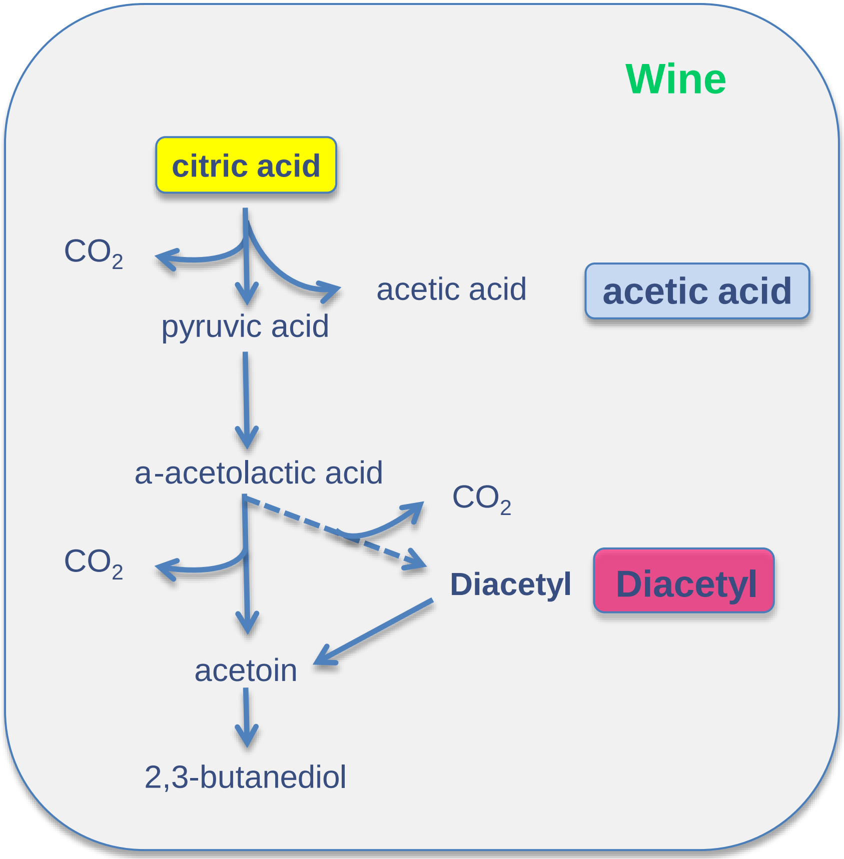
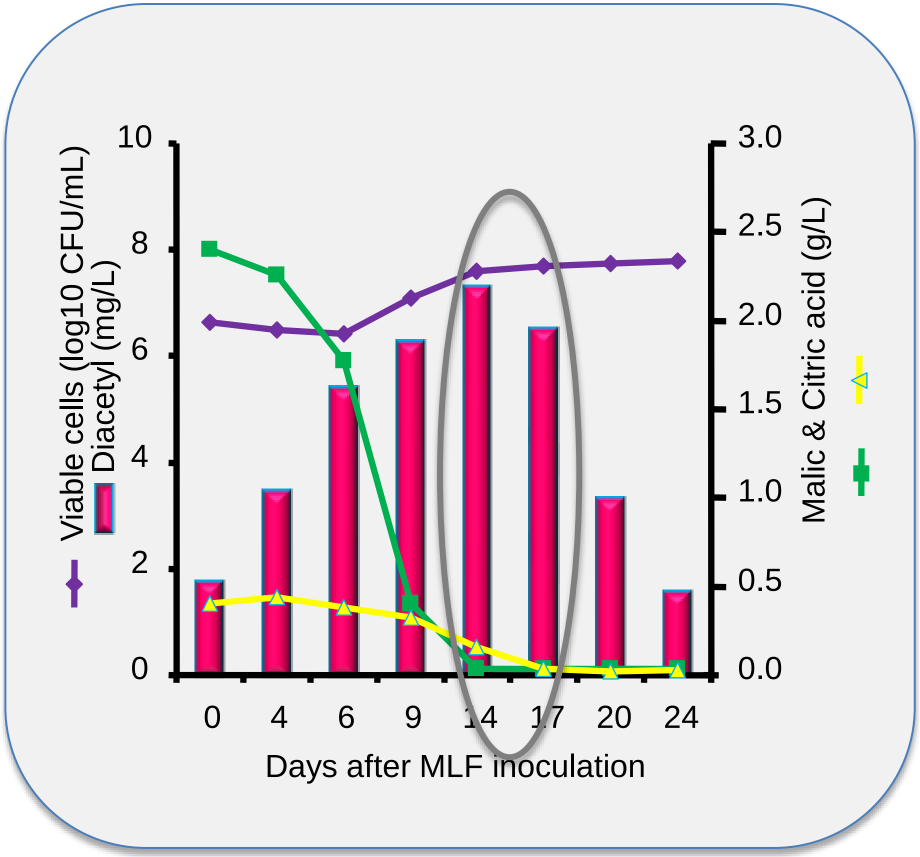
Pathway



Kinetics

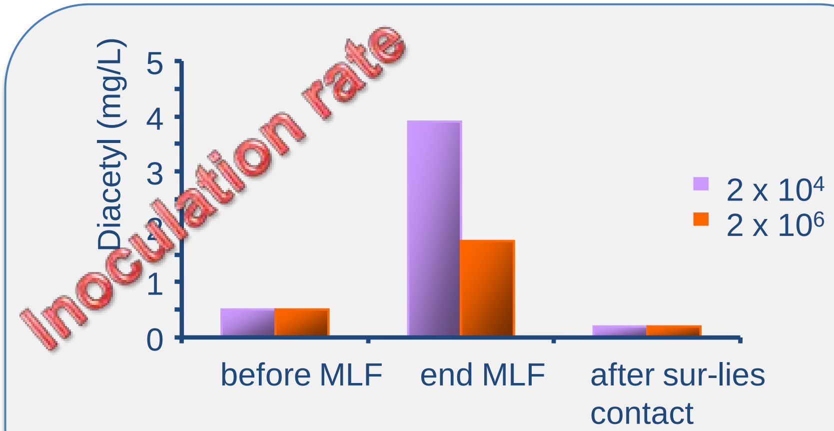


Cabernet Sauvignon, 2000
Barossa & Eden Valley blend
Strain III (12.5 % alcohol)

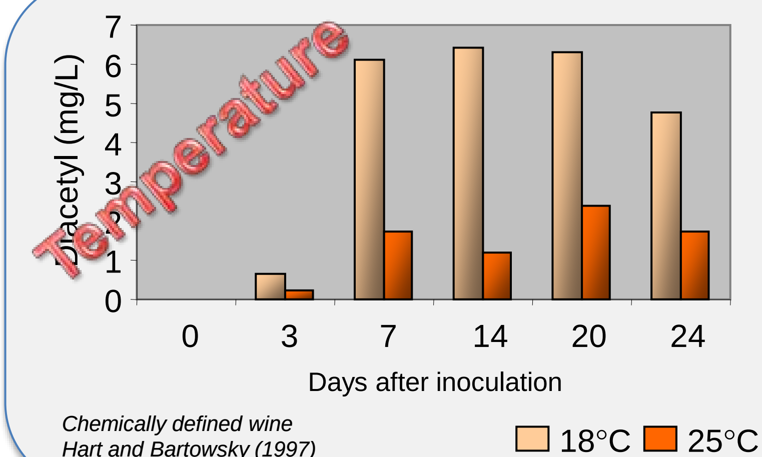
from Bartowsky & Henschke, 2004

Many factors influence Diacetyl

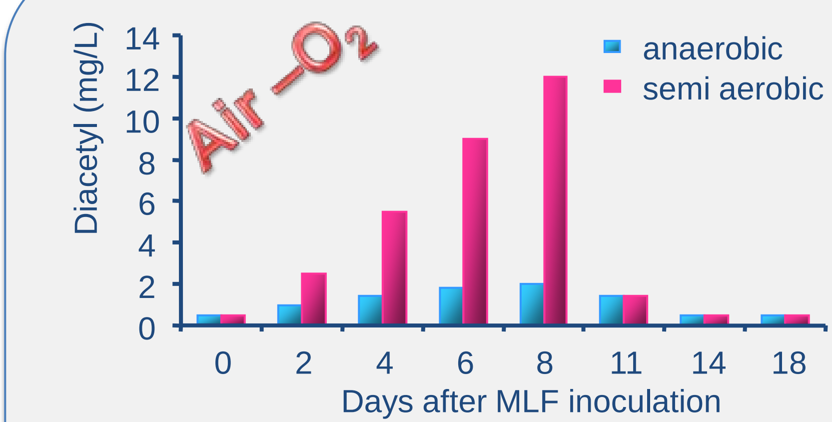
The Australian Wine Research Institute



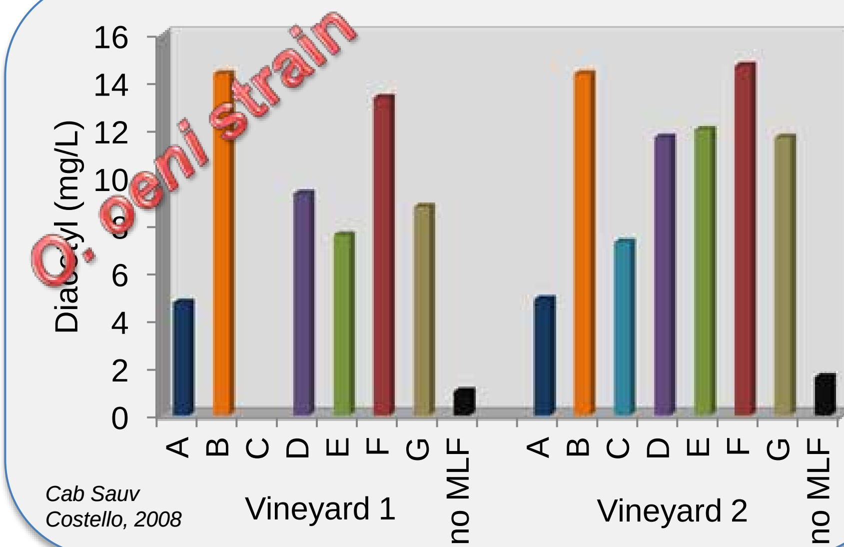
Pinot Noir
Adapted from Krieger et al. (2000)



Chemically defined wine
Hart and Bartowsky (1997)



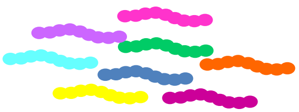
Chardonnay
Adapted from Nielsen & Richelieu (1999)

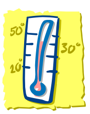


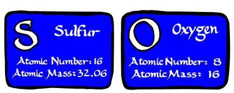
Cab Sauv
Costello, 2008

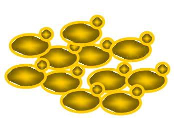
Winemaking parameters & diacetyl

The Australian Wine Research Institute

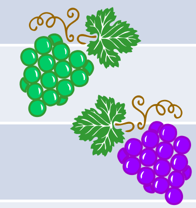


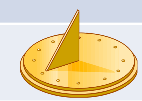


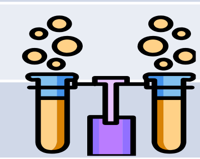




Variable		Effect on Diacetyl
O. oeni strain		Variable
Wine type	White	Lower
	Red	Higher
Inoculation rate	10^4	Higher
	10^6	Lower
Fermentation time	Longer MLF	Higher
Temperature	18˚C	Higher
	25˚C	Lower
SO_2		Binds to diacetyl
Aeration (O_2)	Air	Higher
	Anaerobic	lower
Yeast lees	Long contact	Lower
pH	Lower	May favour production



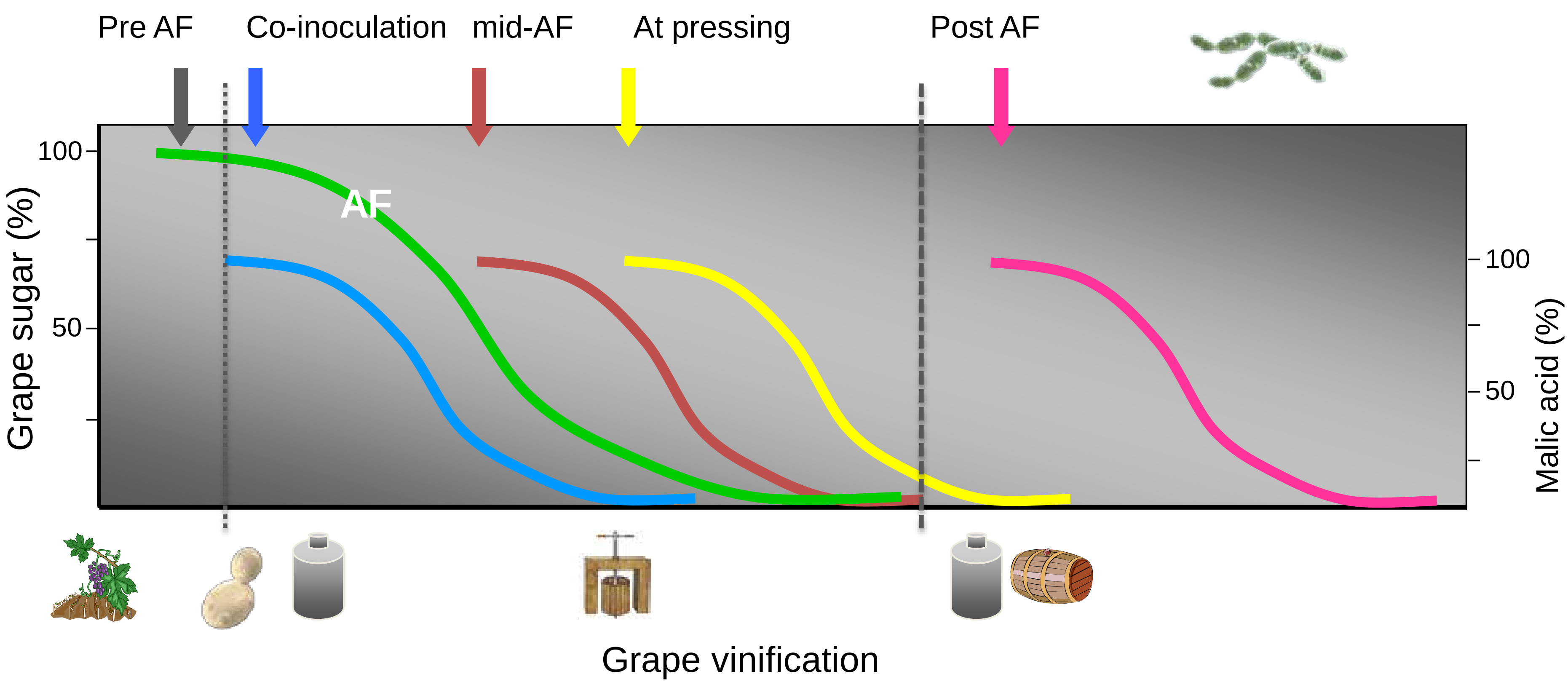




AF + MLF inoculation regime

The Australian Wine Research Institute

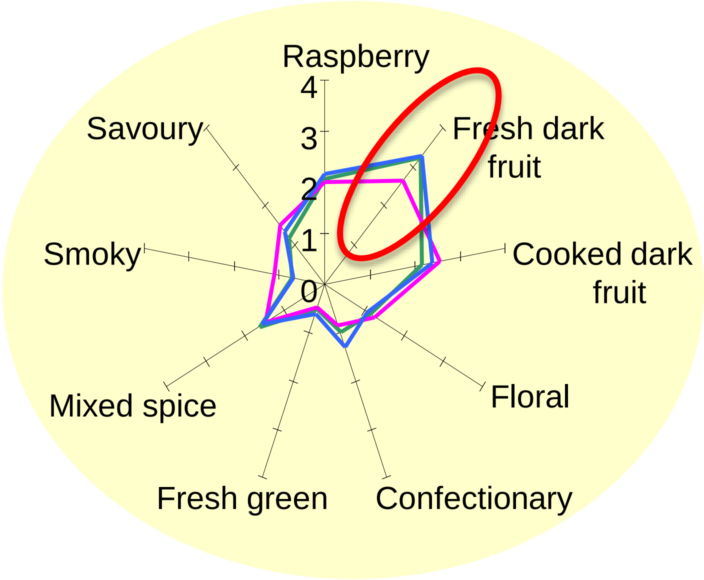
- Time point of bacterial inoculation can influence the wine composition and sensory attributes of red and white wines

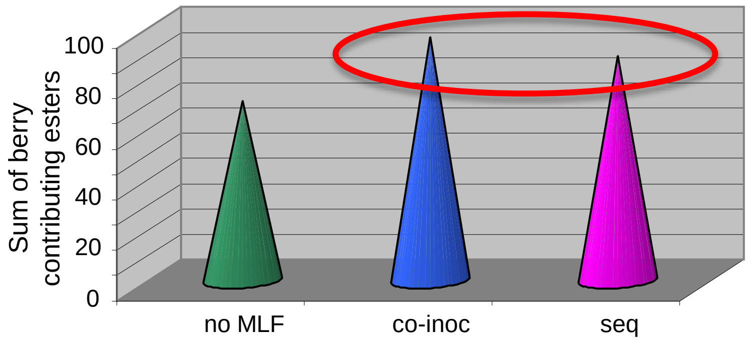


AF/MLF - fruity characters in red wine

The Australian Wine
Research Institute

- Esters associated with berry fruit attributes
  - Summation of berry fruit esters

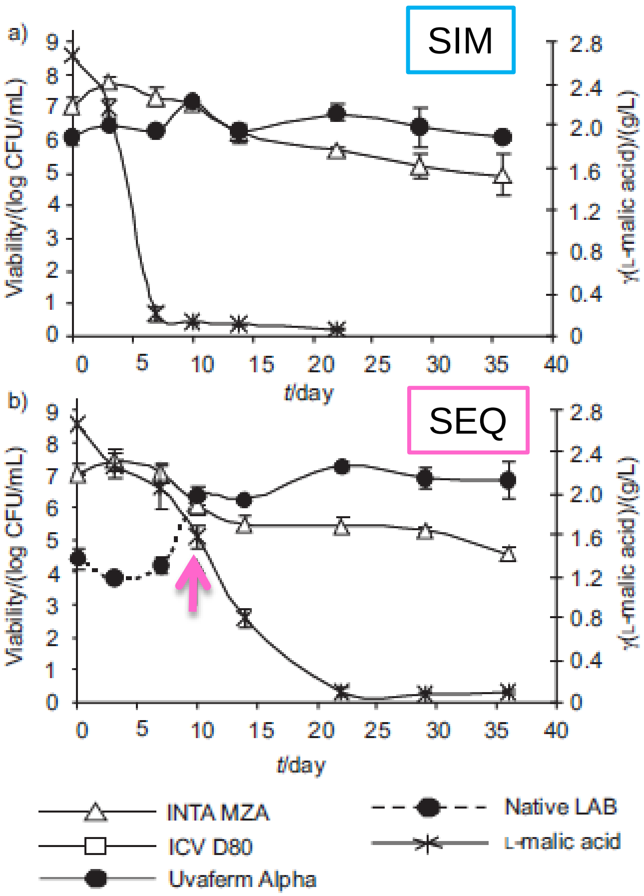




Cabernet Sauvignon
2006
Bordertown

AF/MLF - fruity characters in red wine

The Australian Wine Research Institute



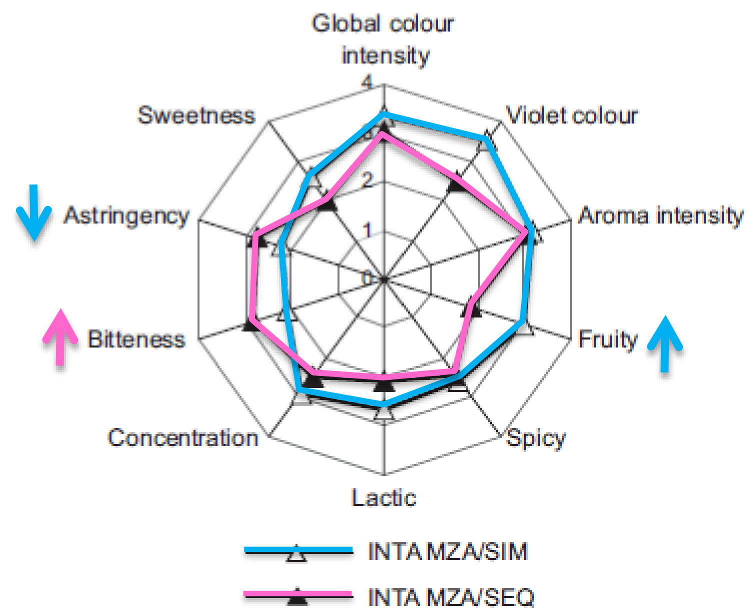


Fig. 5. Sensory descriptors of Malbec wines from must B05 fermented with *S. cerevisiae* strain (INTA MZA) and *Oenococcus oeni* strain (Uvaferm Alpha) in simultaneous (SIM) and sequential (SEQ) inoculations

Malbec (2005)
Mendoza

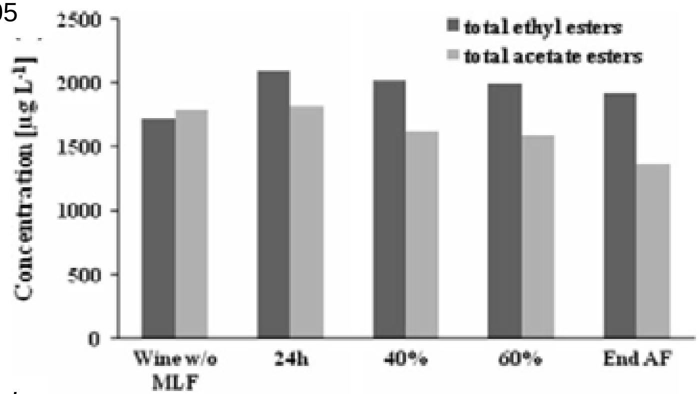
pH 3.6, TA 7.2 g/L
273.5 g/L reducing sugar
2.67 g/L L-malic acid
126 mg/L YAN

Massera et al 2009 Fd Technol Biotechnol

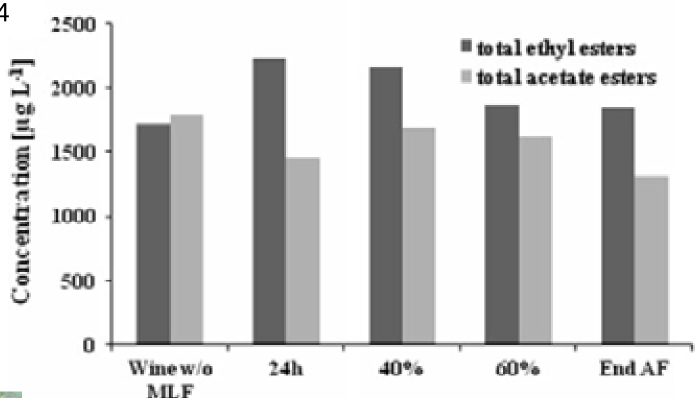
AF/MLF – white wine

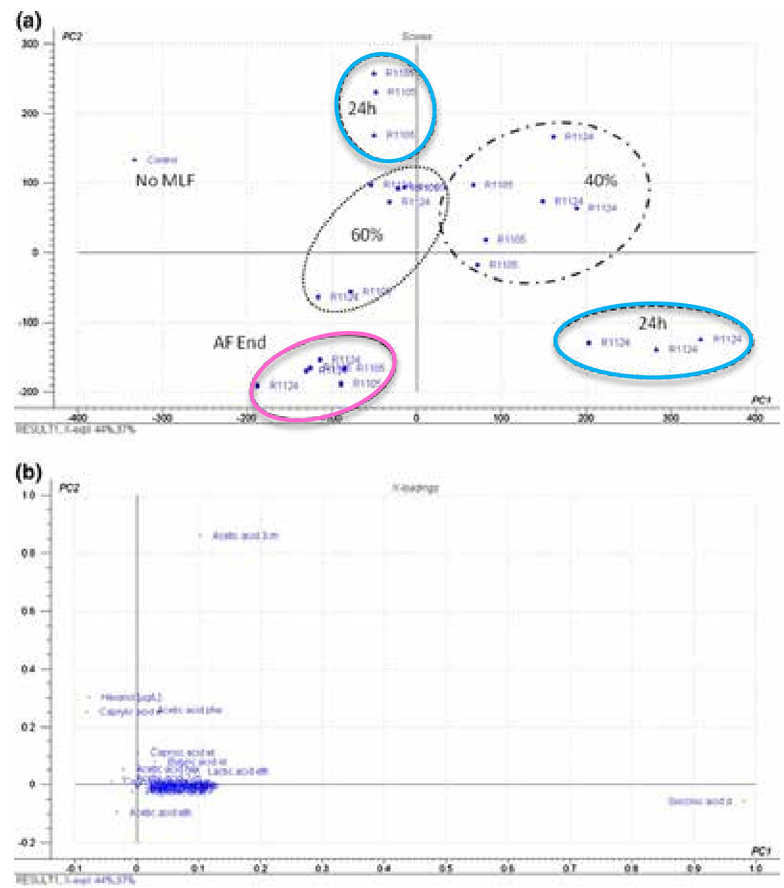
The Australian Wine Research Institute

O. oeni R1105



O. oeni R1124





Riesling (2010)
Rheingau

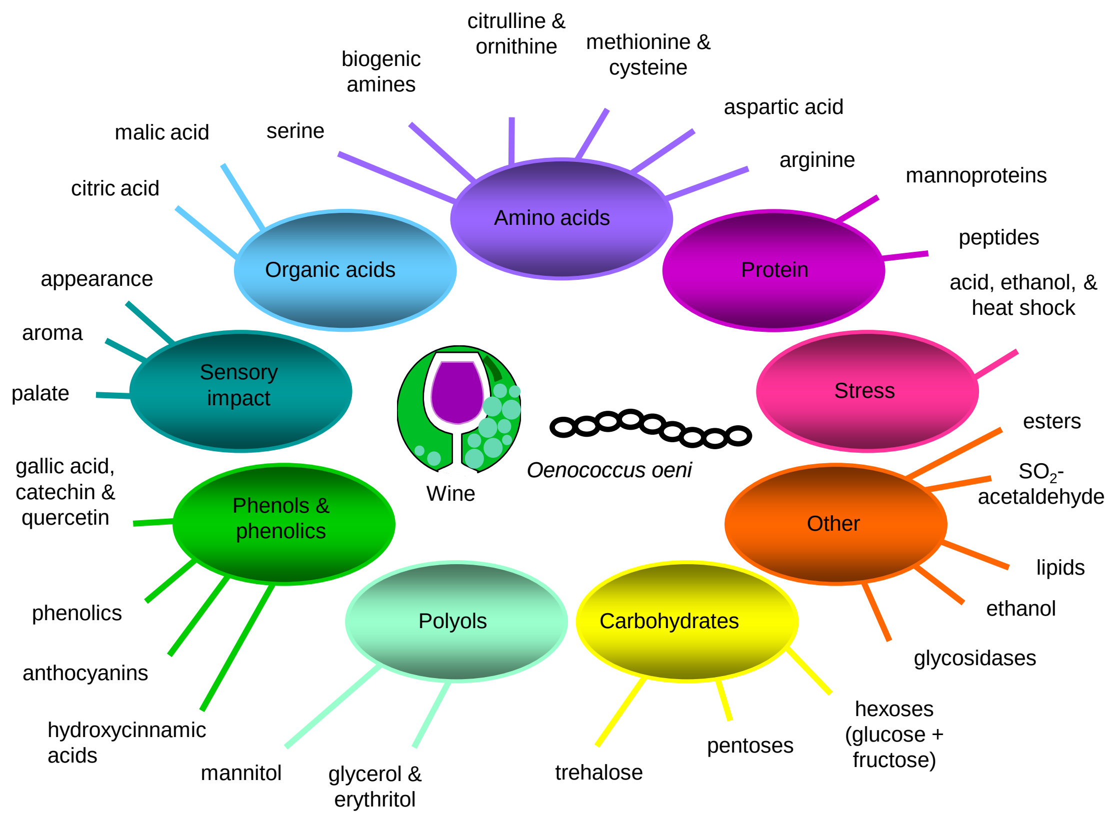
pH 3.1, TA 15 g/L
218.1 g/L reducing sugar
6.5 g/L L-malic acid

Knoll et al. 2012 Wrld J Microbiol Biotechnol

Bacterial metabolism

The Australian Wine Research Institute

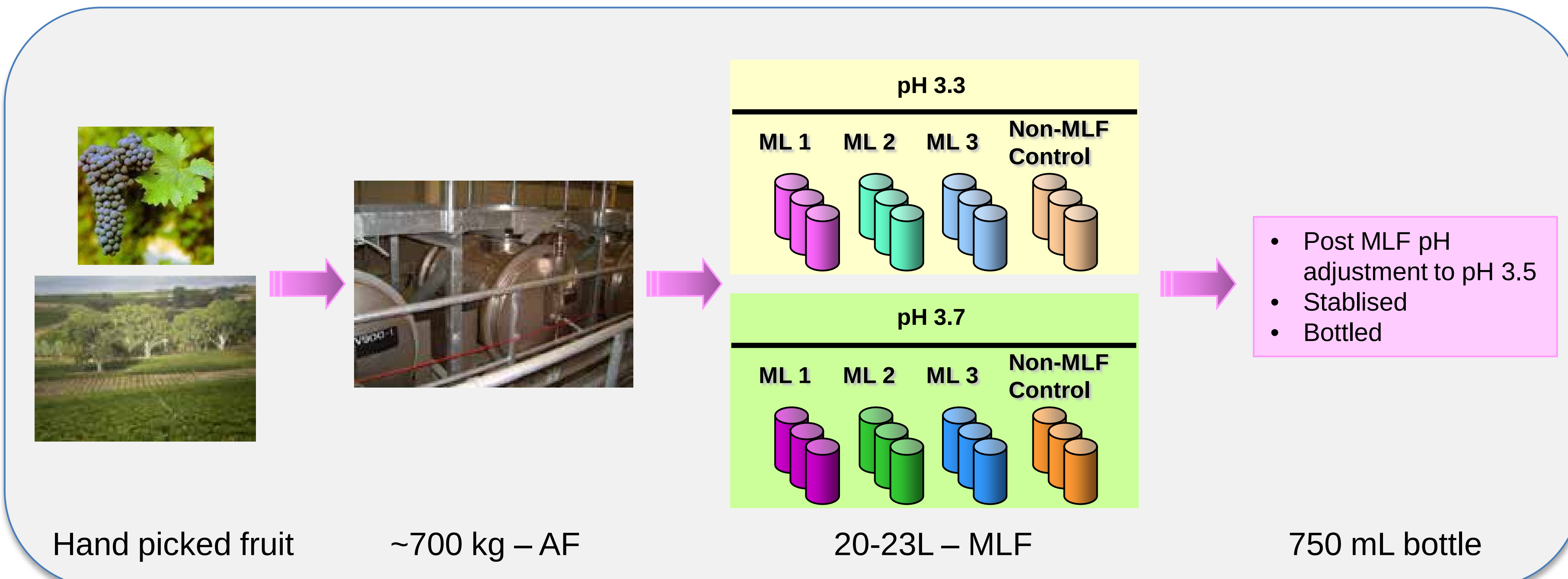
- Factors affecting bacterial metabolism during MLF
  - Wine composition
  - pH



Influence of pH

The Australian Wine
Research Institute

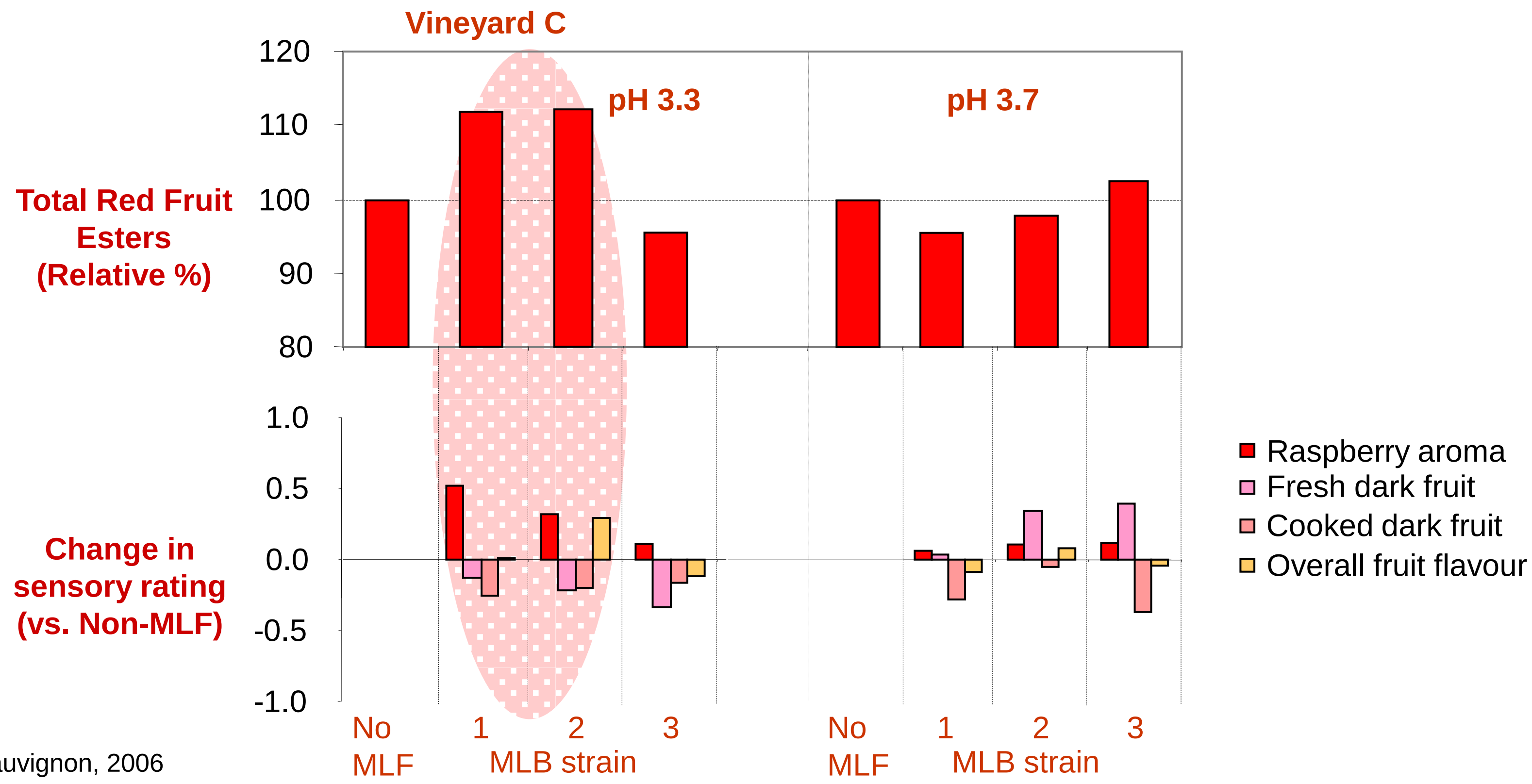
- Influence of wine pH during MLF
- Effect of MLF & ML strains on the development of berry & fruity sensory attributes



Costello et al. 2012 AJGWR

Fruity characters in red wine

The Australian Wine Research Institute

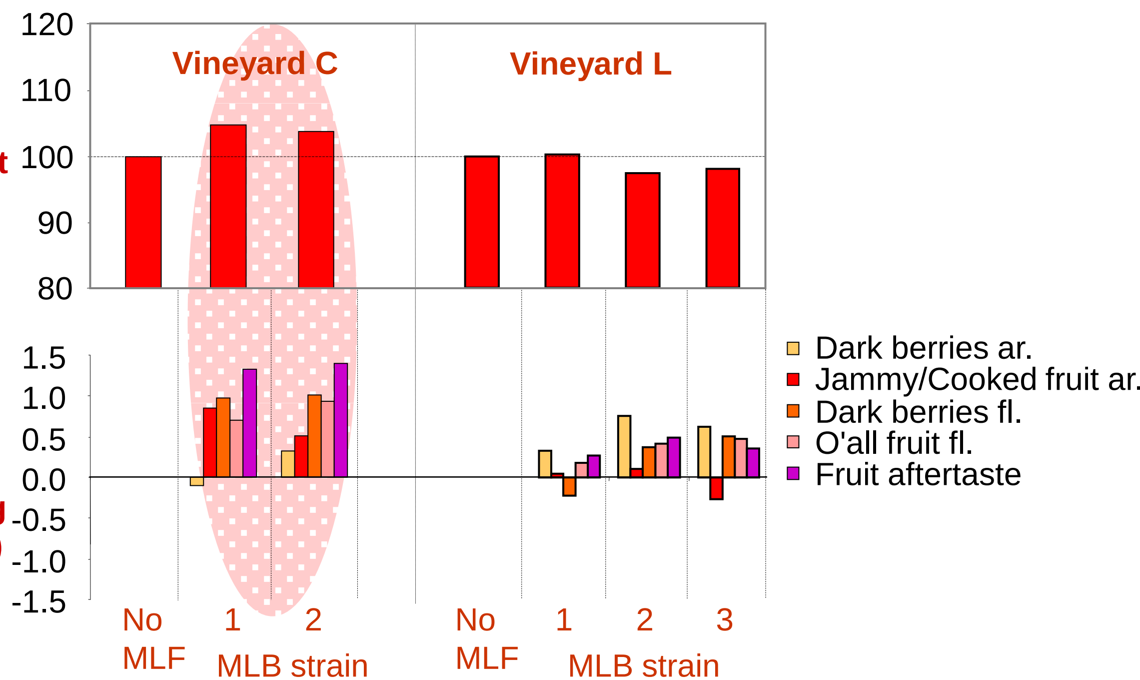


Cabernet Sauvignon, 2006

- *pH influences bacterial metabolism*
- *Increased total red fruit esters correlates with increased berry & fruity Sensory attributes*

Fruity characters in red wine

The Australian Wine Research Institute



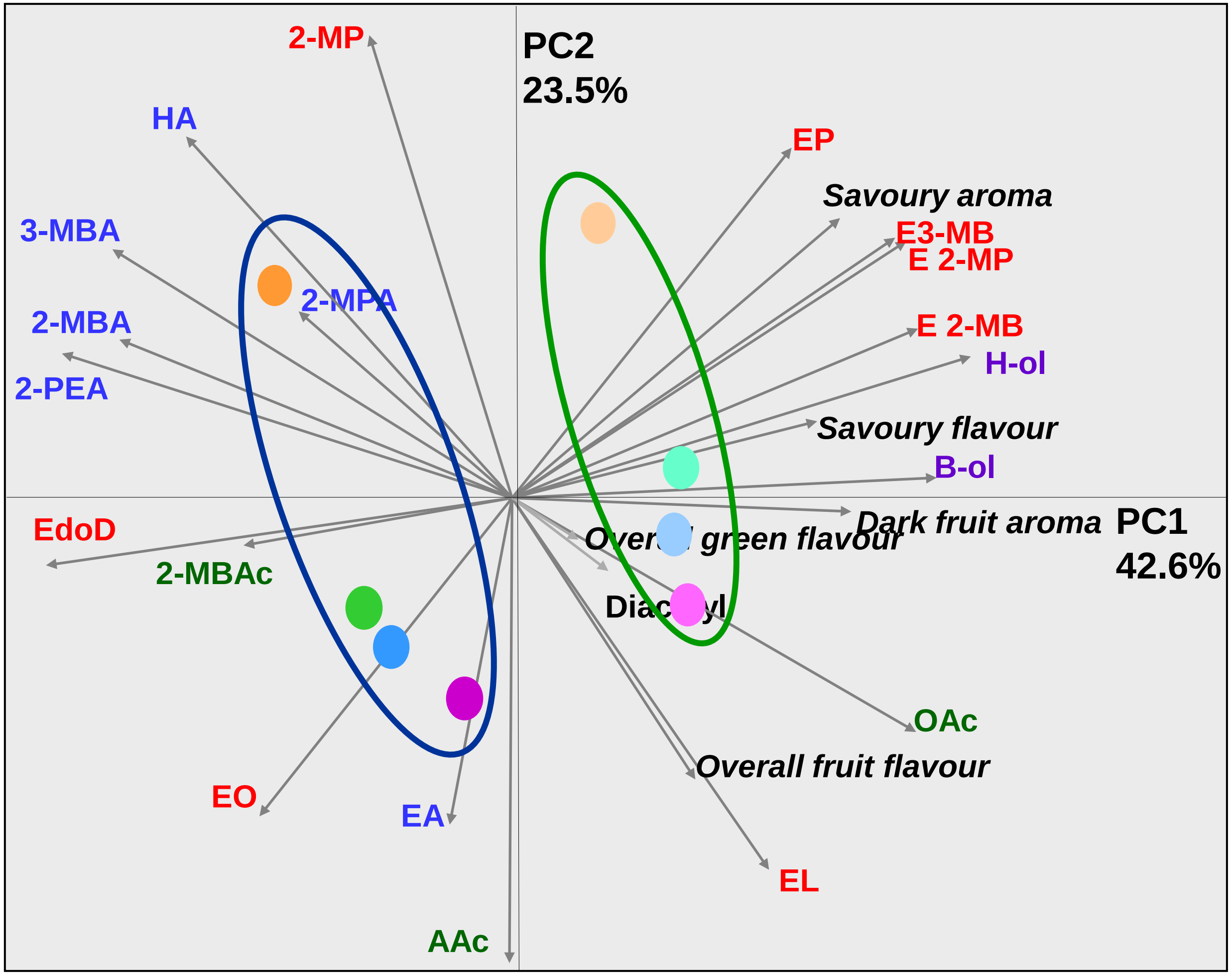
Cabernet Sauvignon, 2008

- *Consistency with ML strains & vineyard over vintages*
- *Differences between vineyards*

from Costello et al, 2012

Wine pH affects *O. oeni* metabolism

The Australian Wine Research Institute



Limestone Coast

pH 3.3

No MLF

MLF

pH 3.7

No MLF

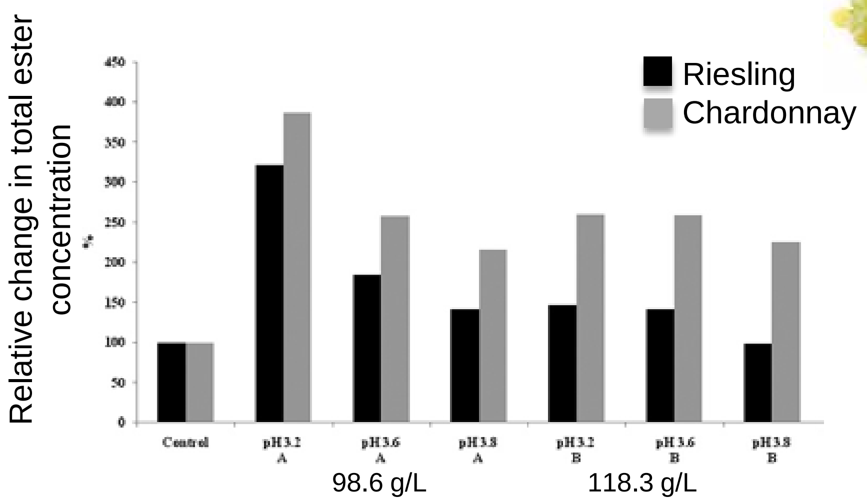
MLF

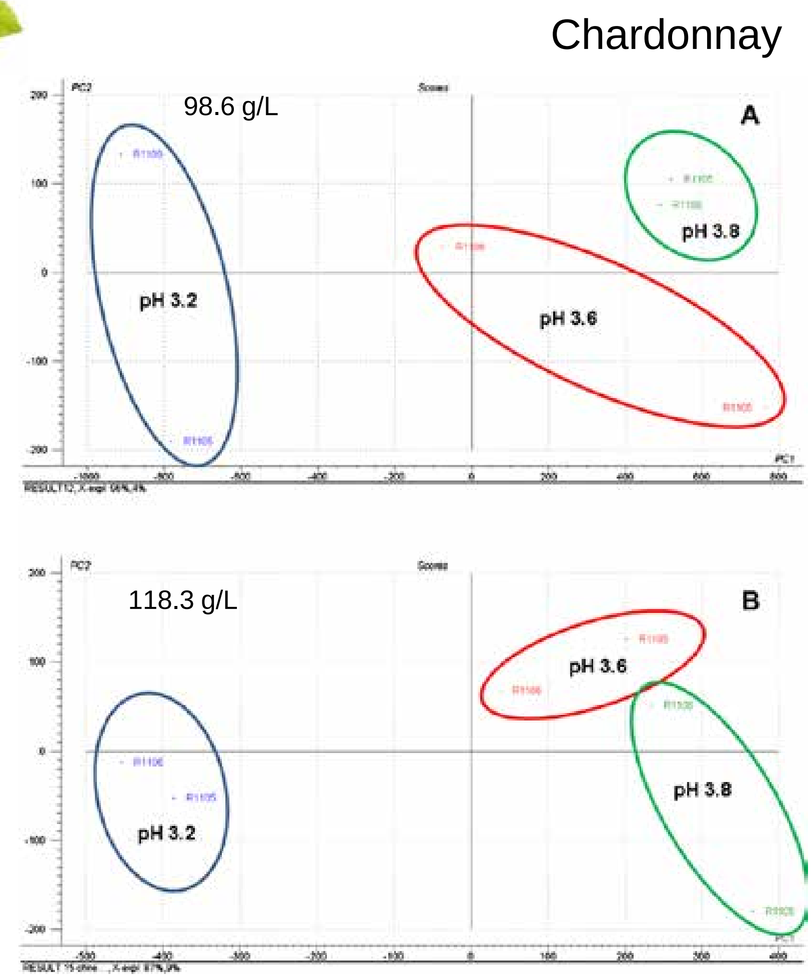
Cabernet Sauvignon,
Limestone Coast 2006

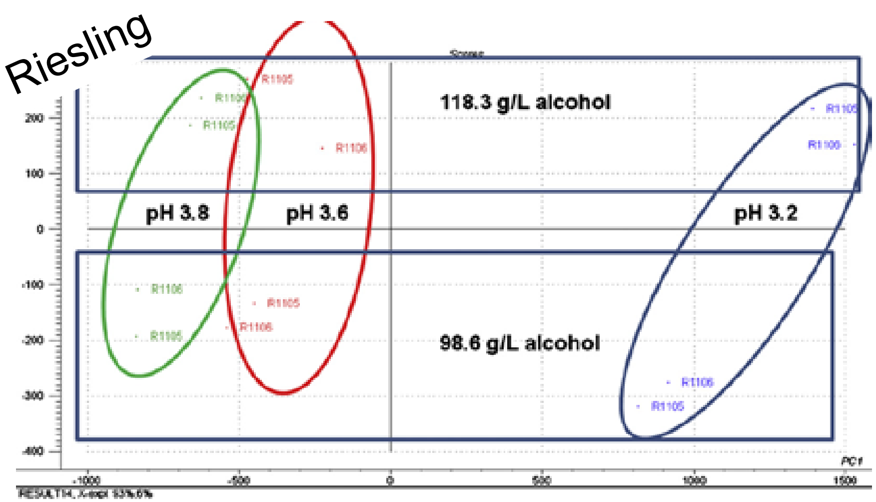
Costello et al. 2012 AJGWR

pH affects *O. oeni* metabolism

The Australian Wine Research Institute



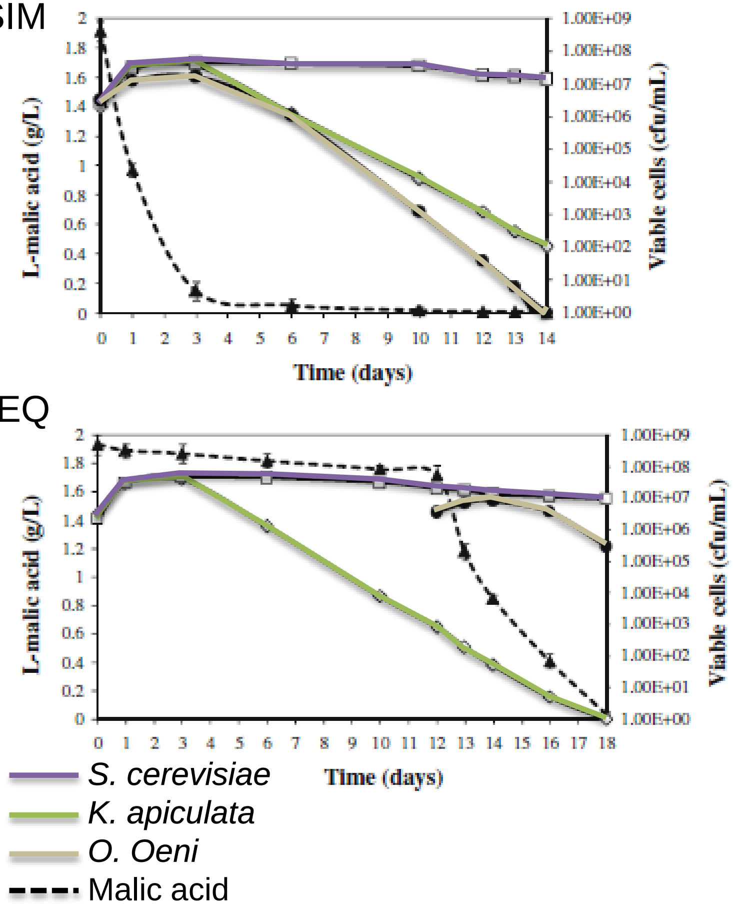




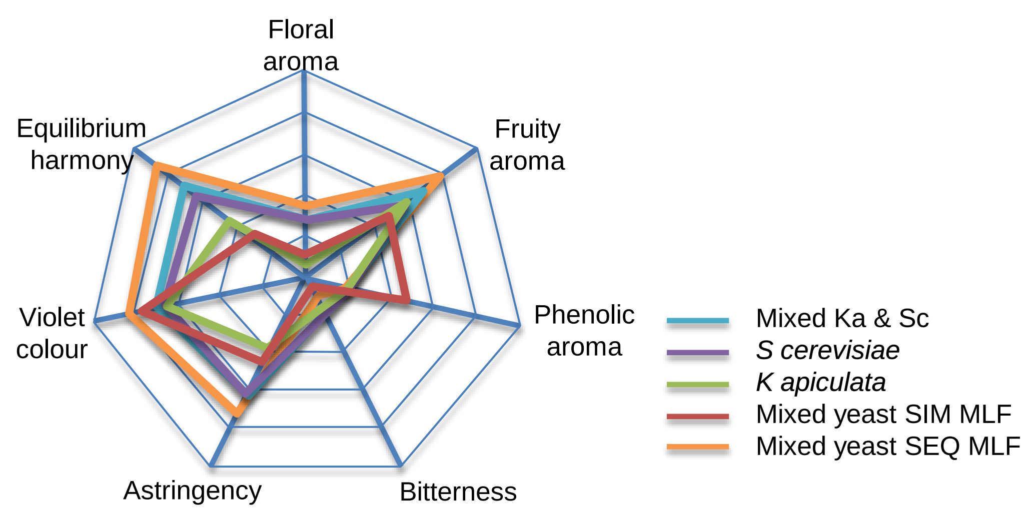
Knoll et al 2011 Fd Sci & Technol

Yeast and bacteria

The Australian Wine Research Institute



		K	S	K+S	K+S+O	K+S+O
Residual sugar	g/L	20.84	0.72	081	086	075
Ethanol	% v/v	8.82	13.91	13.56	12.97	13.38
VA	g/L	0.79	0.45	0.58	1.23	0.61
pH		3.81	3.83	3.81	3.92	4.05
TA	g/L	5.27	5.91	6.22	6.79	4.90
Malic acid	g/L	1.89	1.76	1.81	0.04	0.02
Glycerol	g/L	7.98	8.55	8.43	8.27	8.16
Acetaldehyde	mg/L	37.83	56.54	52.35	17.76	48.14
Colour intensity		1.60	1.62	1.59	1.52	1.70
Colour hue		1.24	1.19	1.22	1.20	1.15



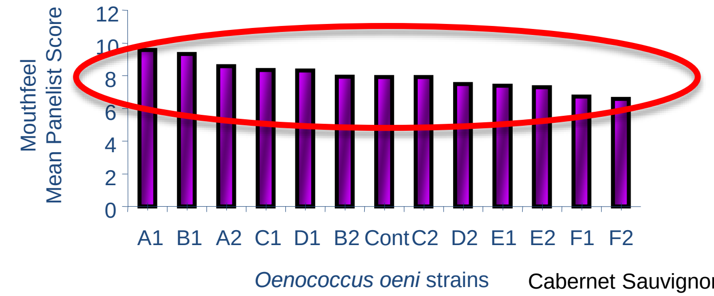
Malbec (2009)
Argentina

pH 4.0, TA 4.7 g/L
245 g/L reducing sugar
1.97 g/L L-malic acid
125 mg/L YAN

Mendoza et al 2011 J Ind Microbiol Biotechnol

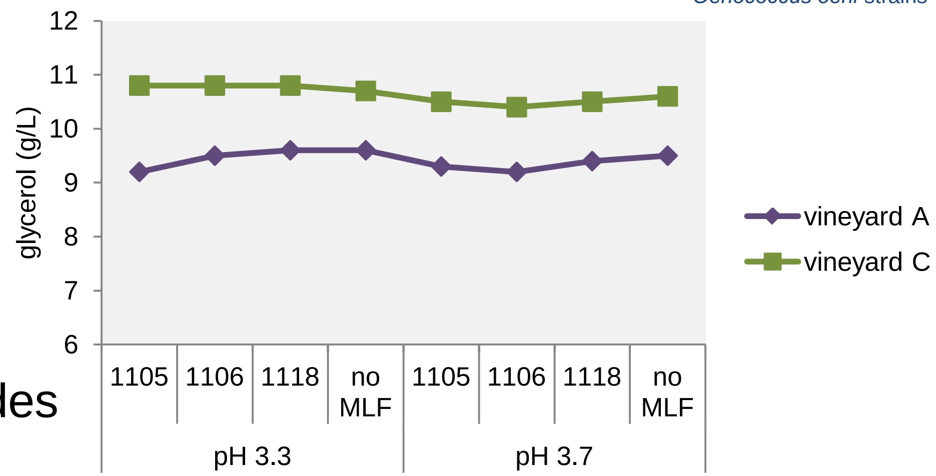
Palate and MLF

- MLF can influence the palate of wine



Cabernet Sauvignon, 1998, NY State, USA
Richardson et al., 1999

- Associated with volume and viscosity
  - Glycerol

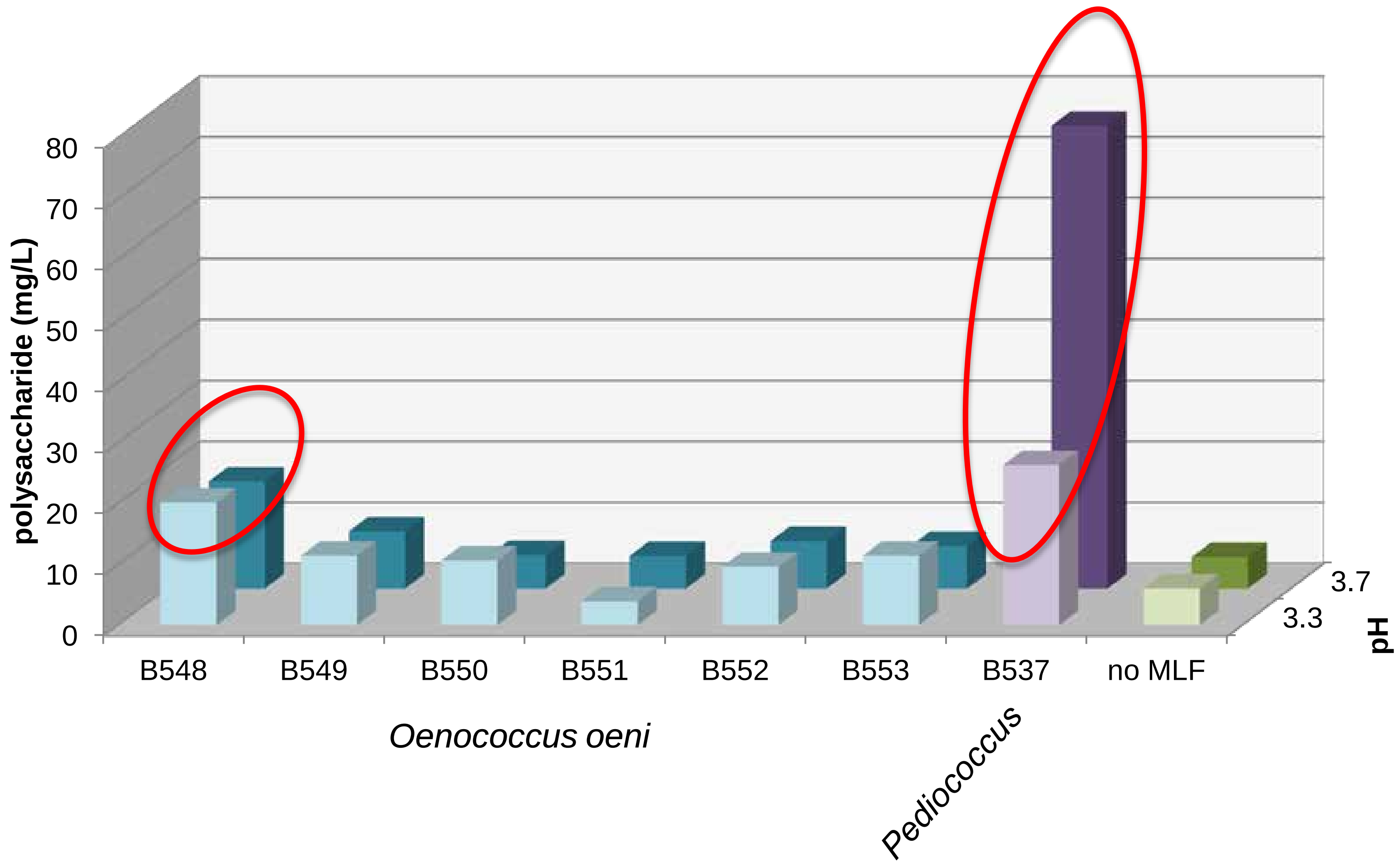


  - Exopolysaccharides

- Recent studies
  - Some strains of *O. oeni* have the genes for exopolysaccharide production

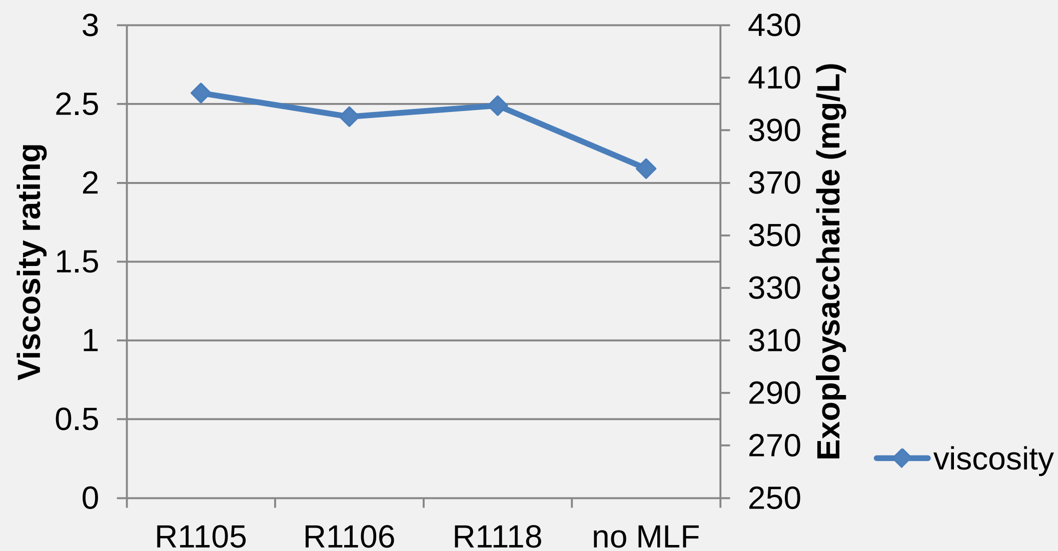
Can *O. oeni* produce exopolysaccharides?

The Australian Wine Research Institute



Sensory & [exopolysaccharide]

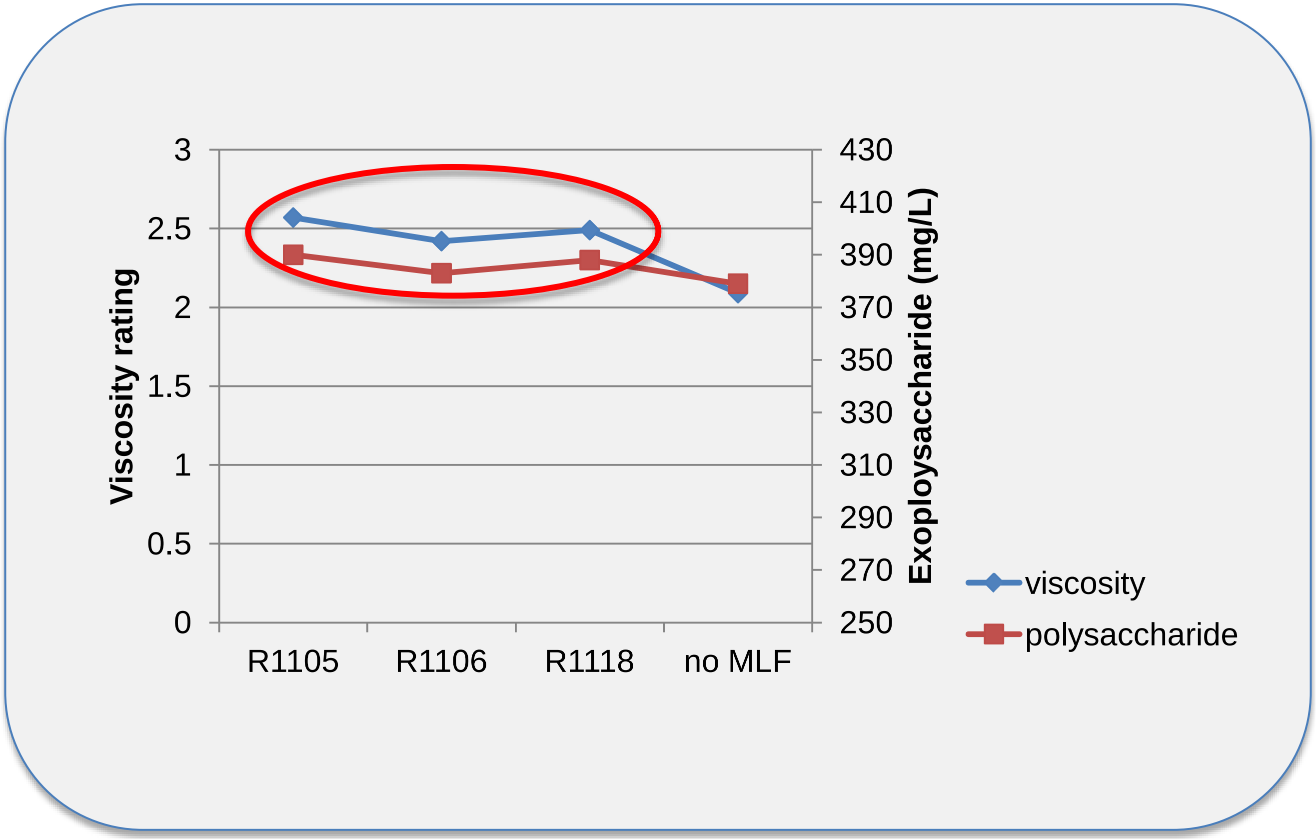
The Australian Wine
Research Institute



Cabernet Sauvignon
Vineyard A
Costello, 2006

Sensory & [exopolysaccharide]

The Australian Wine Research Institute



Cabernet Sauvignon
Vineyard A
Costello, 2006

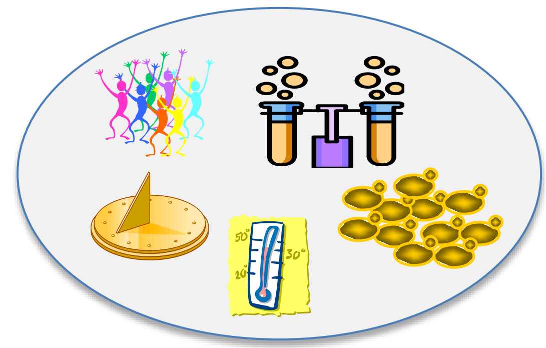
- O. oeni *strains can produce exopolysaccharides*
- *Correlates with viscosity of wine*

MLF: wine aroma & flavour – What can we do?

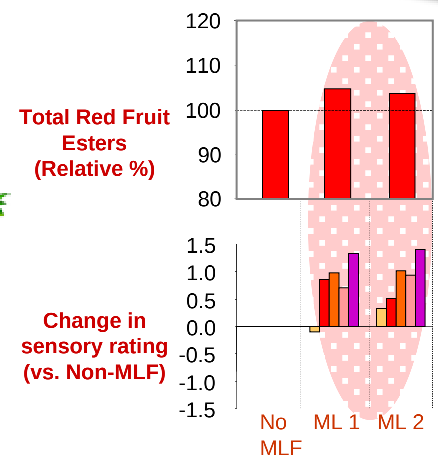
The Australian Wine
Research Institute

- pH can influence bacterial metabolism
  - affects wine aroma and flavour

- Diacetyl – buttery attribute
  - Can be managed through winemaking practices



- Berry & fruity characters in red wine
  - Strain
  - Lower pH



- Mouthfeel
  - Exopolysaccharide production

MLF can be used as a means to influence wine style.

Acknowledgments

The Australian Wine Research Institute

- AWRI Wine Biosciences

  MLF Team
  - Caroline Abrahamse
  - Peter Costello
  - Jane McCarthy

- Wineries: kind donation of grapes & wine for research

- Lallemand: support for berry-fruit research

- This project is supported by Australia's Grapegrowers and winemakers through their investment agency the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government

gwrdc GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Information and online tools available on the AWRI website

www.awri.com.au

The Australian Wine Research Institute

Supporting Australian grape and wine producers

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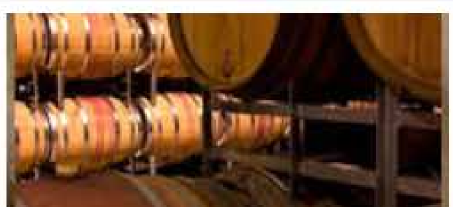

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New resources navigation

The Australian Wine Research Institute

Resources for vineyards

Information on agrochemicals and related analytical services, advice and support, fact sheets and more.

Resources for wineries

Includes permitted additives, winemaking calculators, laboratory setup and method, Frequently Asked Questions, and products and suppliers.

Resources for wine exporters

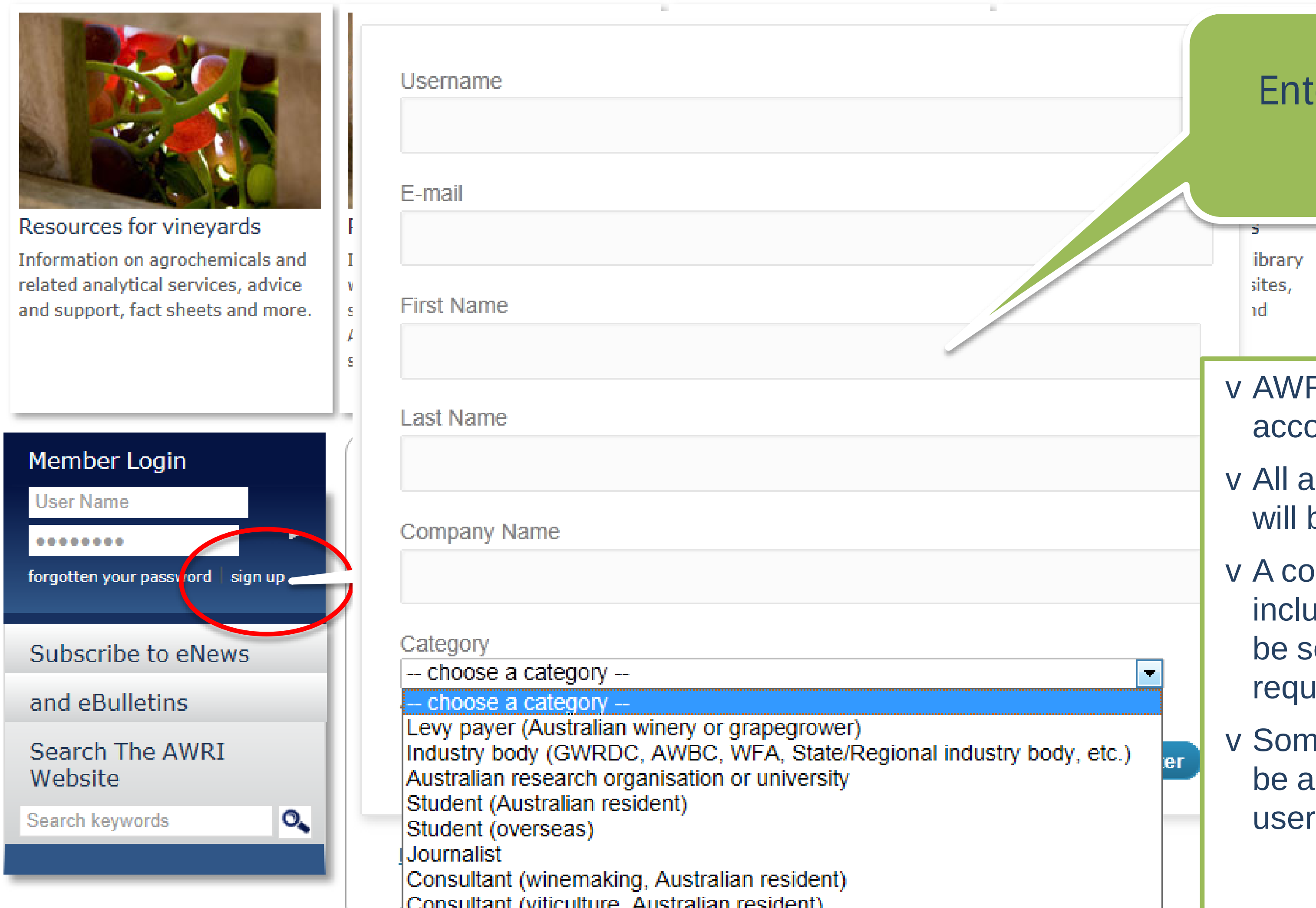
Information for exporters such as factsheets and publications, analytical services and more.

Resources for consumers

Factsheets and publications, library resources, links to other websites, research projects and wine and health

Sign up for new website account

The Australian Wine Research Institute



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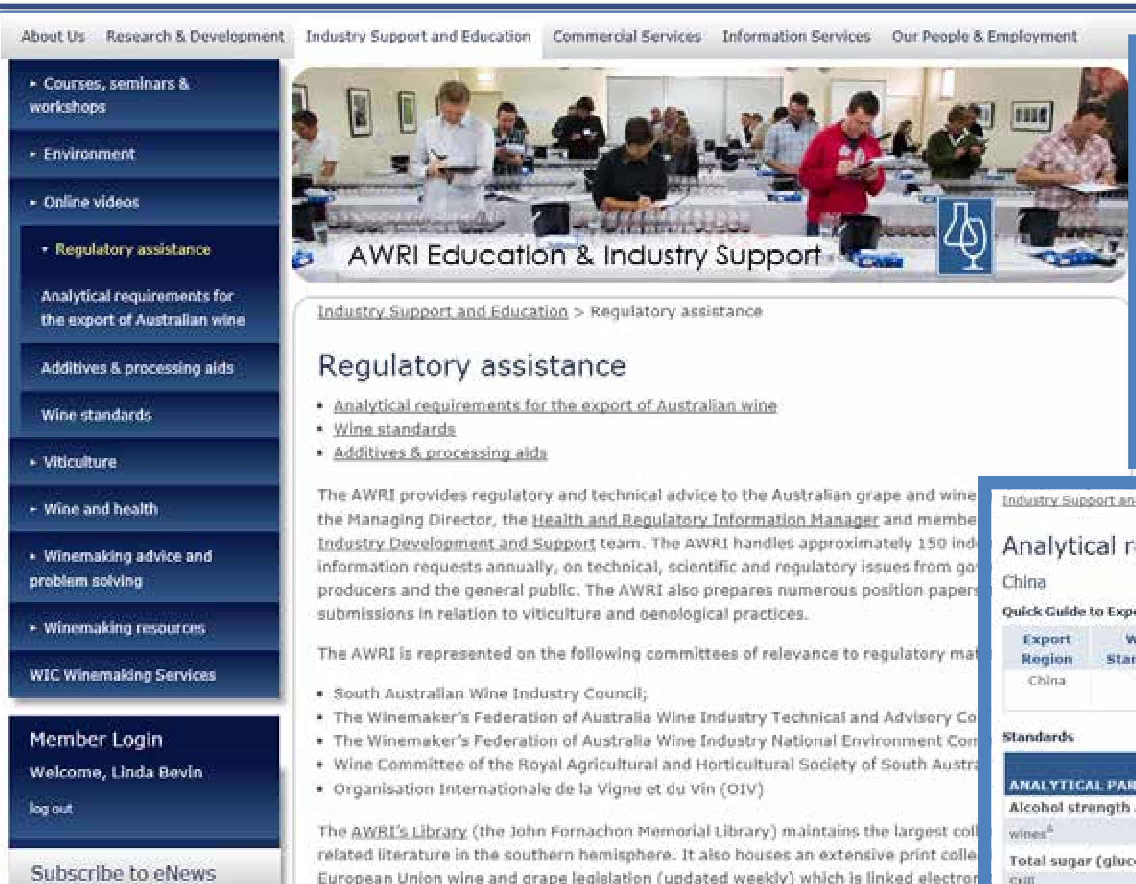
The Australian Wine Research Institute



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- Courses, seminars & workshops
- Environment
- Online videos
- Regulatory assistance
  - Analytical requirements for the export of Australian wine
  - Additives & processing aids
  - Wine standards
- Viticulture
- Wine and health
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AWRI Education & Industry Support

Industry Support and Education > Regulatory assistance

Regulatory assistance

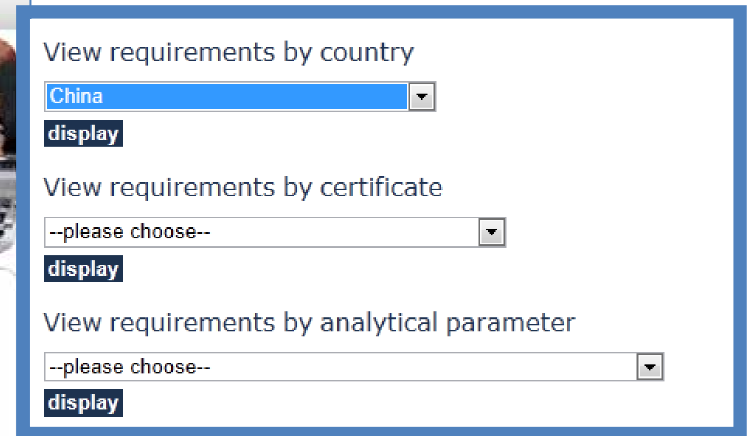
- Analytical requirements for the export of Australian wine
- Wine standards
- Additives & processing aids

The AWRI provides regulatory and technical advice to the Australian grape and wine the Managing Director, the Health and Regulatory Information Manager and membe Industry Development and Support team. The AWRI handles approximately 150 ind information requests annually, on technical, scientific and regulatory issues from go producers and the general public. The AWRI also prepares numerous position papers submissions in relation to viticulture and oenological practices.

The AWRI is represented on the following committees of relevance to regulatory mat

- South Australian Wine Industry Council;
- The Winemaker's Federation of Australia Wine Industry Technical and Advisory Co
- The Winemaker's Federation of Australia Wine Industry National Environment Com
- Wine Committee of the Royal Agricultural and Horticultural Society of South Austr
- Organisation Internationale de la Vigne et du Vin (OIV)

The AWRI's Library (the John Fornachon Memorial Library) maintains the largest coll related literature in the southern hemisphere. It also houses an extensive print colle European Union wine and grape legislation (updated weekly) which is linked electron



View requirements by country

China

display

View requirements by certificate

--please choose--

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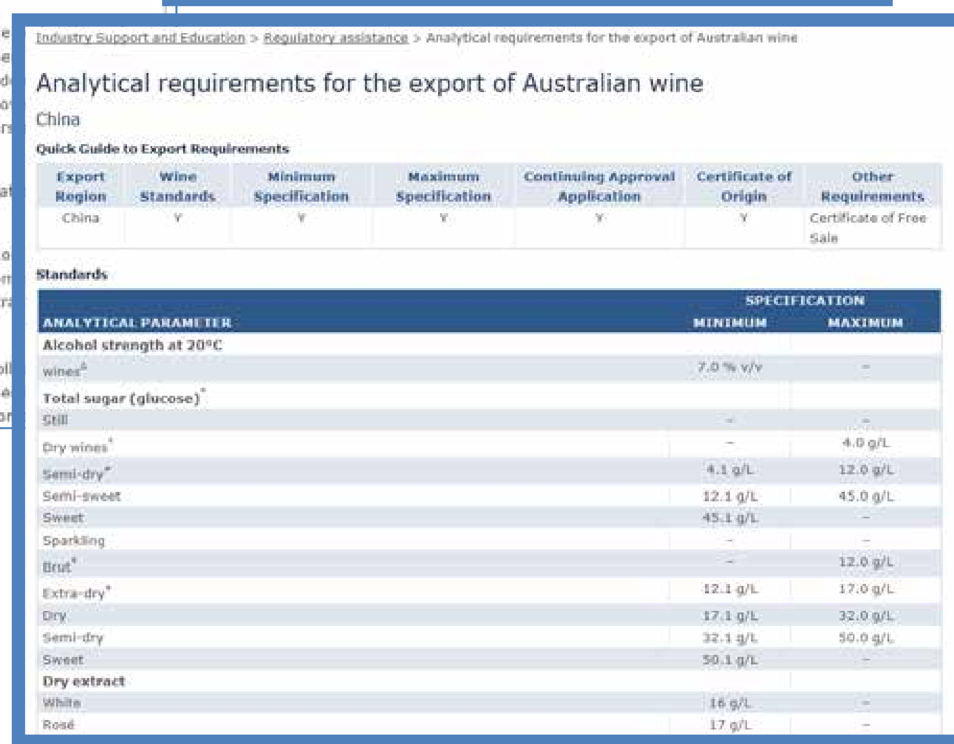
View requirements by analytical parameter

--please choose--

display



Industry Support and Education > Regulatory assistance > Analytical requirements for the export of Australian wine

Analytical requirements for the export of Australian wine

China

Quick Guide to Export Requirements

Export Region	Wine Standards	Minimum Specification	Maximum Specification	Continuing Approval Application	Certificate of Origin	Other Requirements
China	Y	Y	Y	Y	Y	Certificate of Free Sale

Standards

ANALYTICAL PARAMETER	SPECIFICATION MINIMUM	SPECIFICATION MAXIMUM
Alcohol strength at 20°C		
wines[A]	7.0 % v/v	–
Total sugar (glucose)[*]		
Still	–	–
Dry wines[*]	–	4.0 g/L
Semi-dry[*]	4.1 g/L	12.0 g/L
Semi-sweet	12.1 g/L	45.0 g/L
Sweet	45.1 g/L	–
Sparkling	–	–
Brut[*]	–	12.0 g/L
Extra-dry[*]	12.1 g/L	17.0 g/L
Dry	17.1 g/L	32.0 g/L
Semi-dry	32.1 g/L	50.0 g/L
Sweet	50.1 g/L	–
Dry extract		
White	16 g/L	–
Rosé	17 g/L	–

Searchable databases on permitted additives and processing aids, and export analytical requirements

Winemaking calculators

The Australian Wine Research Institute

- Acid addition
- Ascorbic acid addition
- Bentonite addition
- Carbon addition
- Copper sulfate addition
- Crème of Tartar addition
- Deacidification
- Diammonium phosphate additions
- Ferro Cyanide trial
- Fining trial
- Fortification
- Gelatine addition
- General conversion calculators
- Grape juice concentrate (GJC) addition using Pearson Square
- Hydrogen peroxide addition
- Interconversion of acidity units
    - Acetic acid
    - Citric acid
    - Lactic acid
    - Malic acid
    - Sulfuric acid
    - Tartaric acid
    - Tartaric acid (meq/L)
- Isinglass addition
- Laboratory stock solution
- Methanol expressed as proportion of ethanol calculator
- Micro-ox addition
- Molecular sulfur dioxide addition
- Number of standard drinks
- Paired preference
- PMS addition
- PVPP addition
- Same/Different
- Sensory difference test
    - Duo-trio
    - Paired comparison
    - Triangle
- Sorbic acid addition
- Sulfur dioxide addition
- Tannin addition
- Winery stock solution

Number of standard drinks

Suggestions / questions / comments? email the calculator services staff

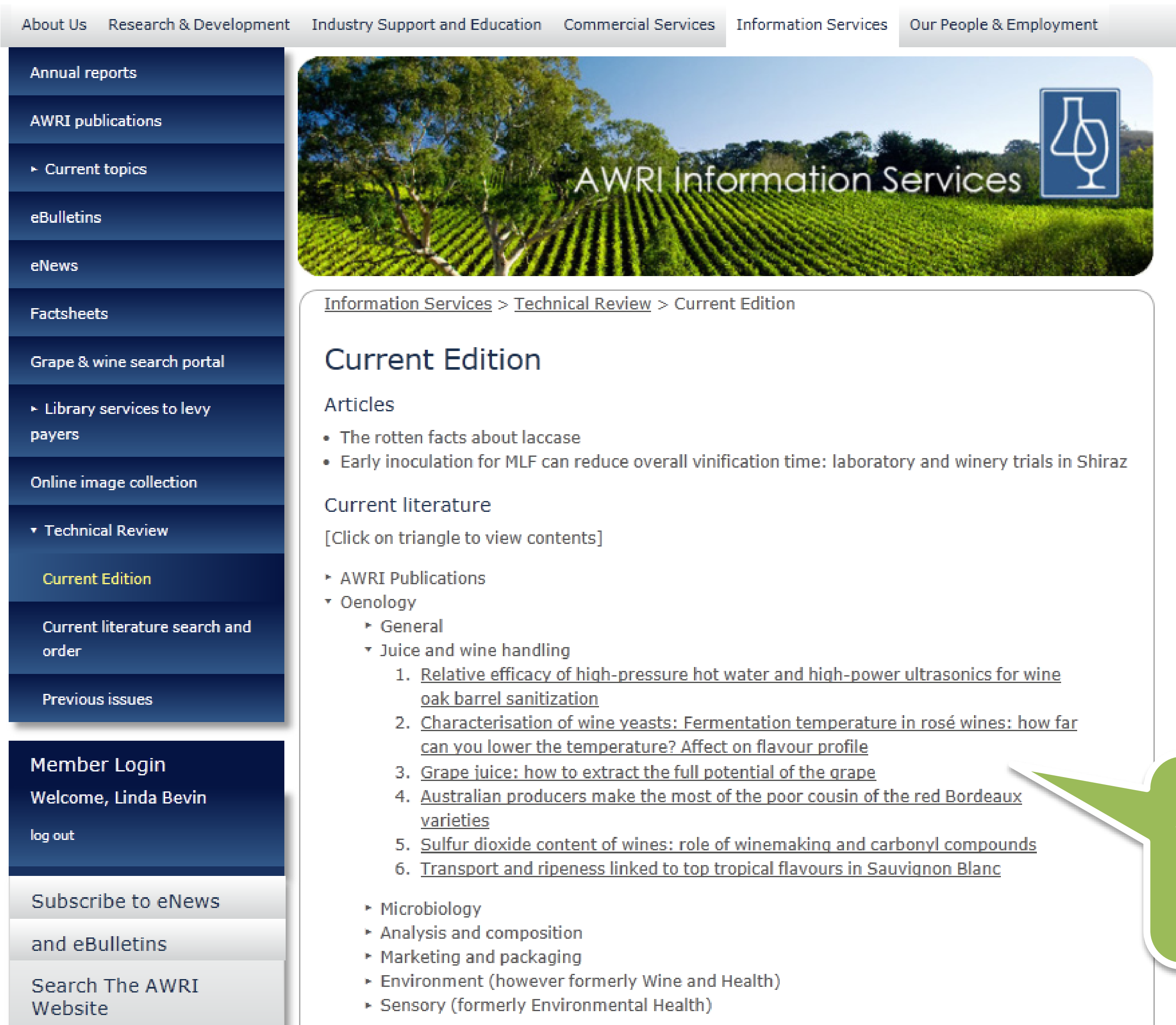
Approximate standard drinks

Container volume	750	mL
Alcohol content	14.5	% v/v
Calculate number of standard drinks	8.6	standard drinks

Clear

Information Services

The Australian Wine Research Institute



- eBulletins
- eNews
- Online image collection
- Grape and wine search portal
- Factsheets
- AWRI publications
- *Technical Review*

Click on article name to view more information and to order a copy of the article

Library Services

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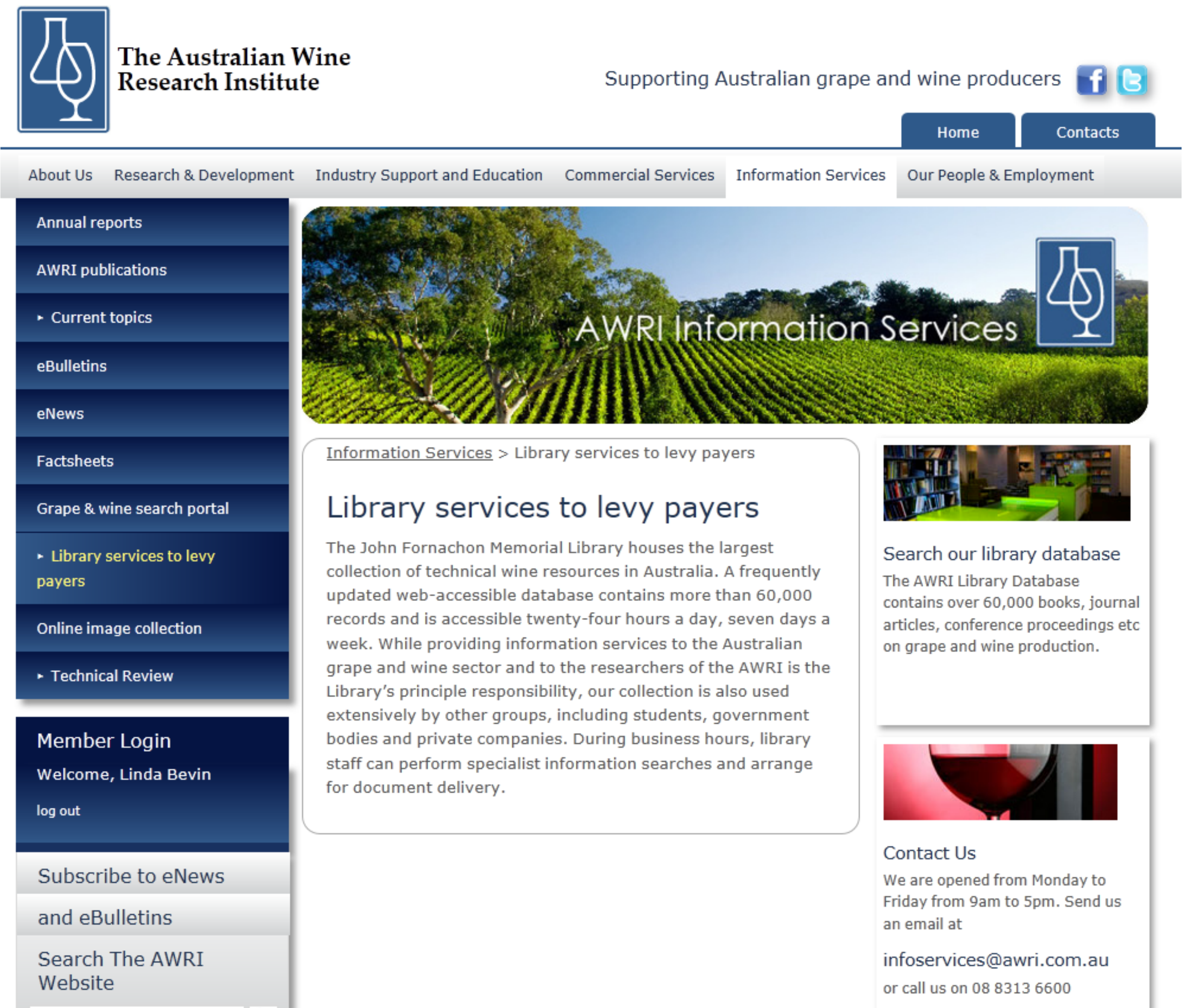


- Free library service to Australian grape and wine producers
- Over 65,000 books, journal articles, conference proceedings etc
- Online library database
- *Fast response time (1 to 3 days)*

Mobile website

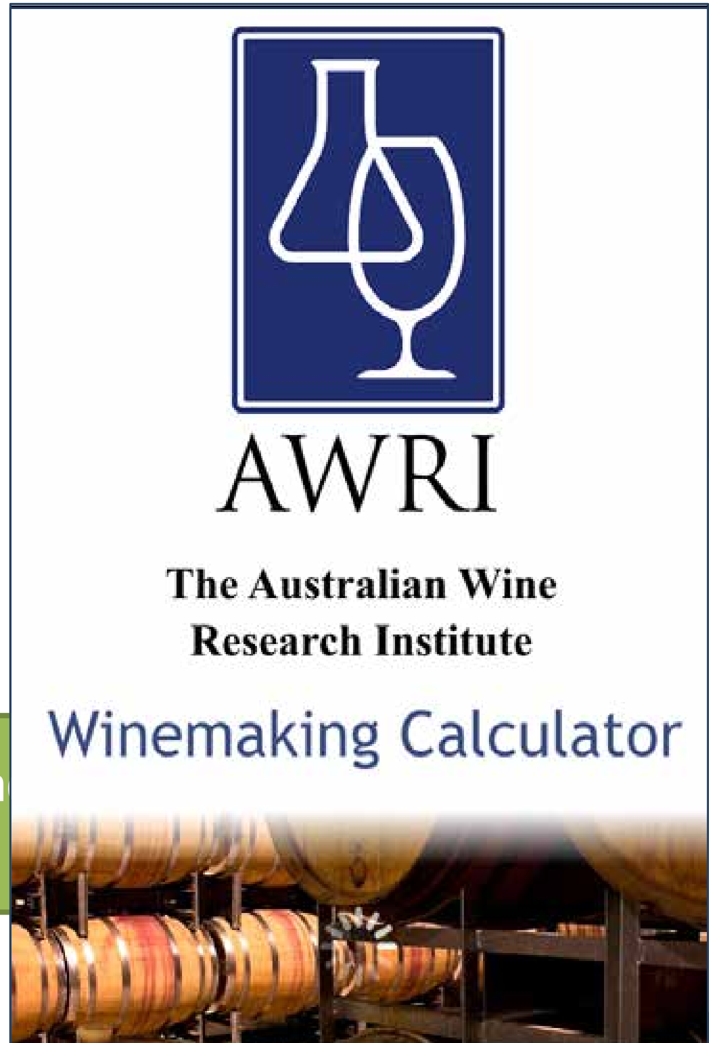
The Australian Wine Research Institute

Mobile-friendly website launched in October 2012.

Provides content to key projects and services offered by the AWRI.

Linked to full website for detailed information.

Winemaking calculator app



Calculators grouped for easy access

Enter the search an
select ‘Calculate’

http://www.awri.com.au/industry_support/winemaking_resources/winemaking-calculators-app/

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The Australian Wine Research Institute

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2013 webinar program

Presentation	Description	Presenter	Date	Register
Optimising your laboratory for the best results	Laboratories are a critical, and often expensive, part of modern wine production. This webinar will highlight a number of areas that are important to not only ensure results are accurate, but to achieve them in an efficient and cost effective manner. Some of the topics that will be discussed include basic lab quality systems; LIMS; lab design; lean systems and troubleshooting common laboratory issues.	Eric Wilkes (The AWRI)	23/07/2013	Register
Strategies for reducing alcohol levels in wine	The AWRI has taken a holistic approach to the development of strategies for the reduction of alcohol concentration in wine. Several viticultural and fermentation practices show considerable promise for the production of good quality reduced-alcohol wines. This session will present our latest findings and point to the need to evaluate a combinatorial approach to reducing alcohol concentration in wine.	Cristian Varela (The AWRI)	30/07/2013	Register
The latest on CMCs	Carboxymethylcellulose is becoming an important part of the winemaker's tool box for white wine tartrate stabilisation. However, like all wine additives, there is more to the successful use of CMCs than sales brochures might suggest. This webinar will look at how CMC works; when it is appropriate to use; what precautions you need to take and the best ways to test the wine when using it.	Eric Wilkes (The AWRI)	6/08/2013	Register
Till death do us part: Cell death in the grape berry as a quality measure	TBA	Steve Tyerman (The University of Adelaide)	20/08/2013	Register
Climate influence and trends for the wine industry	TBA	Darren Ray (Bureau of Meteorology)	27/08/2013	Register

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Date	Event
14 May 2013	AWRI Hunter Valley Seminar *Mercure Resort Hunter Valley, Pokolbin NSW*
14 May 2013	New Technologies in Grapegrowing and Winemaking *Treasury Wine Estates vineyards, Padthaway SA*
15 May 2013	AWRI Barossa Adapting to difficult vintages workshop *Vine Inn, Nuriootpa SA*
21 May 2013	AWRI Clare Adapting to difficult vintages workshop *The Artisan Table, Clare SA*
21 May 2013	AWRI Langhorne Creek and Adelaide Hills Seminar *Langhorne Creek Football Clubrooms, Langhorne Creek SA*
22 May 2013	Regional Smoke Taint Update *Gum San Chinese Heritage Centre, Ararat VIC*
23 May 2013	GWRDC #INseries workshop - China Insights: McLaren Vale
23 May 2013	Regional Smoke Taint Update *Yarra Glen Memorial Hall, Yarra Glen VIC*
24 May 2013	GWRDC #INseries workshop - China Insights: Barossa
24 May 2013	Regional Smoke Taint Update *Oxley Shire Hall, Oxley VIC*
27 May 2013	GWRDC #INseries workshop - China Insights: Hunter Valley
28 May 2013	GWRDC #INseries workshop - China Insights: Yarra Valley
30 May 2013	GWRDC #INseries workshop - China Insights: Margaret River
30 May 2013	Margaret River Wine in Sydney *The Barnet Long Room, Customs House, Circular Quay NSW*

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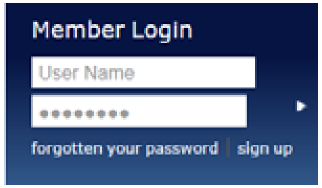

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