

GRAPE & WINE ROADSHOW

Bendigo Seminar

Wednesday 14th August, 2013



Pepper and Spice in Shiraz: what influences rotundone levels in wines?
Leigh Francis

Morning Tea

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Does soil and vine nutrient status affect wine quality?

Mark Krstic



Using MLF to accentuate wine aroma and flavour

Eveline Bartowsky



Carboxymethylcellulose and important tool for white wine tartrate stabilization

Eric Wilkes

Lunch

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Wednesday 14th August, 2013



VESDA – The new risk assessment tool for smoke taint

Ricky James, DEPI Victoria

Afternoon Tea

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Bendigo Seminar

Wednesday 14th August, 2013



Using the timing of MLF inoculation to optimise your winemaking

Eveline Bartowsky



Causes and management of slow and stuck fermentations

Paul Henschke



Features of the AWRI website and closing comments

Mark Krstic

Pepper and spice in Shiraz: what influences rotundone levels in wines?

Leigh Francis

Tracey Siebert

Mark Solomon

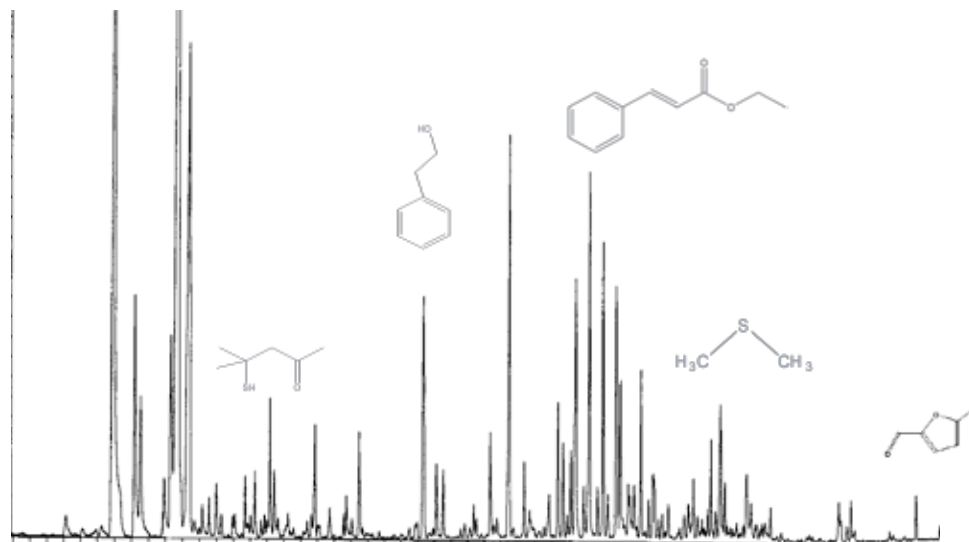
Gerard Logan (University of Auckland)



Gas chromatography-mass spectrometry



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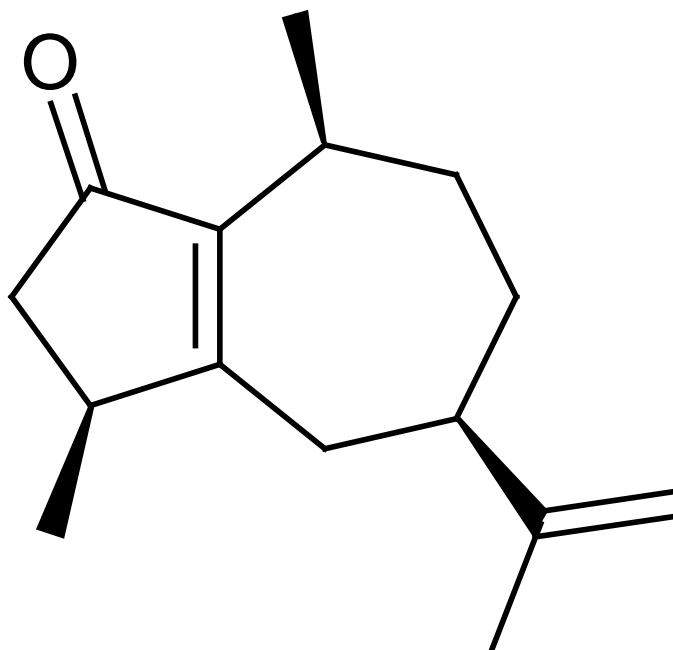




(-)-Rotundone



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- Identity confirmed with reference compound (Symrise)
Cyperus rotundus, nut grass weed
- ^1H and ^{13}C NMR, ORD
- GC-MS-O. co-injections
- Qualitative DA



By GC-MS-O, rotundone was established as the principal aroma impact compound for pepper aroma in grapes and wine.

How potent is rotundone?



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aroma detection threshold



8 ng/L in water



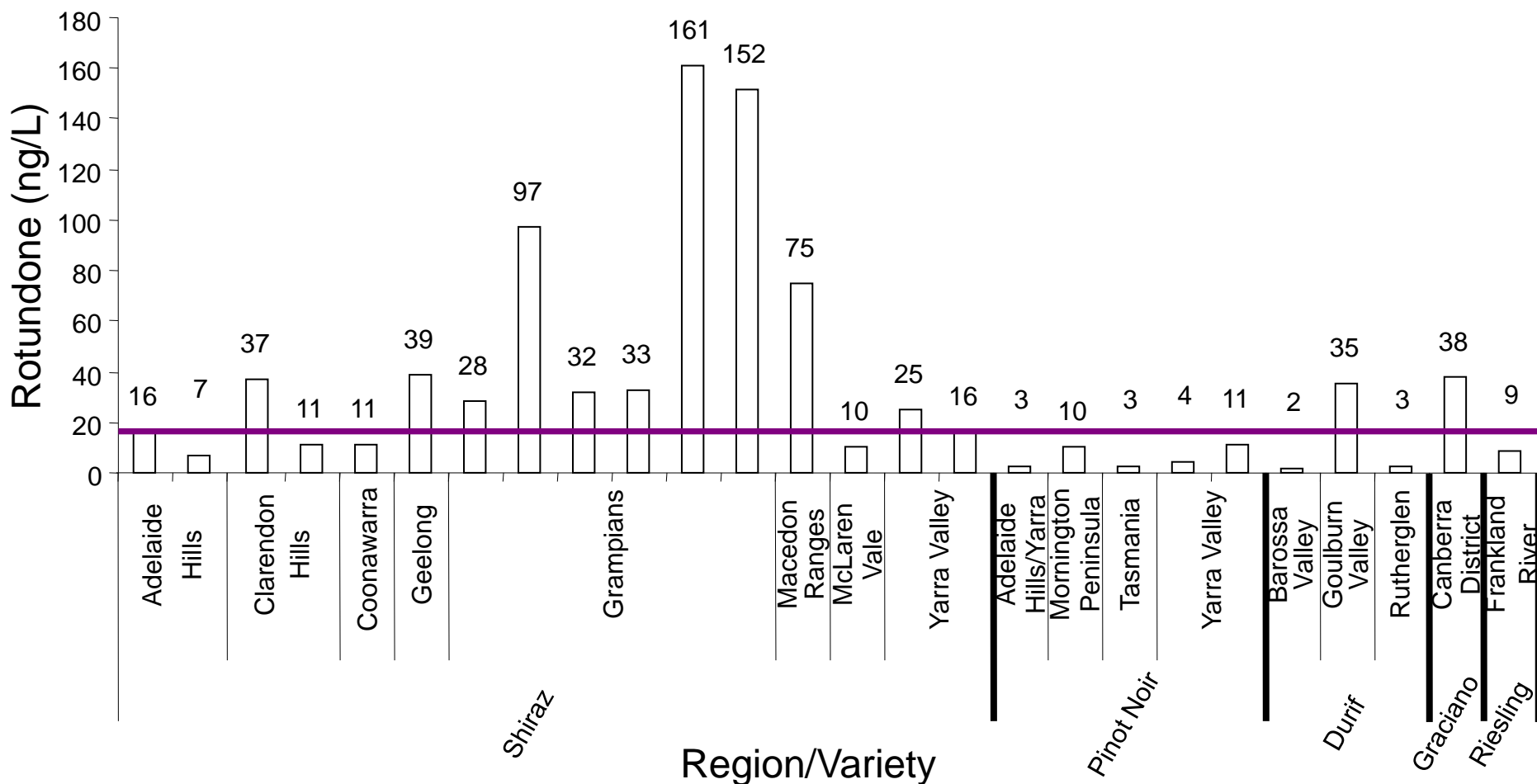
16 ng/L in red wine

20 to 25% of the panellists were anosmic to rotundone

Rotundone in Australian wines



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Australian cool climate Shiraz



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WINE REGIONS OF AUSTRALIA



WESTERN AUSTRALIA

- 1 Swan District
- 2 Perth Hills
- 3 Peel
- 4 Geographe
- 5 Margaret River
- 6 Blackwood Valley
- 7 Pemberton
- 8 Manjimup
- 9 Great Southern

SOUTH AUSTRALIA

- 10 Southern Flinders Ranges
- 11 Clare Valley
- 12 Barossa Valley
- 13 Eden Valley
- 14 Riverland
- 15 Adelaide Plains
- 16 Adelaide Hills
- 17 McLaren Vale
- 18 Kangaroo Island
- 19 Southern Fleurieu
- 20 Currency Creek
- 21 Langhorne Creek
- 22 Padthaway
- 23 Mount Benson
- 24 Wrattenbully
- 25 Robe
- 26 Coonawarra
- 27 Mount Gambier

QUEENSLAND

- 28 South Burnett
- 29 Granite Belt

NEW SOUTH WALES

- 30 New England Australia
- 31 Hastings River
- 32 Hunter
- 33 Mudgee
- 34 Orange
- 35 Cowra
- 36 Riverina
- 37 Hilltops
- 38 Southern Highlands
- 39 Gundagai
- 40 Canberra District
- 41 Shoalhaven Coast
- 42 Tumbarumba
- 43 Pteridocota

VICTORIA

- 44 Murray Darling
- 45 Swan Hill
- 46 Goulburn Valley
- 47 Rutherglen
- 48 Glenrowan
- 49 Beechworth
- 50 King Valley
- 51 Alpine Valleys
- 52 Strathbogie Ranges
- 53 Upper Goulburn
- 54 Heathcote
- 55 Bendigo
- 56 Pyrenees
- 57 Macedon Ranges
- 58 Sunbury
- 59 Grampians
- 60 Henty
- 61 Geelong
- 62 Yarra Valley
- 63 Mornington Peninsula
- 64 Gippsland*

TASMANIA

- 65 Tasmania*



*South Eastern Australia and Gippsland are zones, Tasmania is a state.

www.wineaustralia.com

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New Zealand



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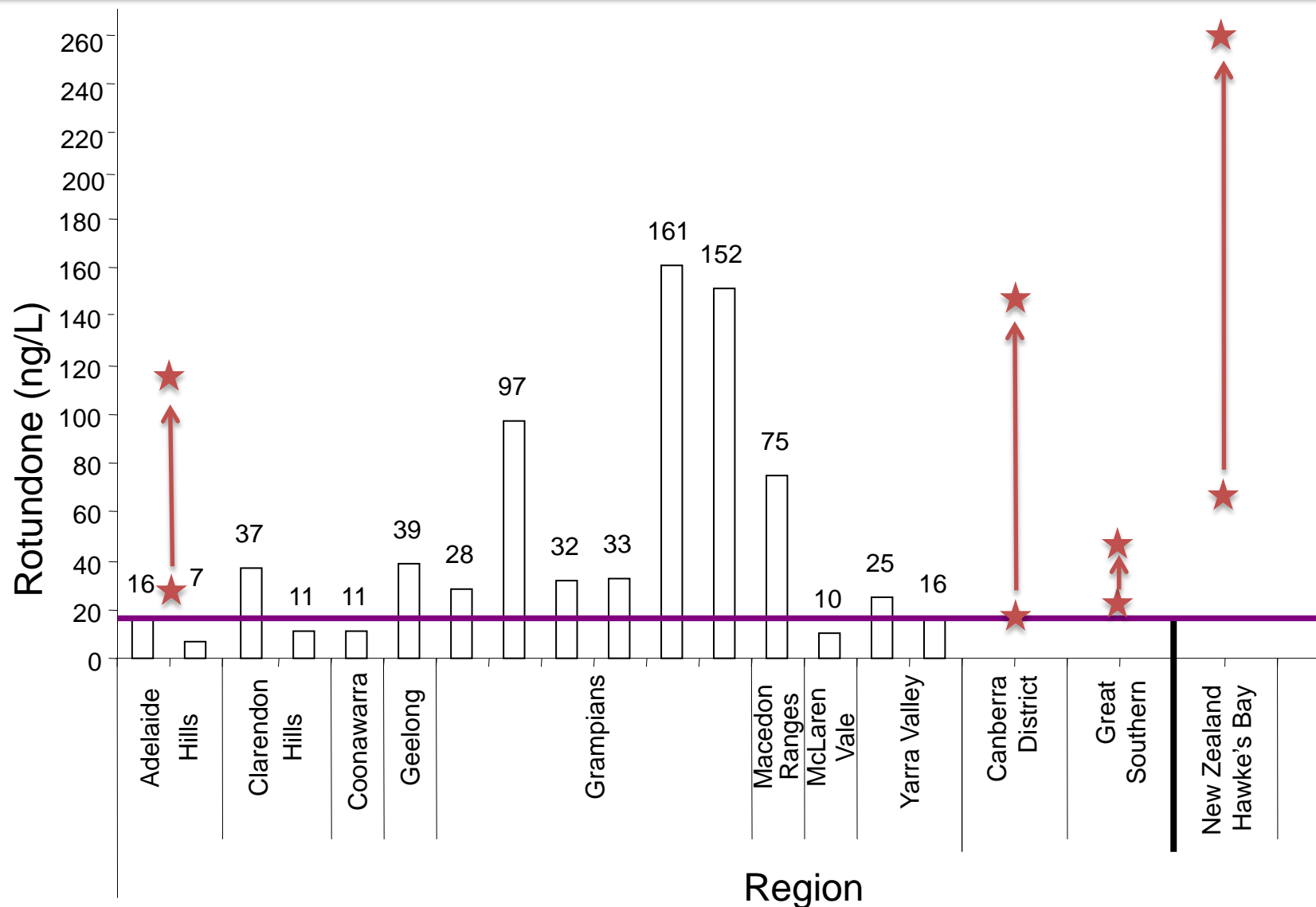
Gimblett Gravels (400 Ha), Hawke's Bay, New Zealand

- 39°37' S, 176°44' E
- 1435 GDD (Base 10°C) (17 year average)
- 803mm rainfall (21 year average)
- 2188 hours of sunshine/year
- 14.5°C mean temperature all year
- Omahu Gravel Soil
- Maritime with a little continentality

Rotundone in other commercially available Shiraz wines



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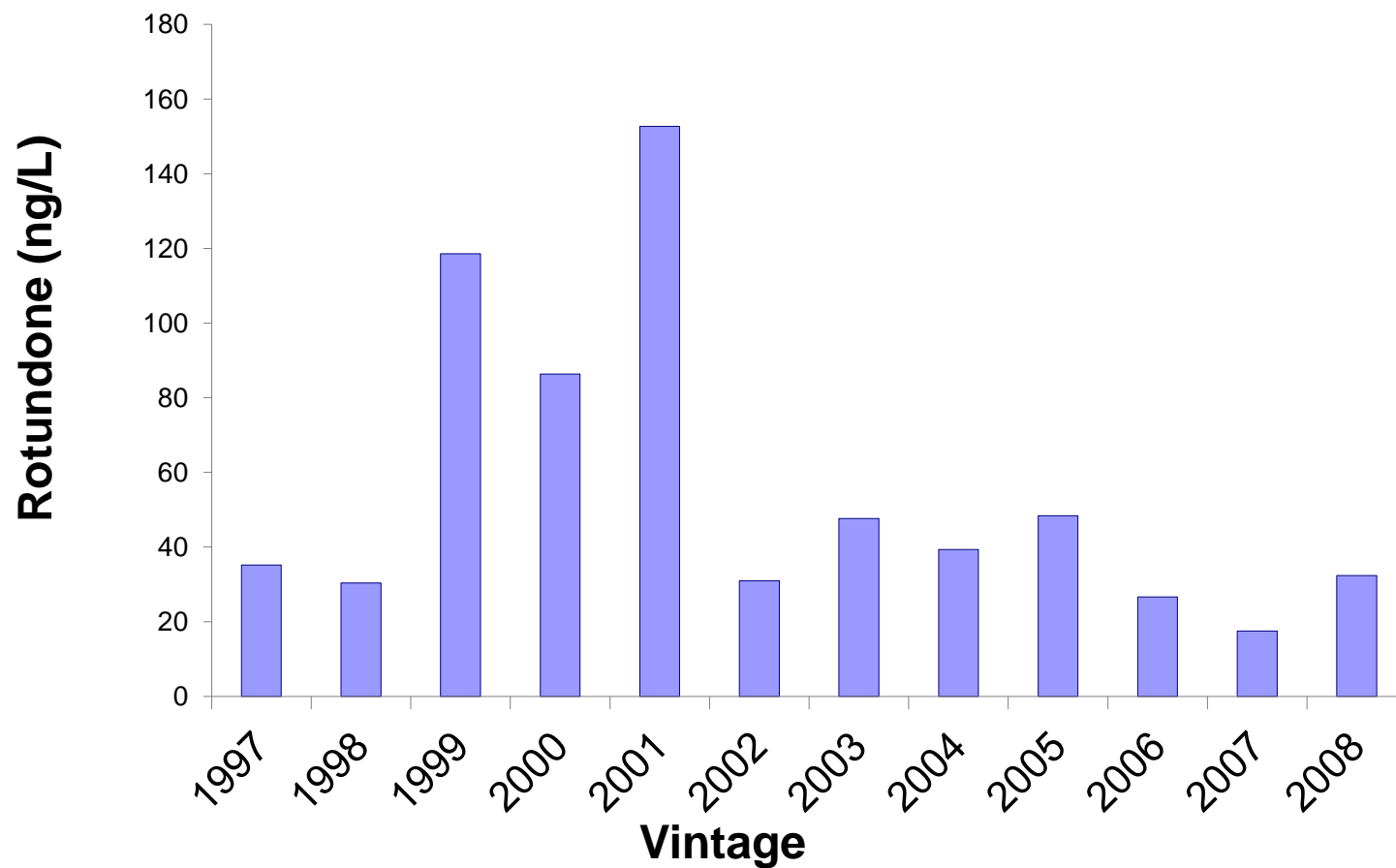


Variability across vintages: Canberra District



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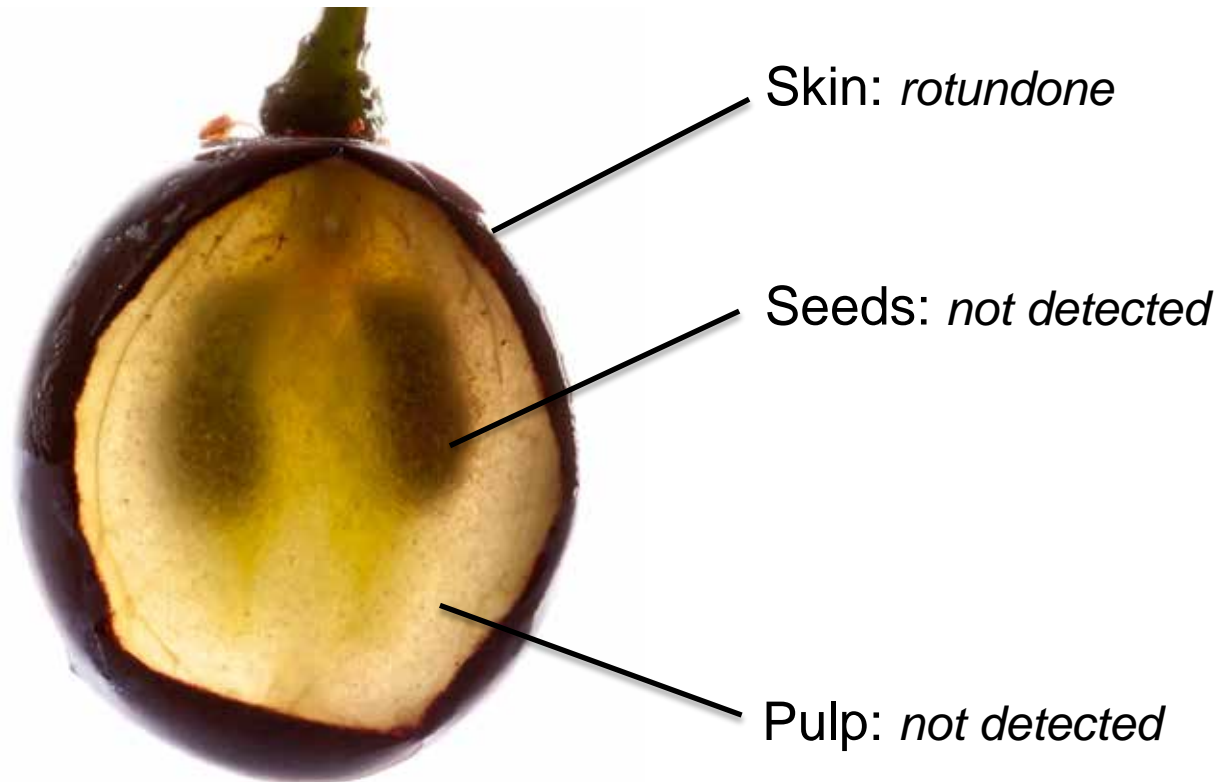
Shiraz/Viognier wines - same winery & style



Rotundone is only present in the skin



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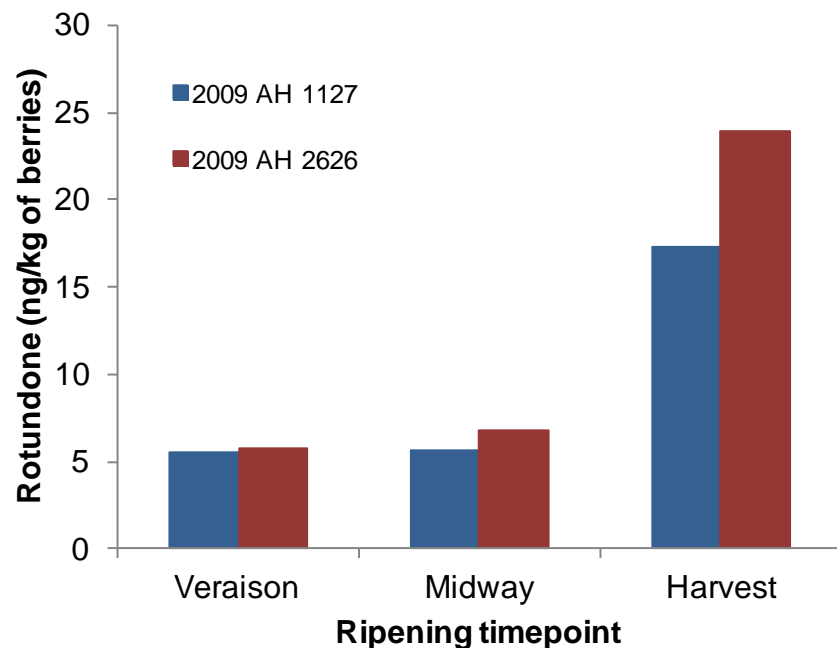
Photograph by Eric Wilkes

Rotundone increases during late stage ripening



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Adelaide Hills Shiraz berries during ripening

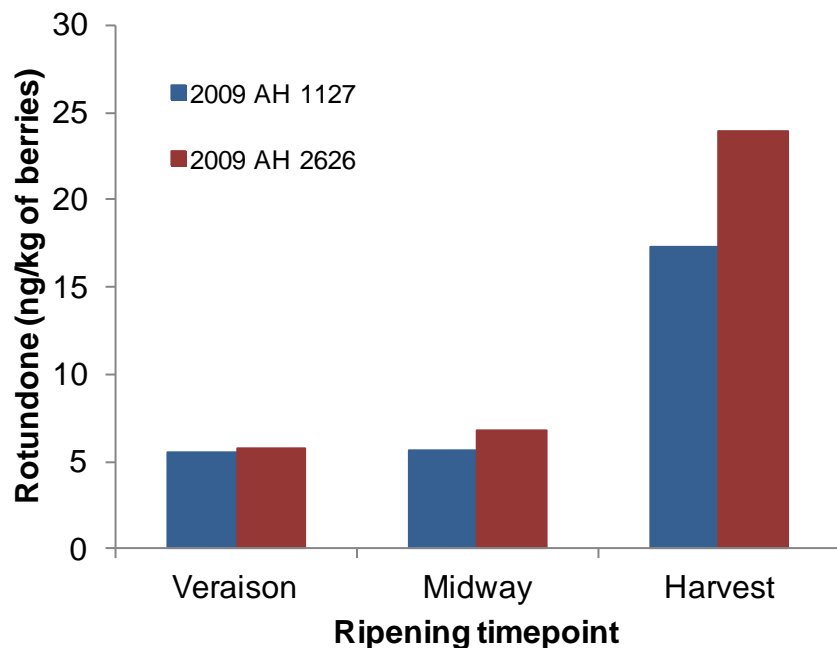


Rotundone increases during late stage ripening

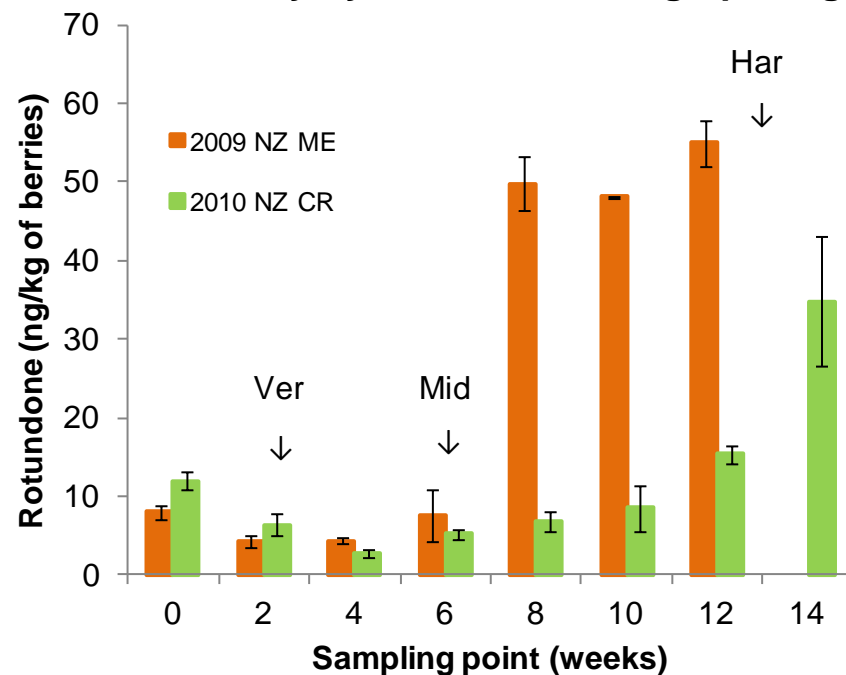


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Adelaide Hills Shiraz berries during ripening



NZ Hawke's Bay Syrah berries during ripening



Does vine management affect rotundone levels?



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- ✓ Fruit exposure
- ✓ Leaf removal time
- ✓ Crop load
- ✓ Vine vegetative vigour
- ✓ Clones

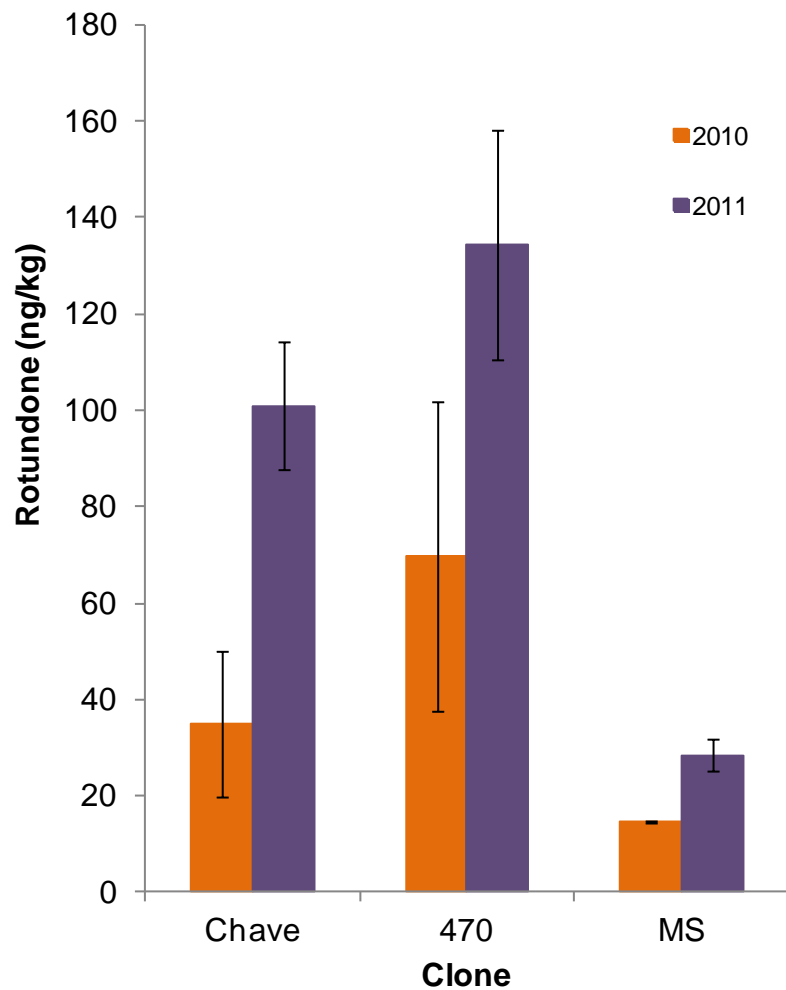


Clone and crop load

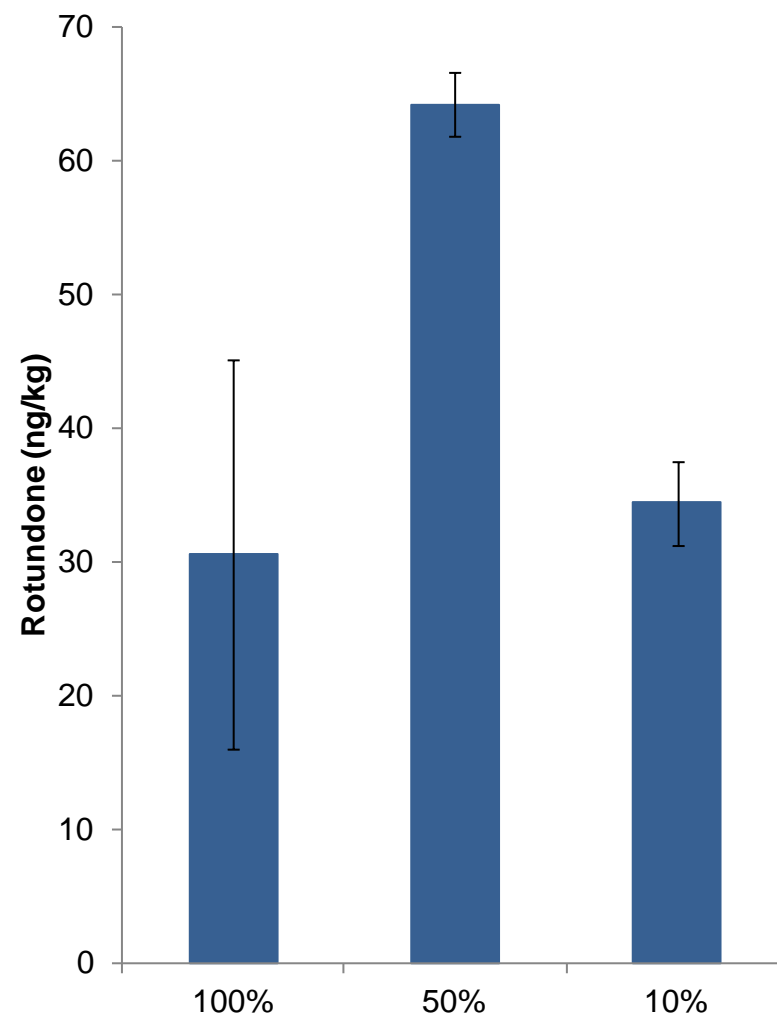


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NZ Hawke's Bay Syrah Clones



NZ Hawke's Bay Syrah 2011 - Crop Load

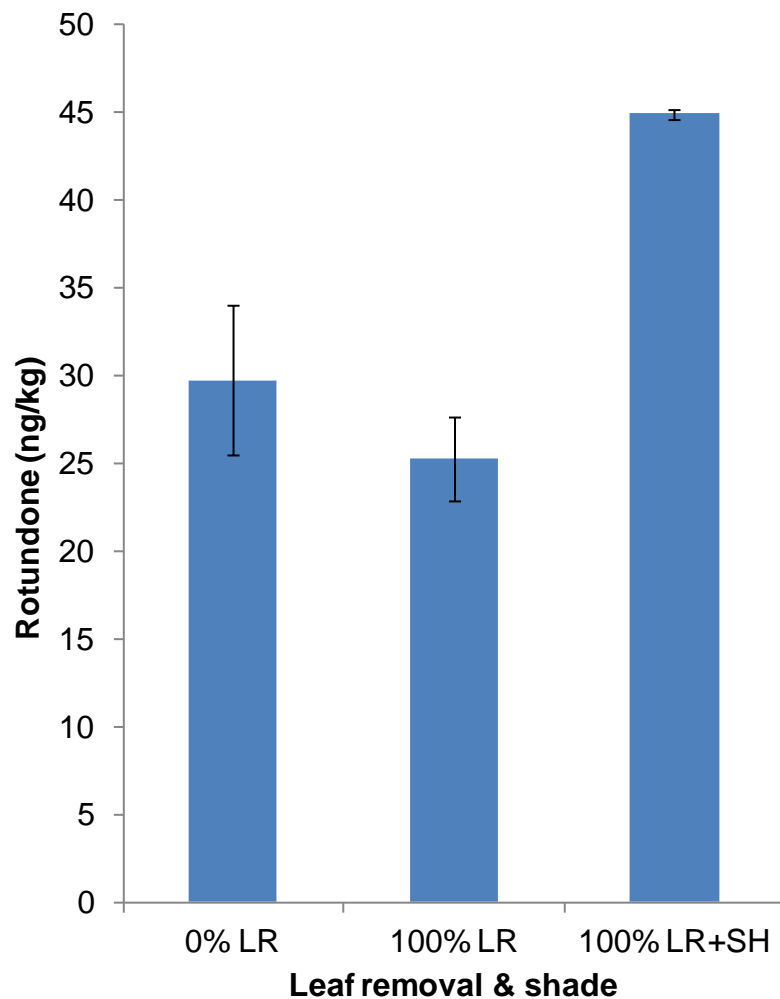


Leaf removal

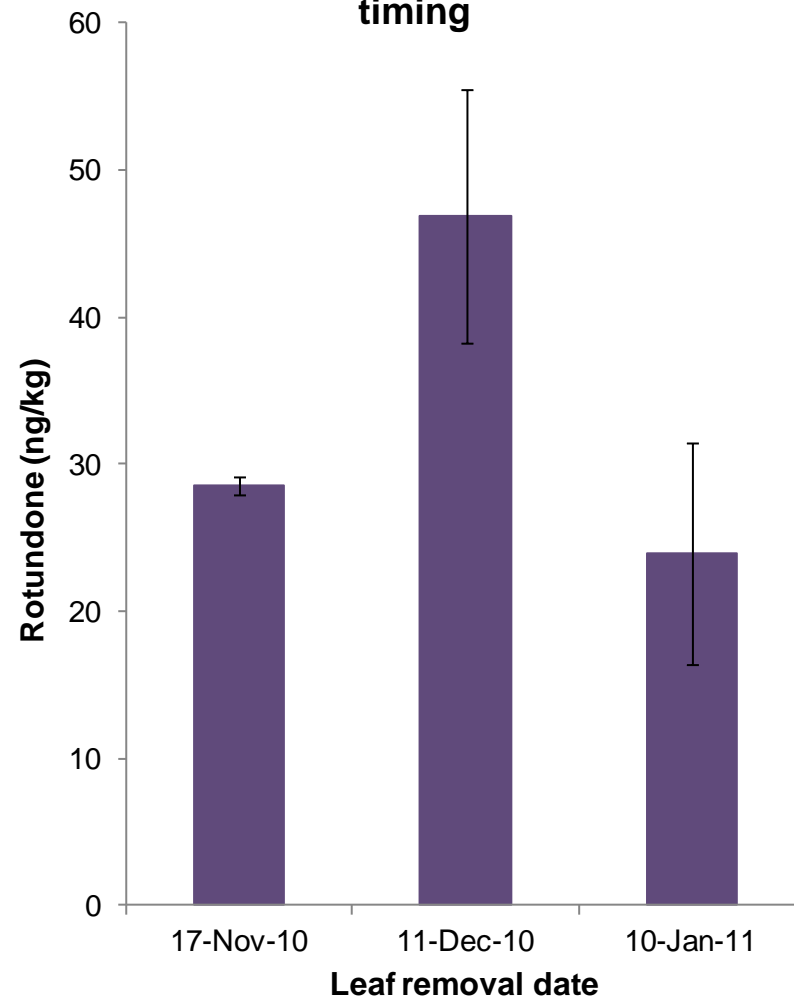


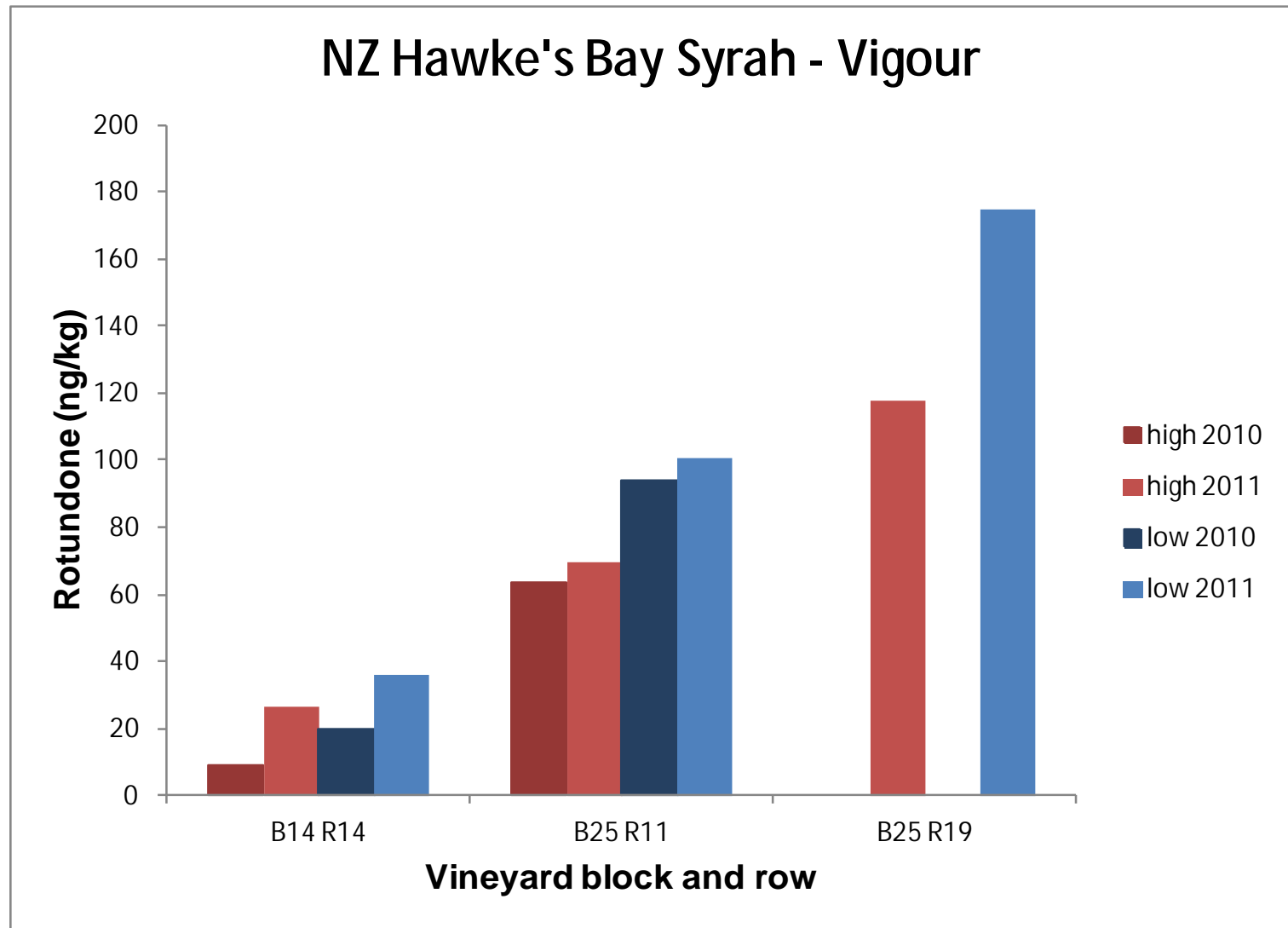
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NZ Hawke's Bay Syrah 2011 – Leaf removal



NZ Hawke's Bay Syrah 2011 – Leaf removal timing



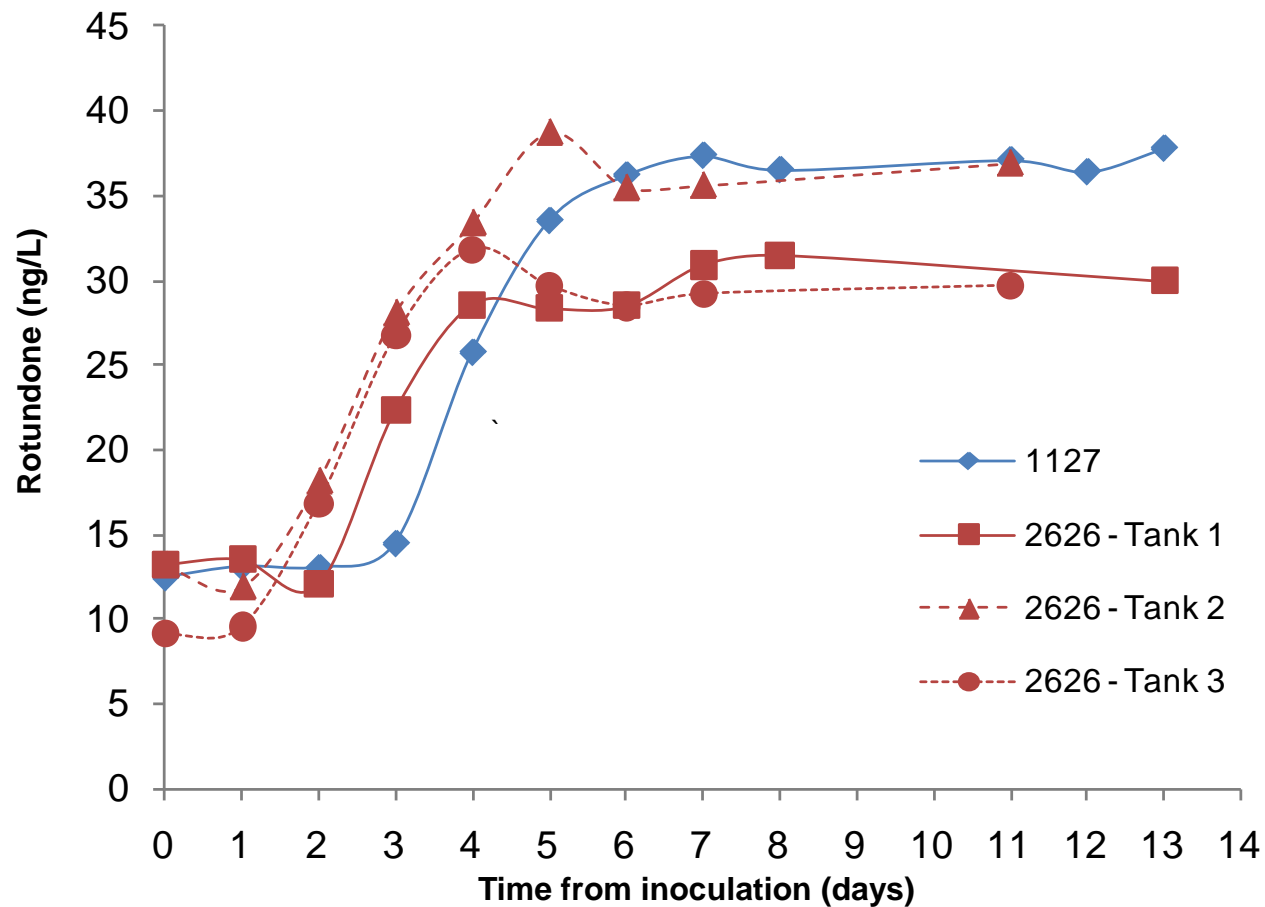


Rotundone extraction during winemaking



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Adelaide Hills Shiraz commercial fermentation 2009

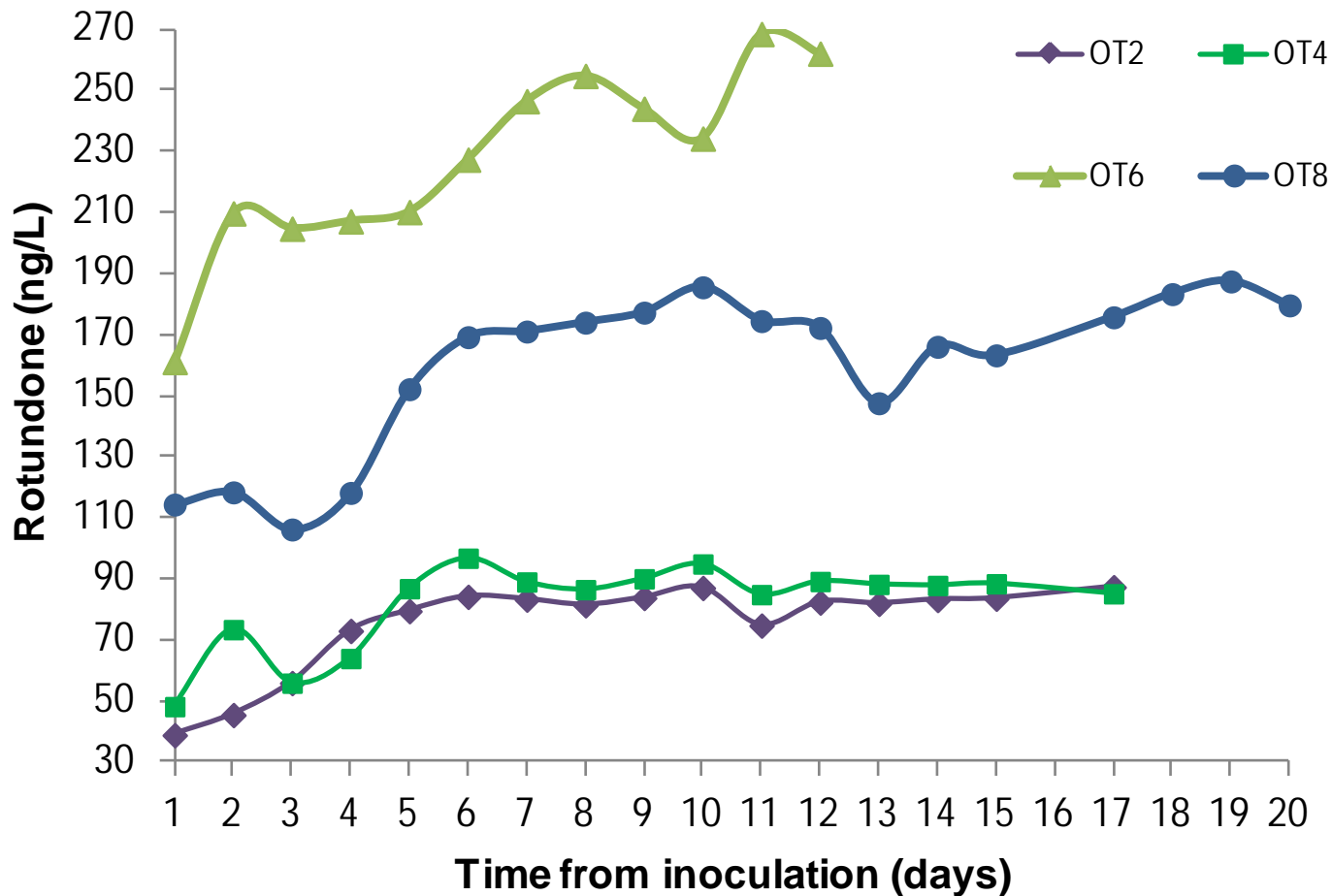


Rotundone extraction from berries during winemaking



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NZ Hawke's Bay Syrah commercial fermentation 2011

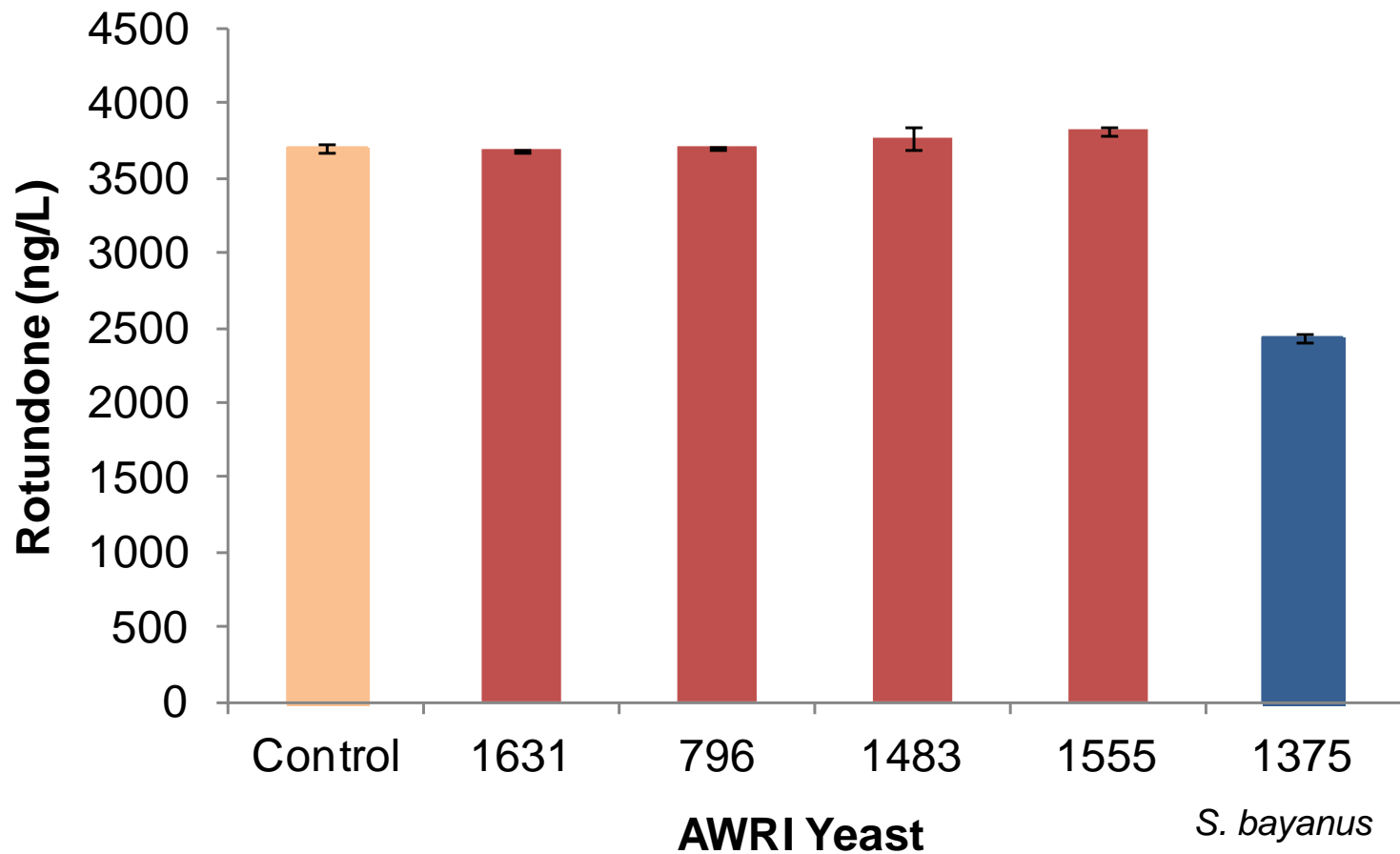


Can yeast affect rotundone levels during fermentation?



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Fermentation in defined juice medium 2011



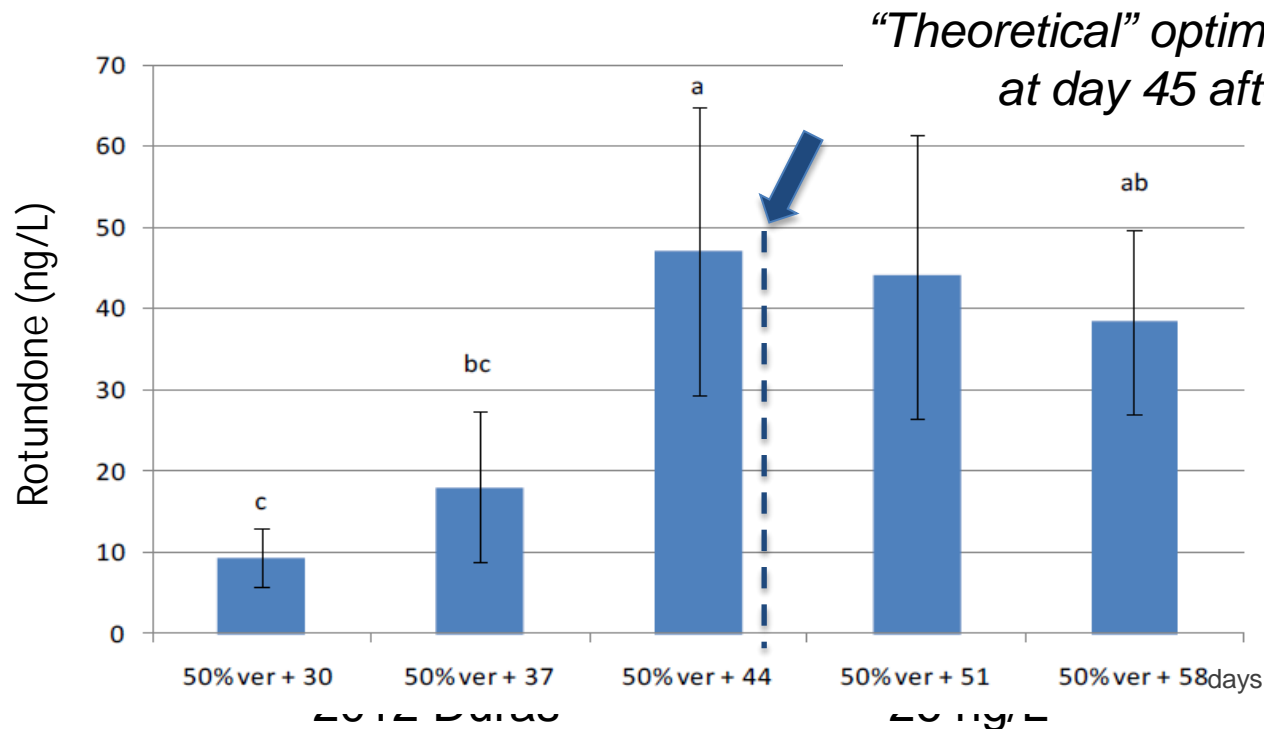
Rotundone in French Pyrenees wines



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Olivier Geffroy, IFV Sud-Ouest

2011 Duras microvinification at 5 levels of maturity



IFV viticulture trials:

Irrigation / Elicitor / crop load

Control

Leaf removal

2011

43-48 ng/L

37 ng/L

12 ng/L

2012

29-36 ng/L

27 ng/L

12 ng/L

Conclusions and future directions



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- ✓ Viticulture parameters affected rotundone levels:
Picking date, clone, vigour, leaf removal & crop load
- ✓ Why does rotundone occur in Shiraz more often than other cultivars?
- ✓ Can rotundone be modulated during winemaking?



Acknowledgements



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- Darryl Catlin, Winemaker, and the winery and laboratory staff of Shaw and Smith Wines
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- Symrise, Germany

AWRI

- Mango Parker, Claudia Wood
- Flavour & Sensory Teams
- Radka Kolouchova
- The University of Auckland & EIT Hawke's Bay
- Mission Estate Wines
- Craggy Range Vineyards



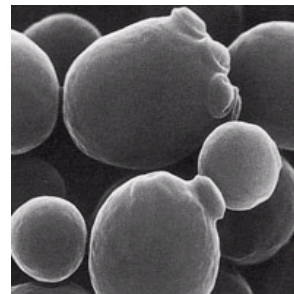
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NEW ZEALAND



The AWRI, a member of the Wine Innovation Cluster in Adelaide, research is financially supported by Australia's grape growers and winemakers through their investment body the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government.

Does soil and vine nutrient status affect wine quality?

Mark Krstic



Does soil nutrient status affect wine aroma and flavour?



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- § in popular press, the 'terroir effect' is often attributed to specific minerals that are alleged to confer typicity to wine
- § soils with rock appear to elicit allusions of minerality





“ But what separates Beechworth from so many other regions,, is minerality. It’s there, you can see it, with minerals glistening in the sun—slate and shale and great boulders of granite.....

While some disagree, you can taste minerality and it’s there in the wines”

Jeni Port, The Age

Feb 2012

What are the facts?



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- § Geological minerals are complex chemical compounds that are mainly tasteless
- § Vine roots can only take up ions in solution
- § Membranes only allow certain ions to be taken up
- § Even then not all ions taken up by roots will end up in fruit

What are the facts?



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- § Some wine is said to be reminiscent of smell of wet rocks following rain
- § Actually is scent of organic compounds released from plants during dry periods and 'captured' by rocks (= petrichor) (Bear and Thomas 1964)

What are the facts?



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- § What about 'minerality' in wine?
- probably a descriptor for acidity



What are the facts?



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§ Rocks affect physical structure of soil and water relations



Does soil nutrient status affect wine aroma and flavour?



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- Many studies – no correlation between wine quality and soil content of any nutritive element with exception of N (and salt)



Red wine quality: negatively correlated with vine N

- particularly when water not limiting

§ low soil N best for red wine quality

White wine: moderate soil N best for quality

§ Low N ® decreased aromatic precursors and increased tannin

§ High N ® increased Botrytis

NITROGEN



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- § Of all mineral nutrients, N has greatest effect on growth, yield and fruit composition
 - \uparrow soil N \rightarrow \uparrow photosynthesis \rightarrow \uparrow sugar
- § As for water, excess N can have negative effect
 - eg increased canopy size



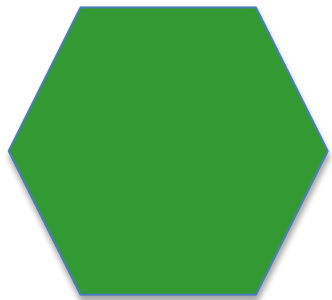


- § Demand for N greatest from **budburst to flowering**
 - But most uptake from soil after flowering
 - Overwintering reserves thus very important (like starch)
- § Storage reserves are lowest at flowering
 - Therefore plant is vulnerable to deficiency if insufficient N in soil after flowering

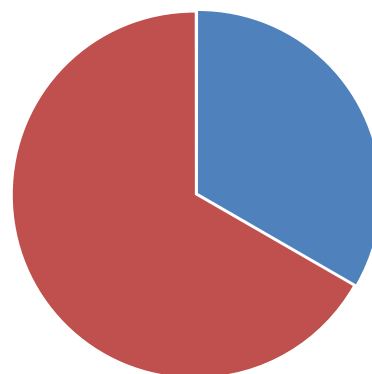
Total berry NITROGEN



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LEAVES



FRUIT

What is effect of N fertilisation on vine performance?



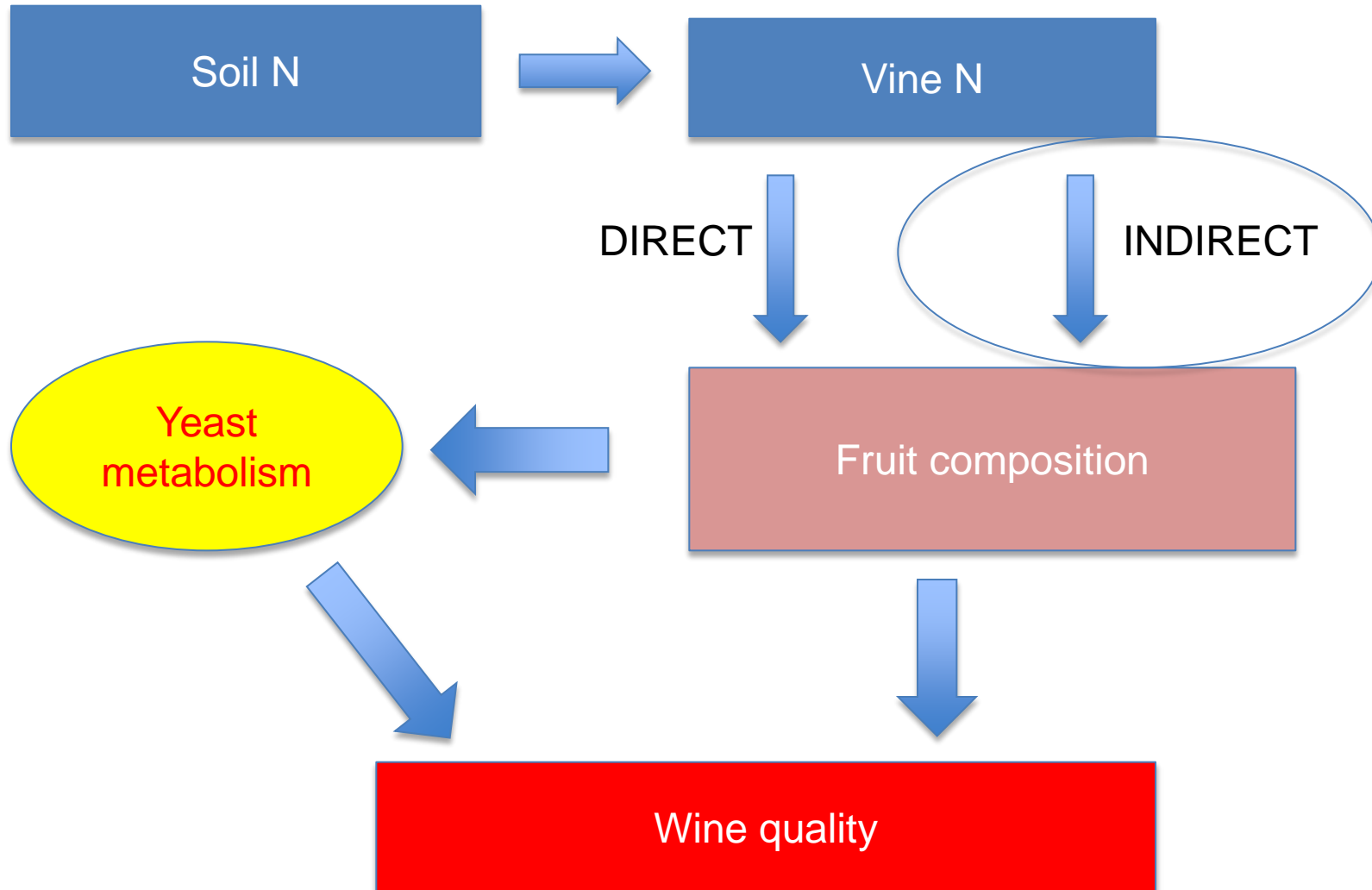
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- Ø **Deficit to marginal status** (based on tissue analysis)
 - Ø N fert. generally has a positive effect
- Ø **Adequate to high status** (based on tissue analysis)
 - Ø N fert. may have negative effect
 - Ø Disrupt balance
 - Ø Increases vegetative growth
 - Ø Increases shading
 - Ø Decreases net photosynthesis
 - Ø assimilates diverted from fruit to shoots

Nitrogen effect on fruit composition and wine quality



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N impact on wine quality: Indirect effect



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§ Excess → ↑ vegetative growth ↑ canopy density

- More bunch zone shading → fruit composition
 - e.g. increased methoxypyrazine concentration in Cab Sauv in response to N fertilization





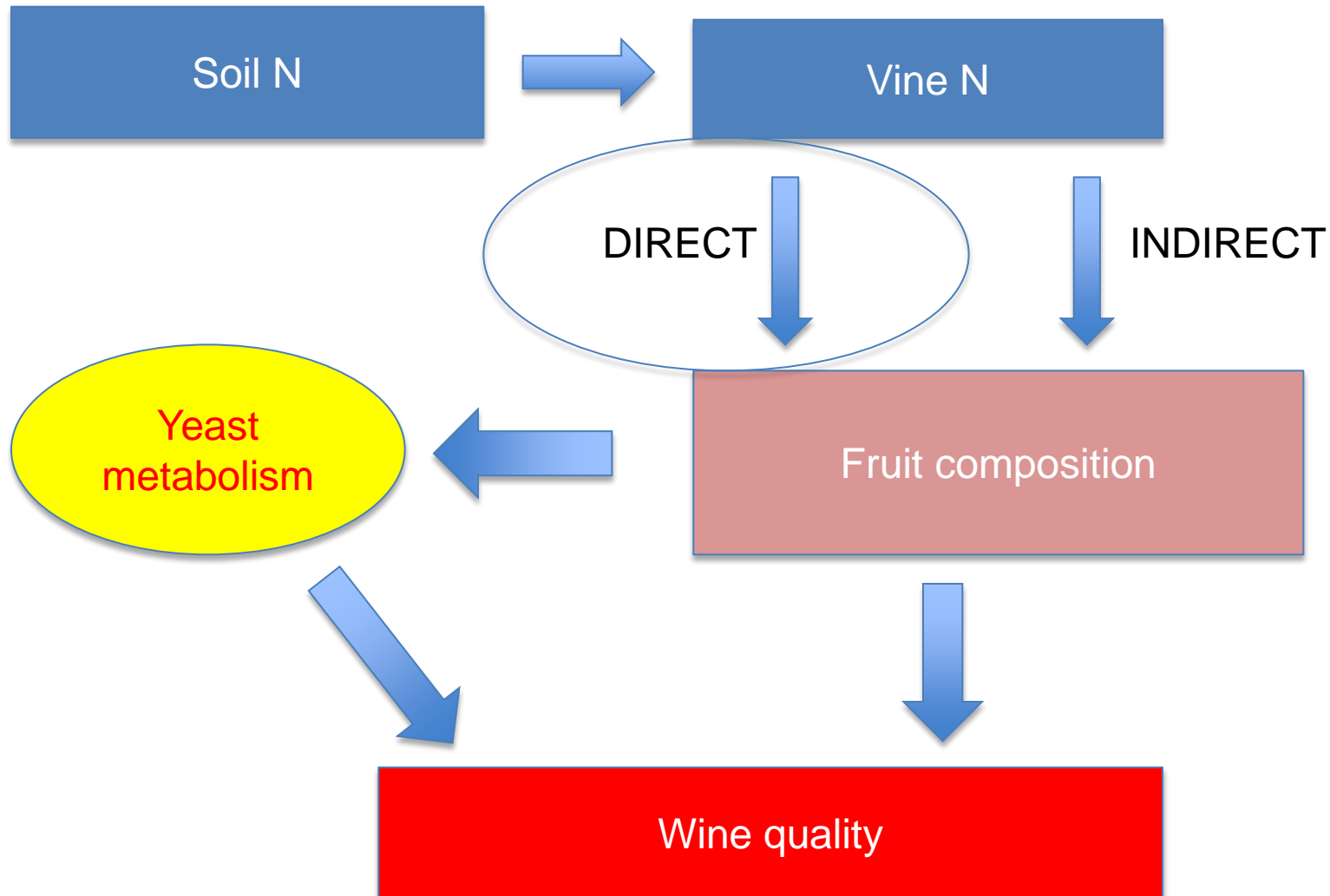
§ Excess → ↑ vegetative growth ↑ canopy density

- More bunch zone shading → fruit composition
 - generally decreased monoterpenes in response to N fert.
- **More disease**
- **Growing tips compete with fruit for assimilate**

Nitrogen effect on fruit composition and wine quality



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N impact on wine quality: Direct effect



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- § Nitrate uptake → reprogramming of gene expression
- § High nitrate suppresses genes involved in phenolic production
- § Also high nitrate → ↑ organic acid production
↑ amino acid

Overall effect is decreased phenolics



- Response to N fertilisation depends on starting point
 - less than adequate level:
 - \emptyset may increase anthocyanins
 - adequate or more:
 - \emptyset may decrease anthocyanins



§ Can the negative effect of shading caused by high N be overcome by leaf removal in bunch zone etc?

- Not necessarily – high N and low flavonol make berries more susceptible to sunburn

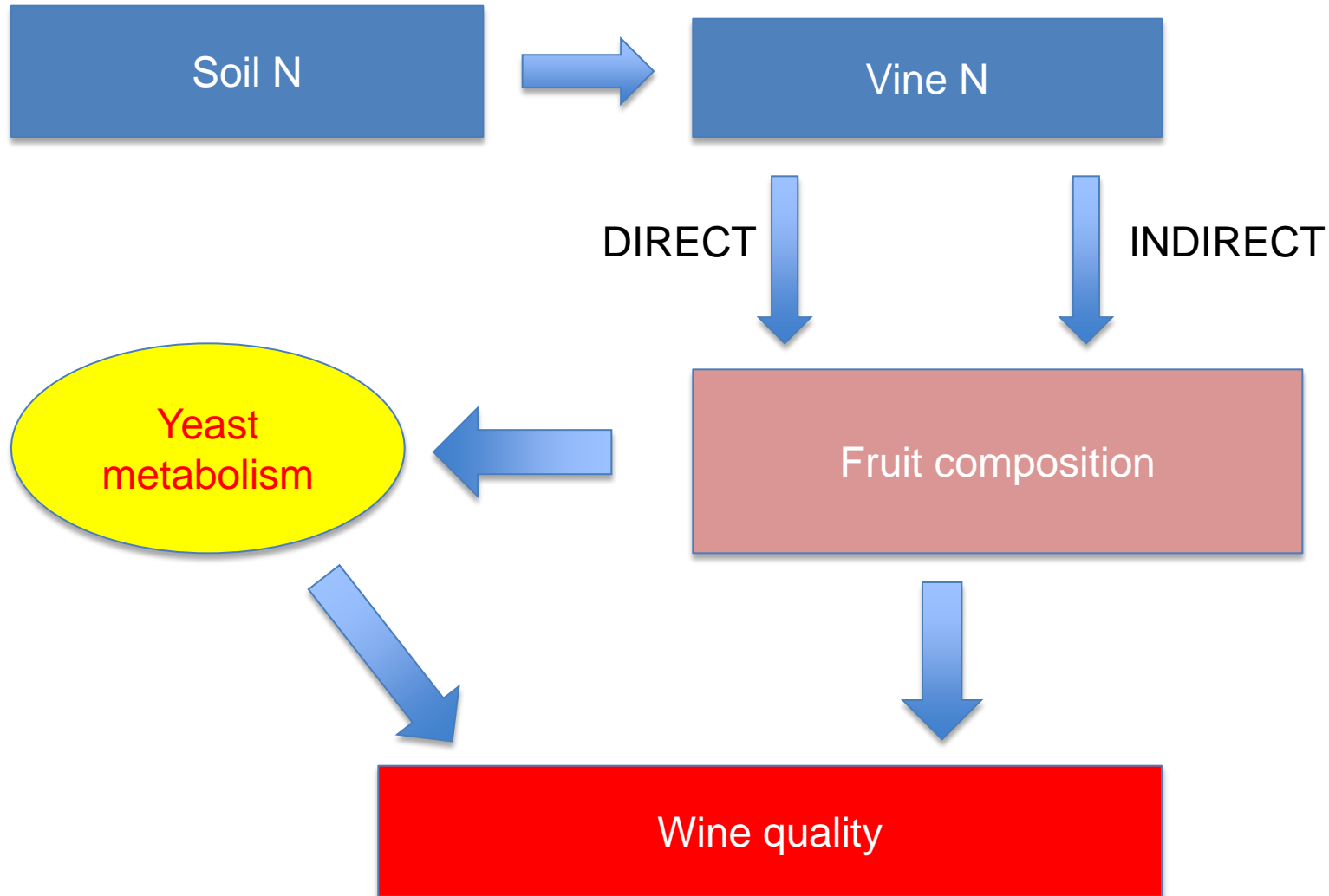
§ Or hedging?

- This may waste resources because removes young leaves and retains old inefficient leaves
- Also diversion of assimilates away from fruit

Nitrogen effect on fruit composition and wine quality



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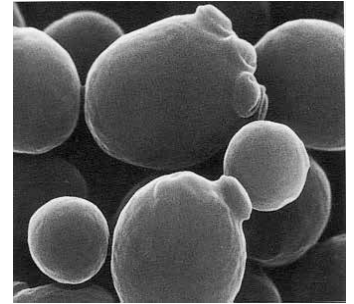
N and fermentation



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Nitrogen affects

- Ø Yeast growth
- Ø Metabolic activity
 - Ø Fermentation rate
 - Ø Flavour active compounds (fermentation bouquet)



Grape nitrogen: effect on yeast



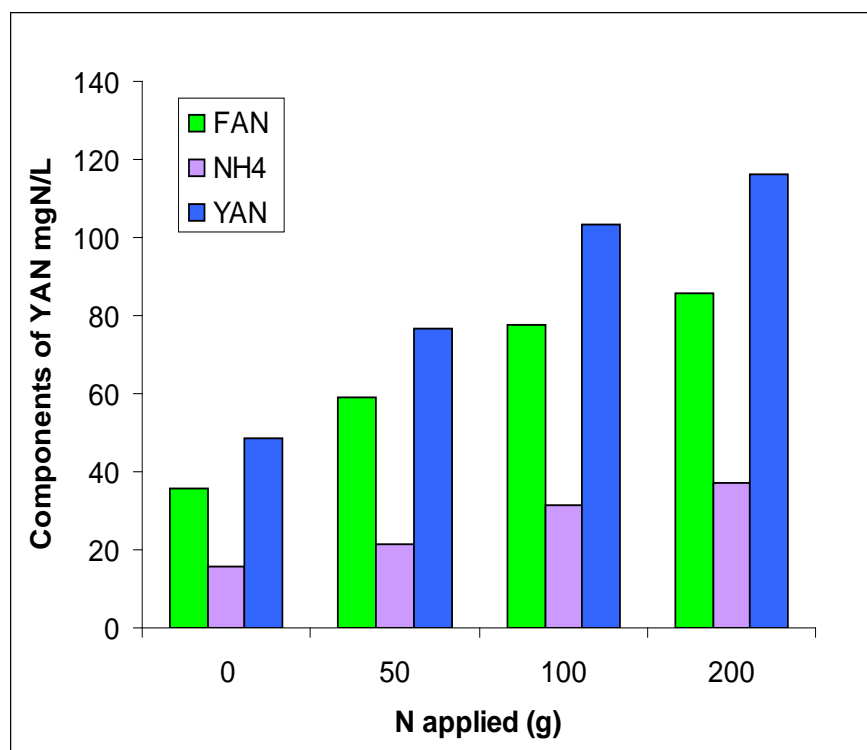
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- Ø Total Nitrogen in juice is mainly
 - Ø Ammonia
 - Ø Free Amino Acids
- Ø Yeast assimilable N (YAN)
 - = free amino N (FAN) + ammonia N ($\text{NH}_3\text{-N}$)
- Ø Yeast will use ammonium N initially,
then most assimilable amino acids
- Ø If YAN too low → stuck or slow ferments

Does N fertilization affect YAN in grapes?



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Grape N that yeast can use

AWRI fermentation study

- Ø Filtered Chardonnay juice
- Ø Low N (YAN = 160 mg/L)
- Ø High N demand yeast (AWRI 796)
- Ø Wine analysis and descriptive sensory analysis

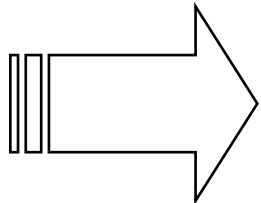
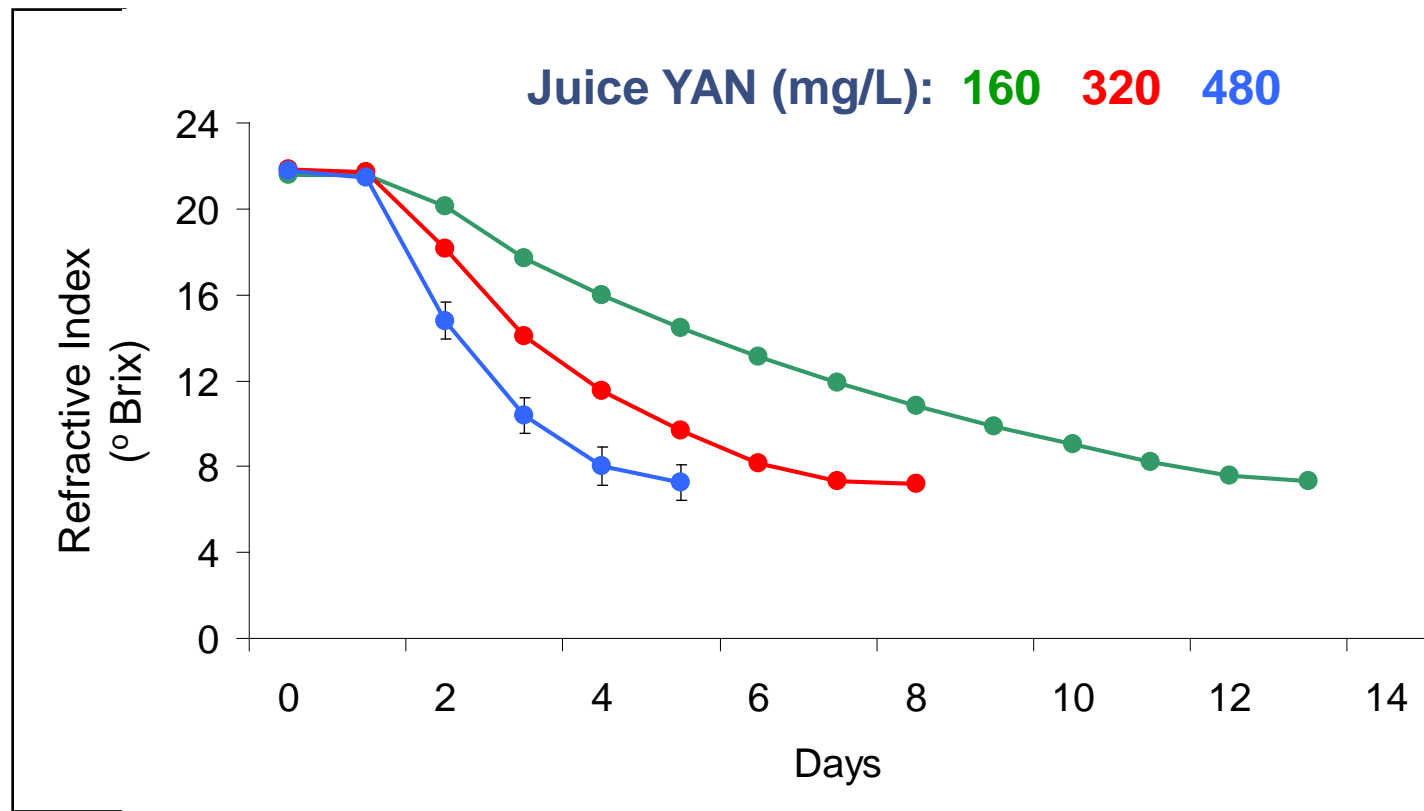


Diego Torrea

Effect of Juice N on Fermentation



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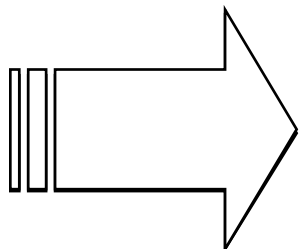
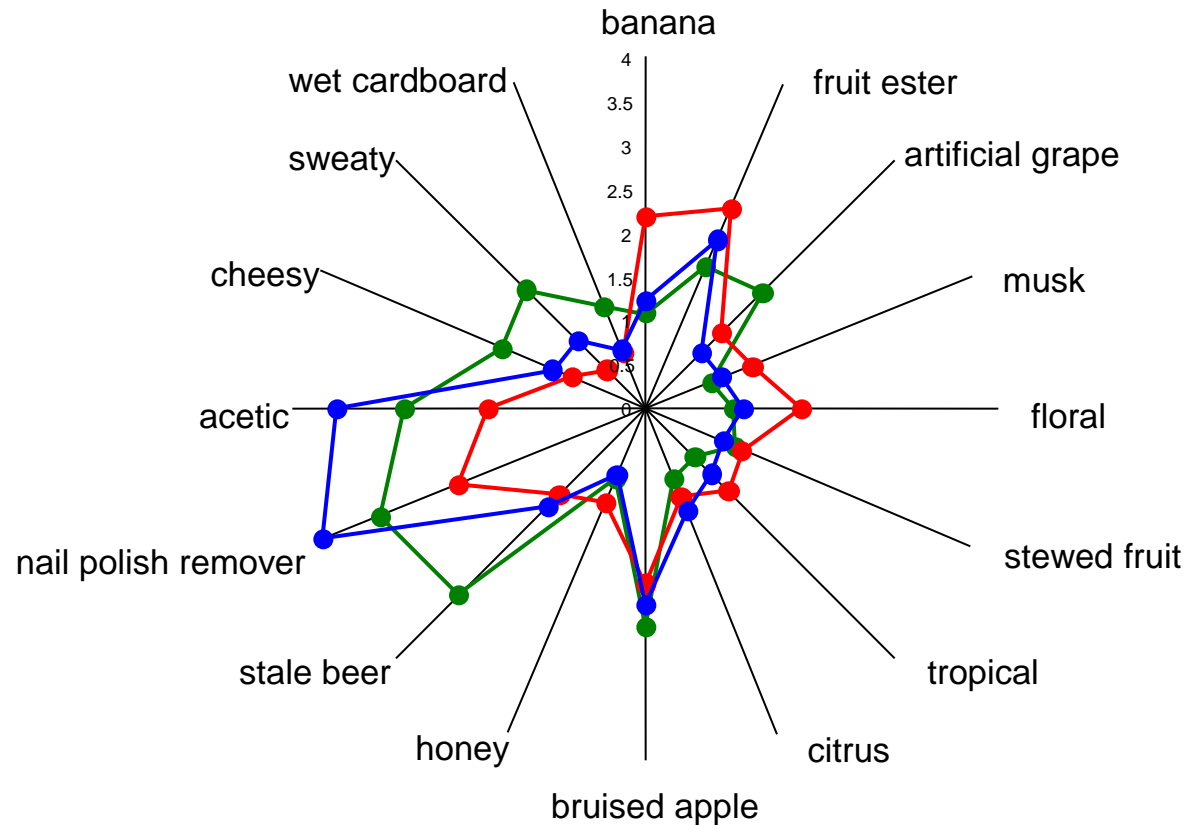


Juice YAN affects: i) yeast growth,
ii) fermentation, and
iii) fermentation duration

Effect of juice N concentration on wine aroma profile



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Low & High N: Negative effects
Moderate N: Positive effects

Juice YAN (mg/L): 160 320 480

- § increased PR proteins with increased plant N
- → haze and increased need for bentonite fining





✓ High K in juice

- § → decreased concentration of free acids ??partic. tartaric (and \uparrow pH)
- § → may decrease rate of degradation of malic acid

What factors determine how much
K ends up in juice?



- ✓ No evidence for direct effect of soil K on wine quality
 - § Except K deficiency may impair sugar accumulation

- ✓ K fertilisation effect on juice K concentration?
 - § No consistent results

- ✓ Factors such as rootstock type, irrigation, canopy management etc much more influential than K status of soil
 - ✓ Reduced irrigation → reduced juice K concn
 - ✓ Shoot trimming → increased leaf blade K concn

Impact of K movement from leaves to fruit



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EXPOSED CANOPY

Less potassium
moves from leaves to
the berries

Shading → ↑ K in leaves at veraison
→ ↑ K in berries at maturity
Therefore more K in wine, higher pH

SHADED CANOPY

More potassium moves from
leaves to the berries





✓ Direct or indirect effect?

✓ Direct

§ Rootstock type affects:

a) uptake by roots \ddot{O}

b) transport from roots to shoots \ddot{O}

c) transport from leaves to fruit ?

✓ Indirect

§ Rootstock type affects shoot vigour, canopy shading



Take home messages



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- Ø Minerality of wine is unlikely to be related to soil nutrient status
- Ø N is the only soil nutrient that has a significant impact on wine quality
- Ø N has both direct and indirect effects on fruit composition and wine quality
- Ø Only use N fertiliser to correct a deficiency or to maintain adequate levels (timing is important)
- Ø Measure must YAN before fermentation



Can DAP addition make up for N deficient grapes?



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Ø Perhaps

Ø But some evid that not entirely cos C backbone of AA
is metab to higher alcs so AA not just source of N



Using MLF to accentuate wine aroma and flavour

Eveline Bartowsky
Senior Research Microbiologist

Malolactic fermentation

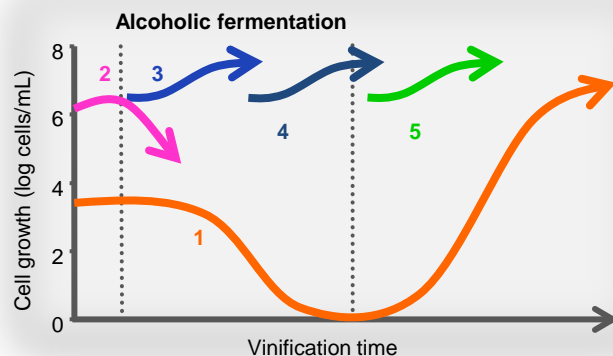
✓ MLF ...

- § 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 than AF

It's all about aroma & flavour

✓ Talk will concentrate on sensory aspect of MLF

✓ Sensory

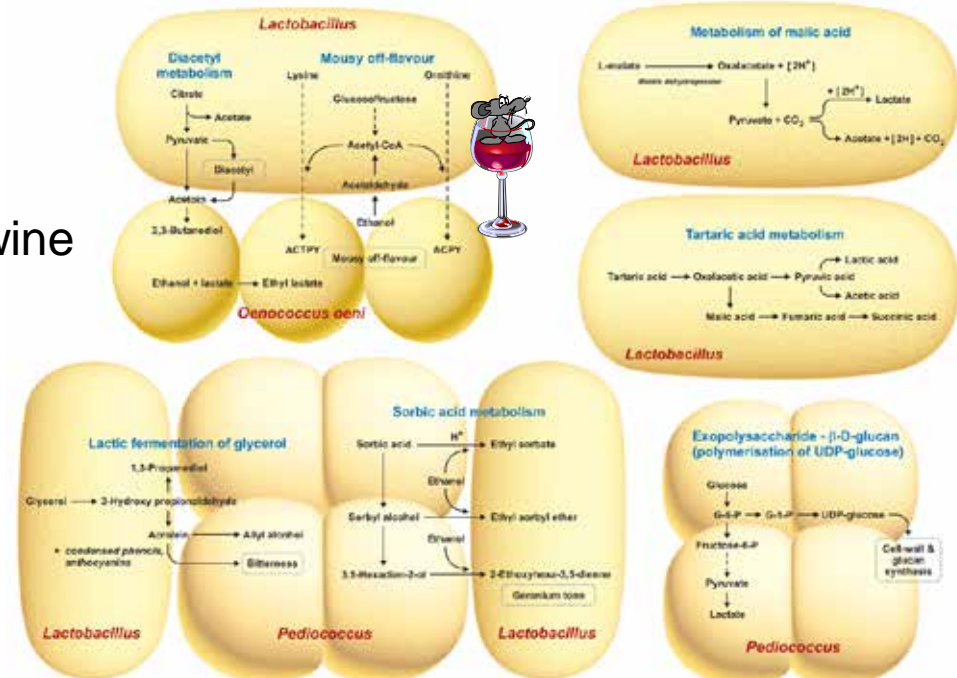


Diacetyl



Berry-fruit characters in red wine

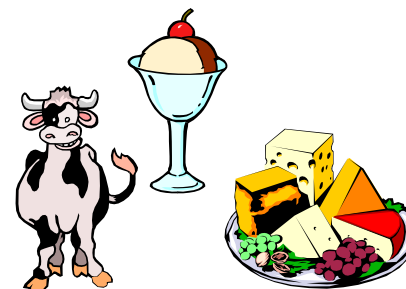
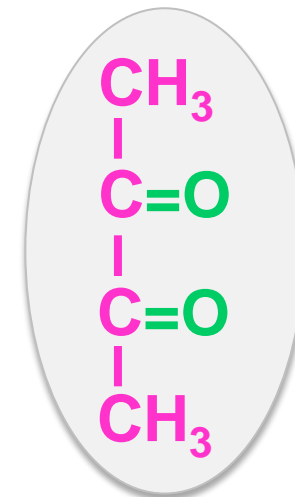
§ Polysaccharides (mouthfeel)





Buttery aroma - Diacetyl

- ✓ *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

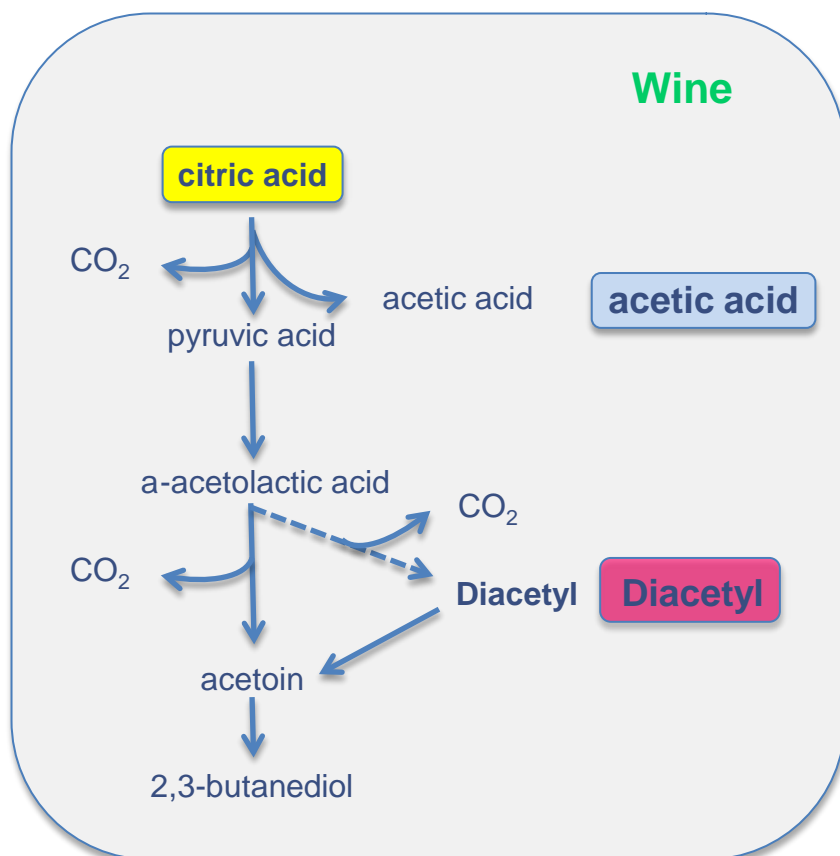


2 important considerations for diacetyl producⁿ

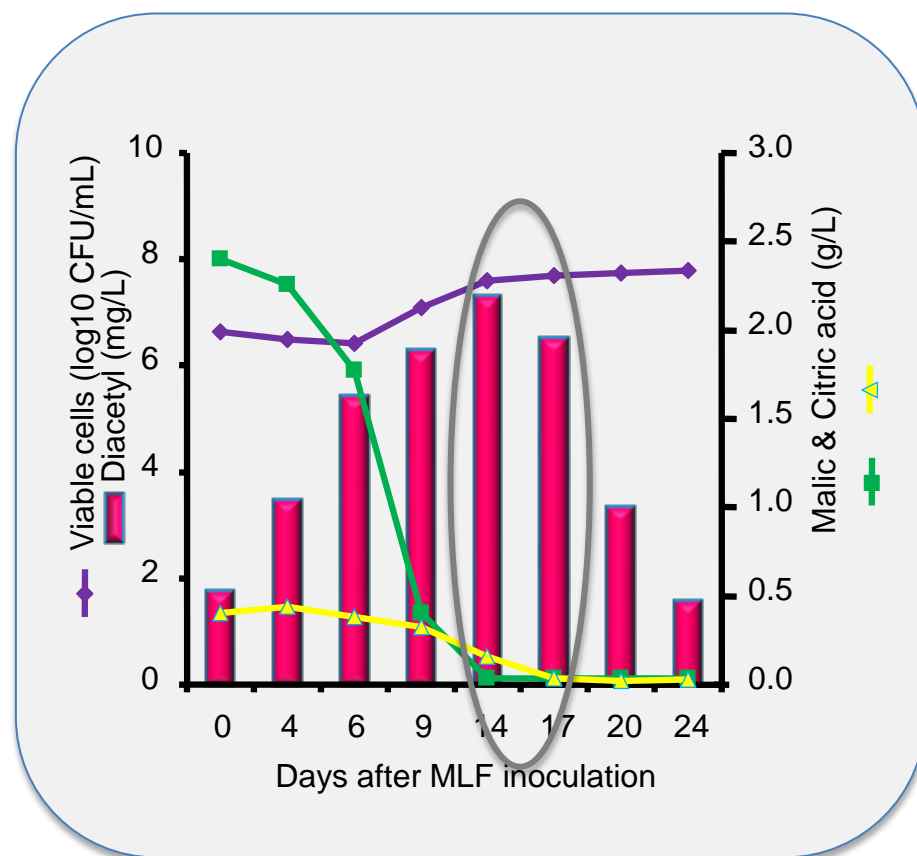


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Pathway



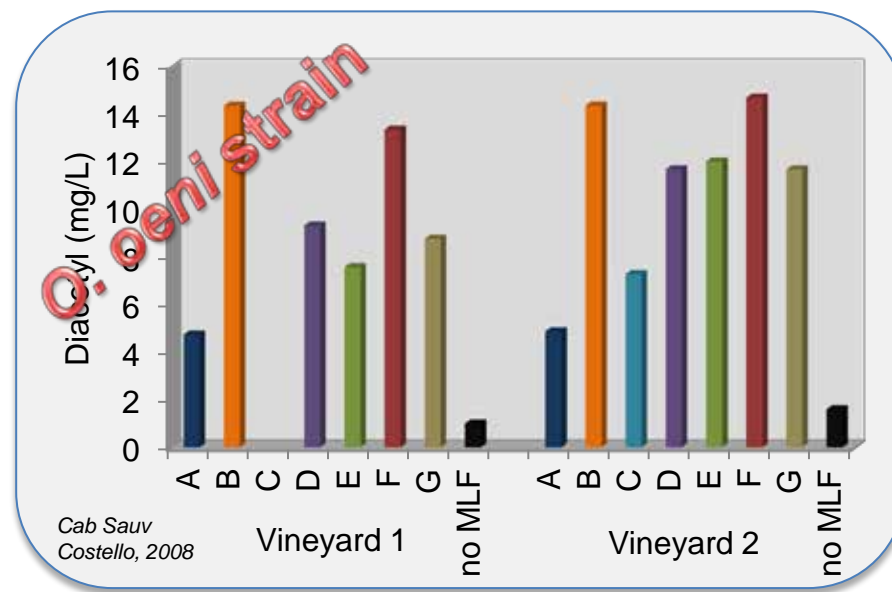
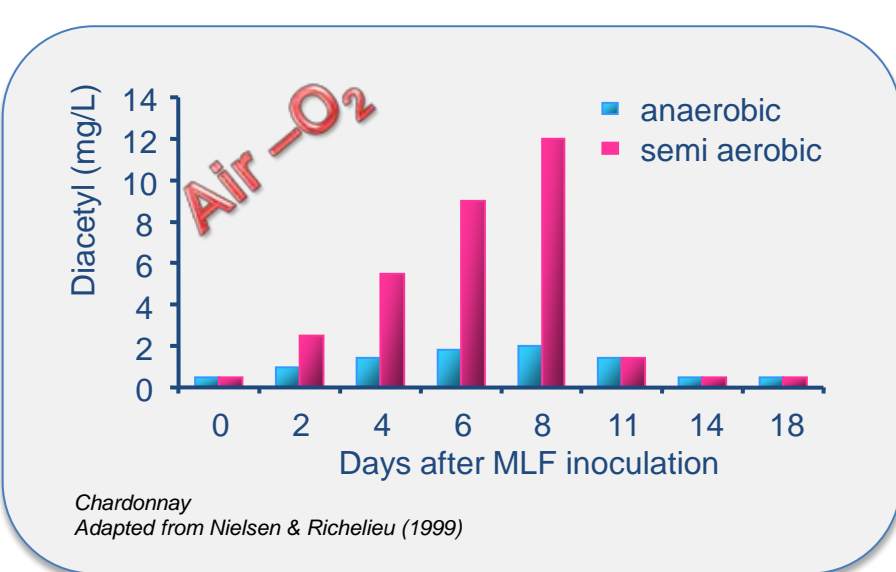
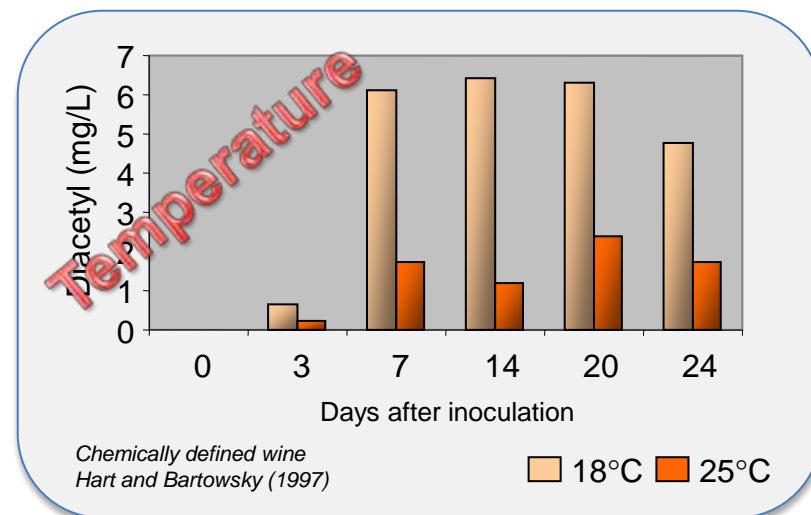
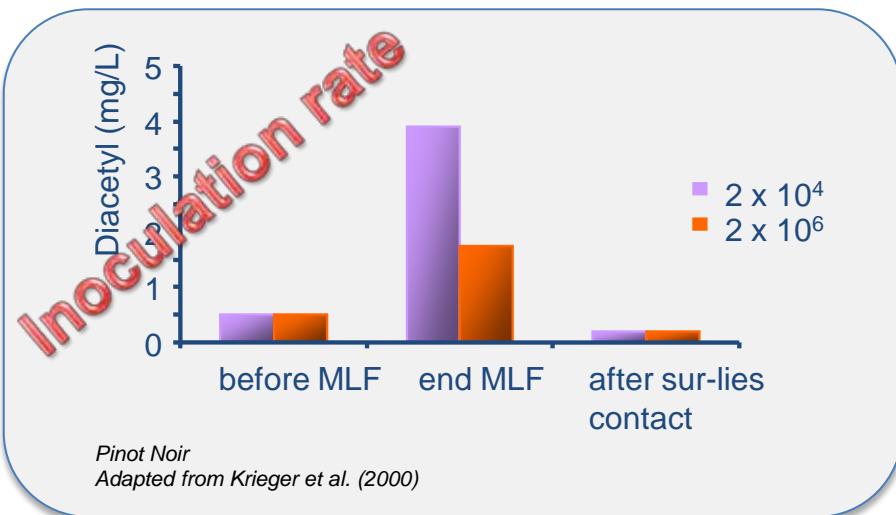
Kinetics



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

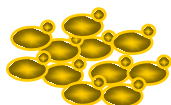
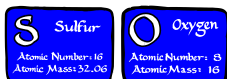


Many factors influence Diacetyl

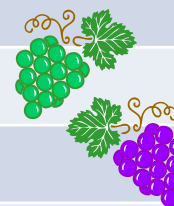




Winemaking parameters & diacetyl

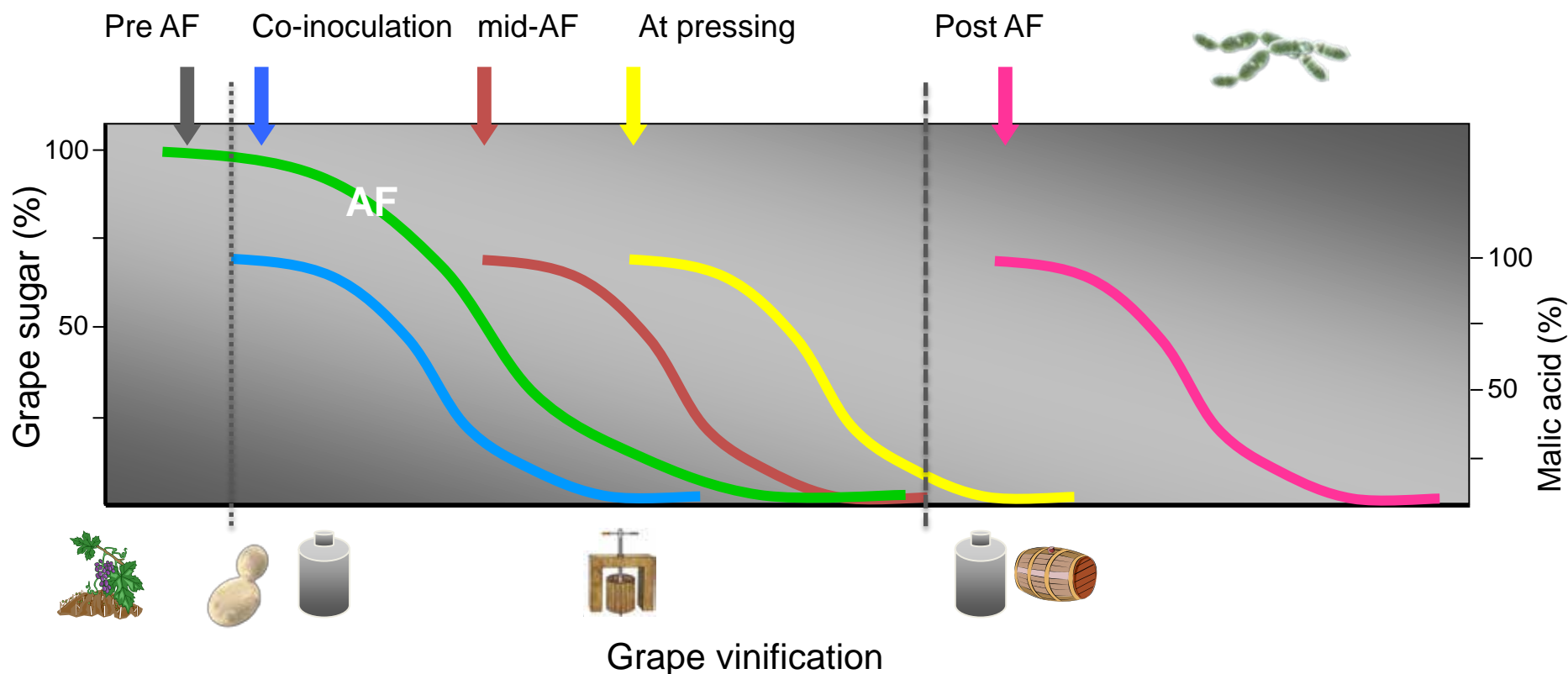


Variable		Effect on Diacetyl
<i>O. oeni</i> strain		Variable
Wine type	White	Lower
	Red	Higher
Inoculation rate	10^4	Higher
	10^6	Lower
Fermentation time	Longer MLF	Higher
Temperature	18°C	Higher
	25°C	Lower
SO ₂		Binds to diacetyl
Aeration (O ₂)	Air	Higher
	Anaerobic	lower
Yeast lees	Long contact	Lower
pH	Lower	May favour production



AF + MLF inoculation regime

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



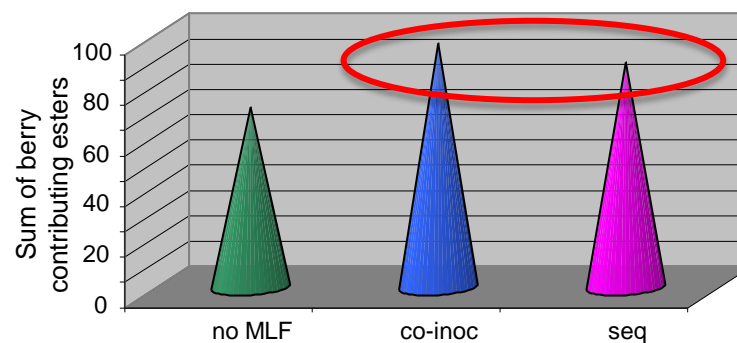
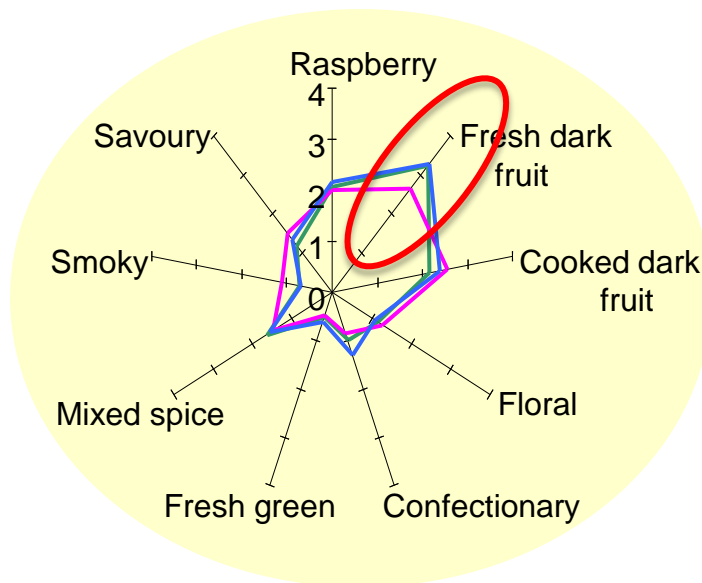
AF/MLF - fruity characters in red wine



The Australian Wine
Research Institute

✓ Esters associated with berry fruit attributes

§ Summation of berry fruit esters



AF/MLF - fruity characters in red wine



The Australian Wine Research Institute

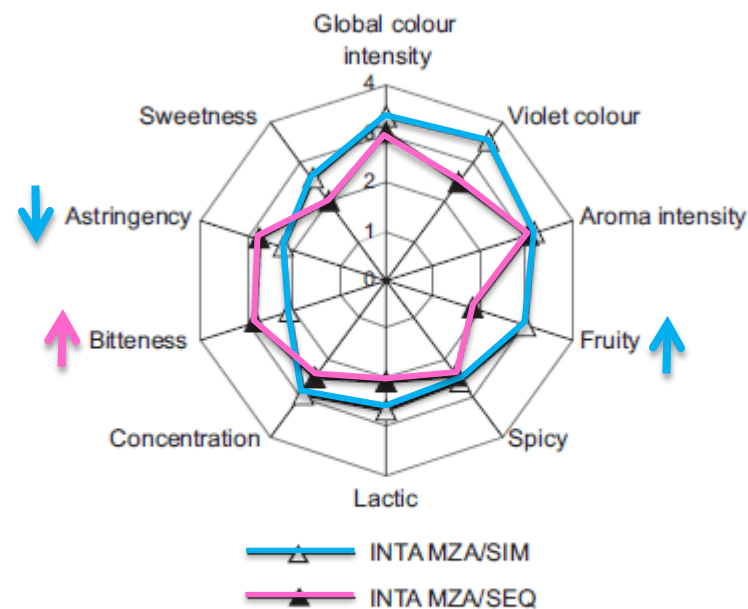
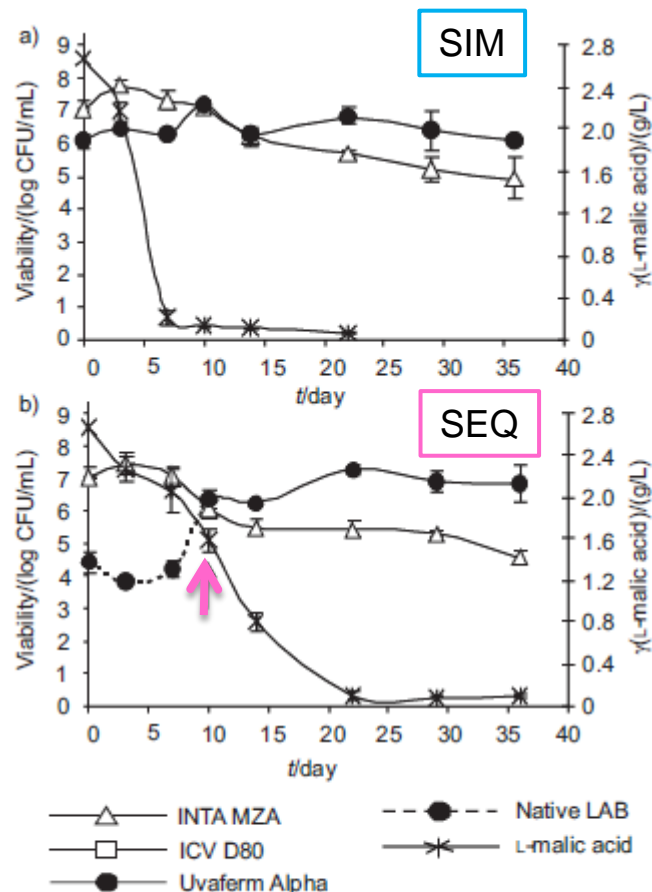


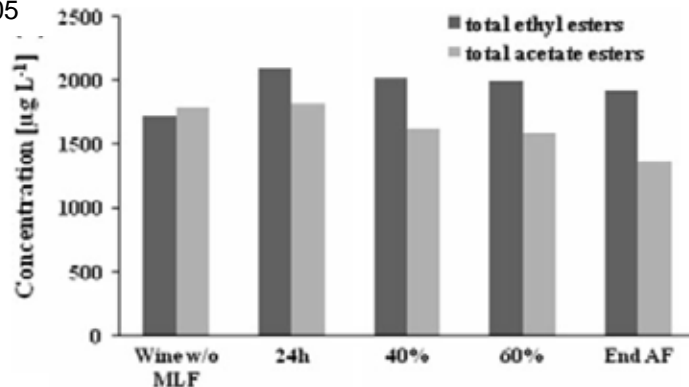
Fig. 5. Sensory descriptors of Malbec wines from must B₆ fermented with *S. cerevisiae* strain (INTA MZA) and *Oenococcus oeni* strain (Uvaferm Alpha) in simultaneous (SIM) and sequential (SEQ) inoculations

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

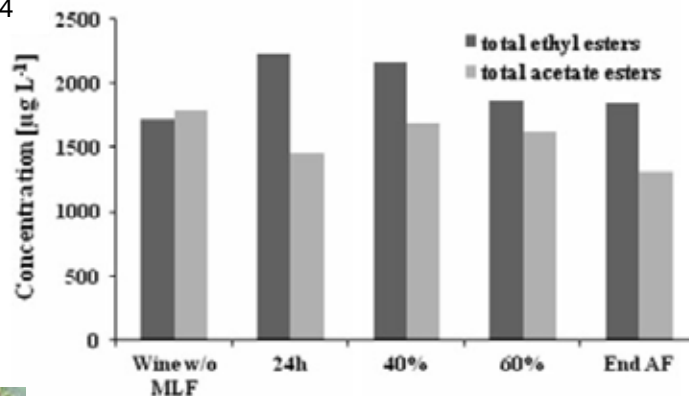


AF/MLF – white wine

O. oeni
R1105

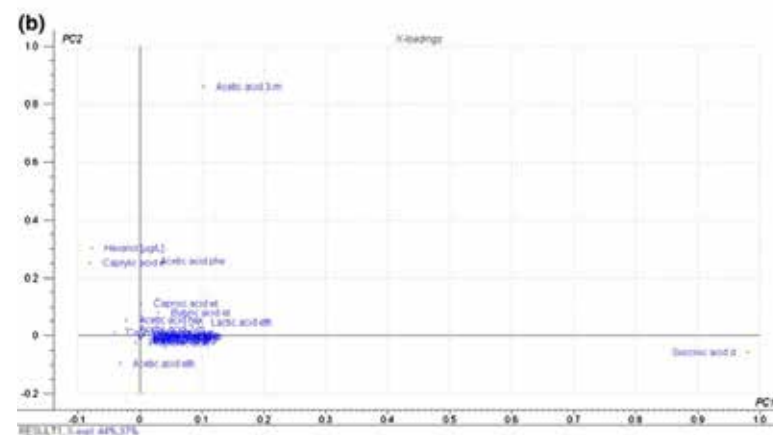
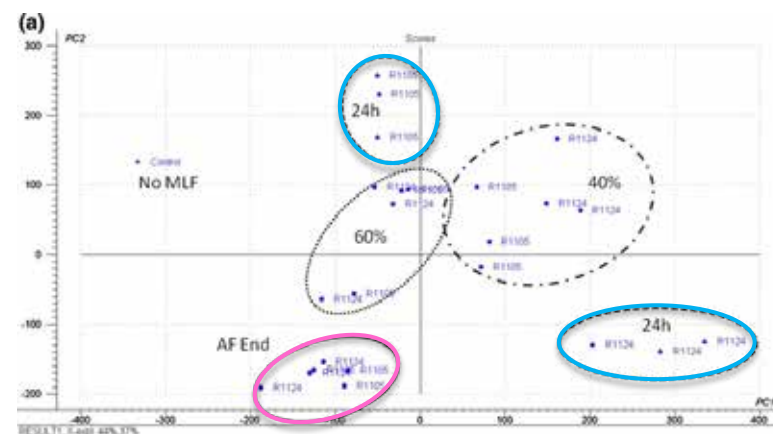


O. oeni
R1124



Riesling (2010)
Rheingau

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



Bacterial metabolism

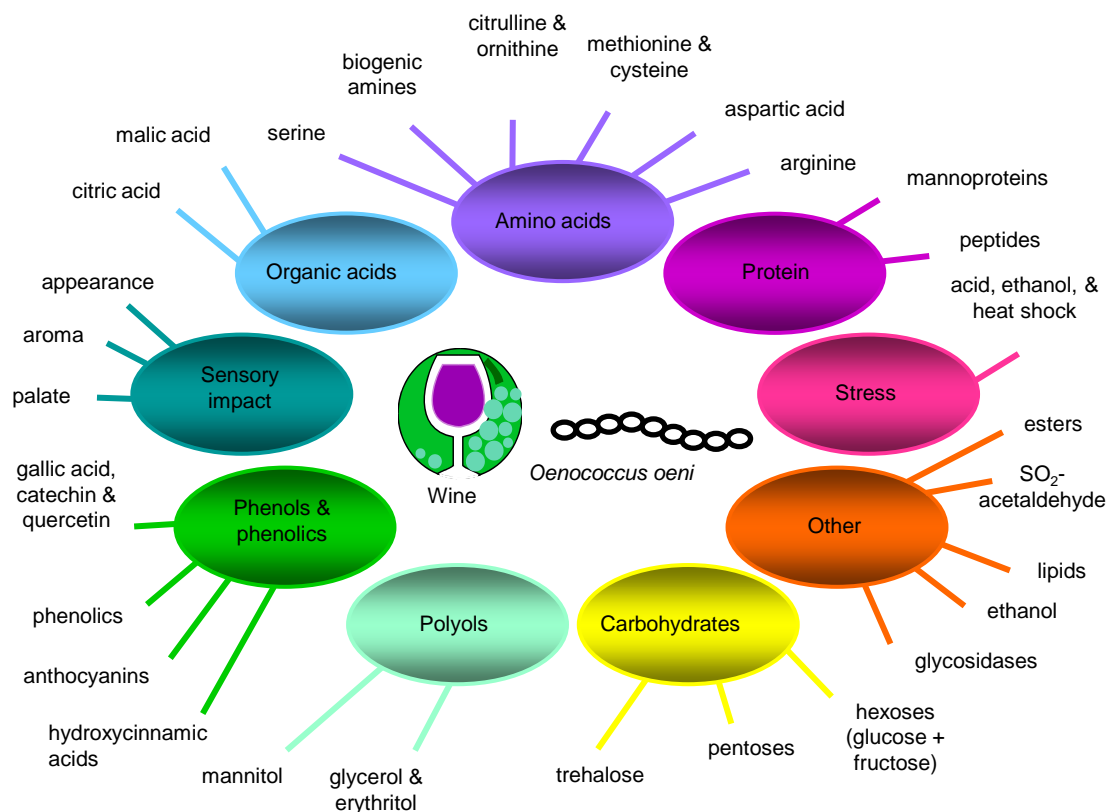


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∨ Factors affecting bacterial metabolism during MLF

§ Wine composition

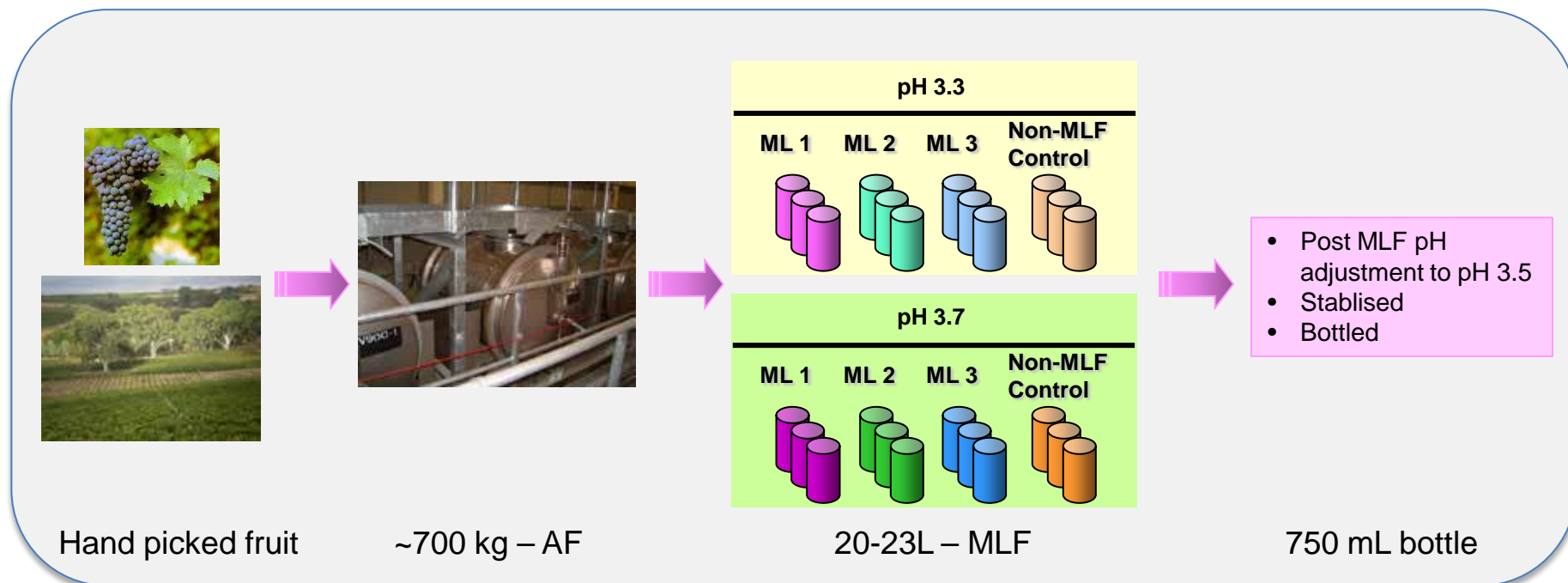
§ pH





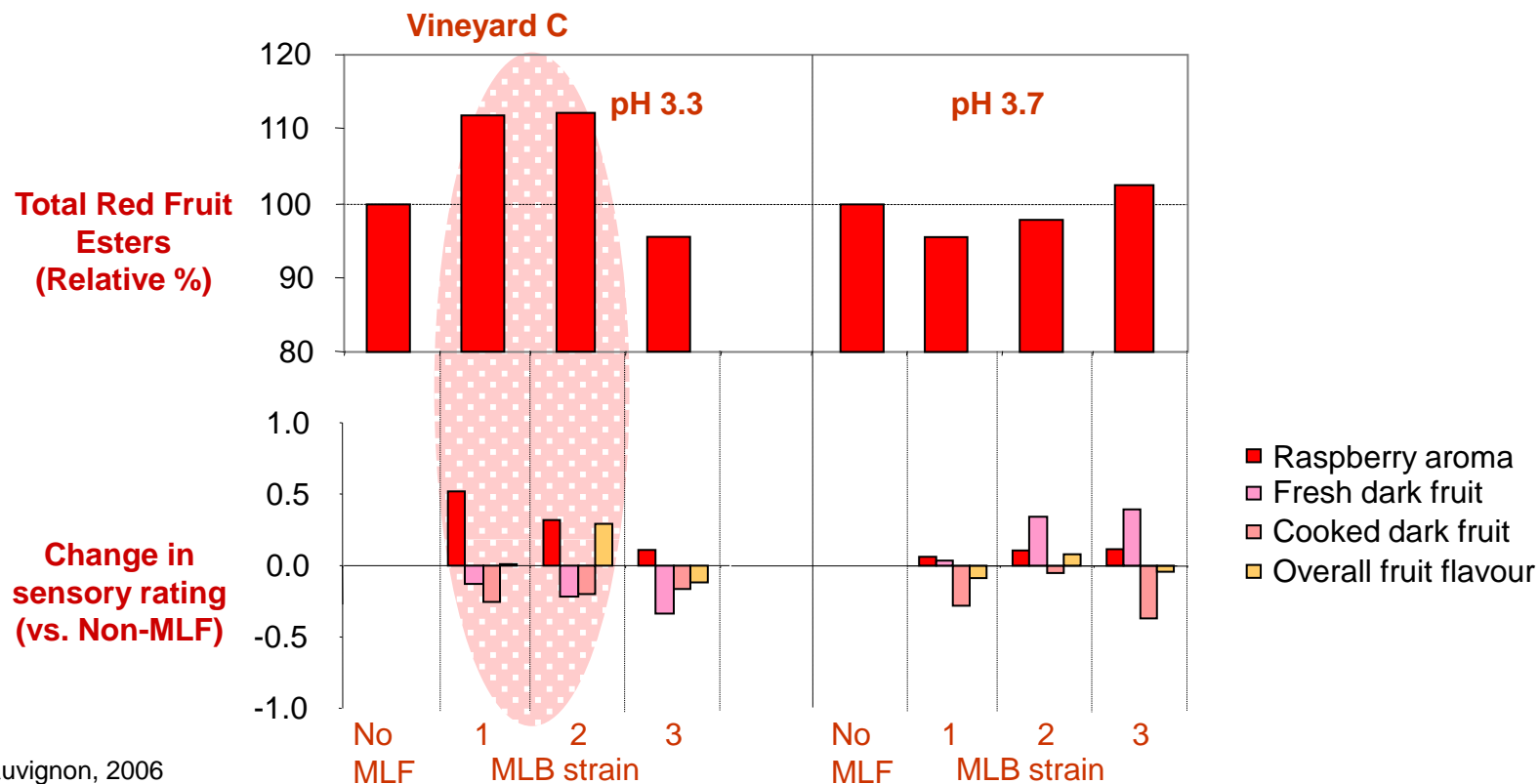
Influence of pH

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





Fruity characters in red wine



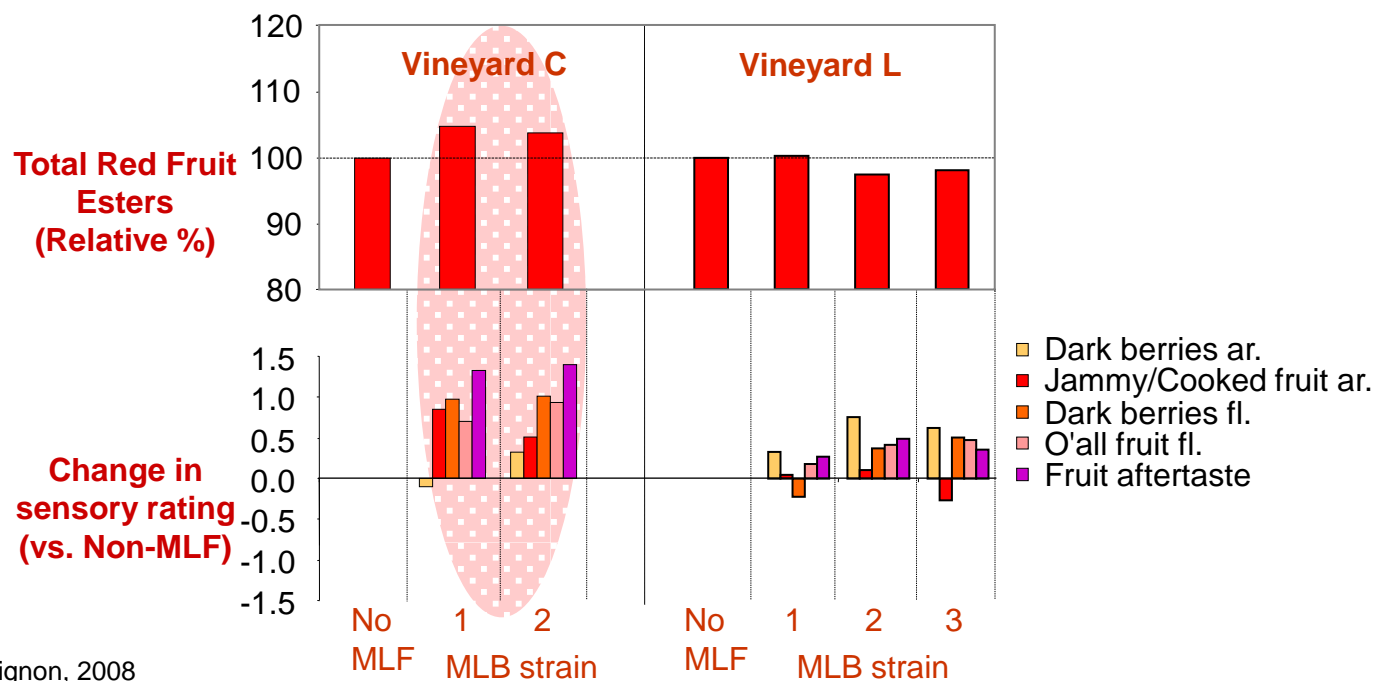
Cabernet Sauvignon, 2006



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



Fruity characters in red wine



Cabernet Sauvignon, 2008

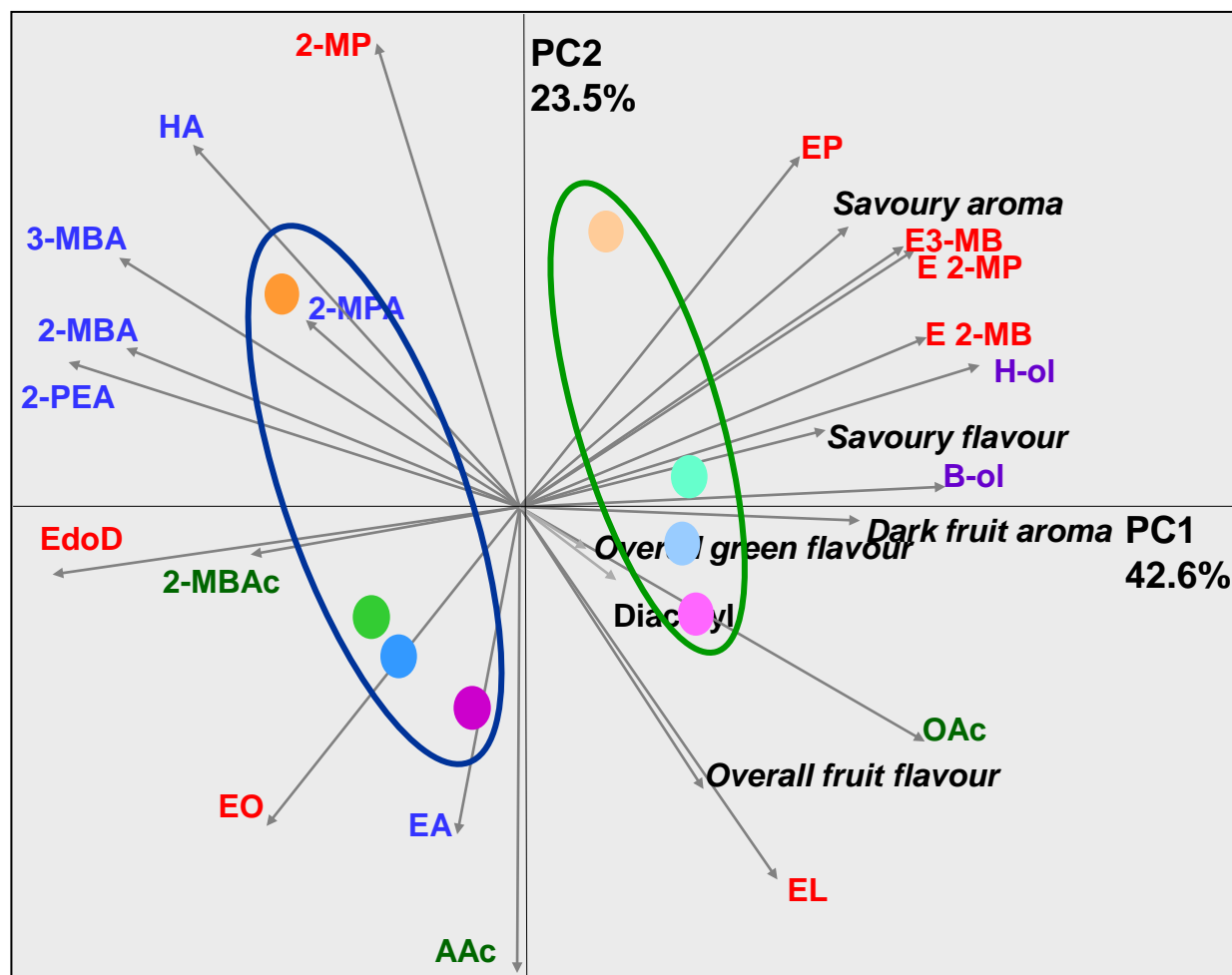


- Consistency with ML strains & vineyard over vintages
- Differences between vineyards

Wine pH affects *O. oeni* metabolism



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Limestone Coast

pH 3.3

No MLF

MLF

pH 3.7

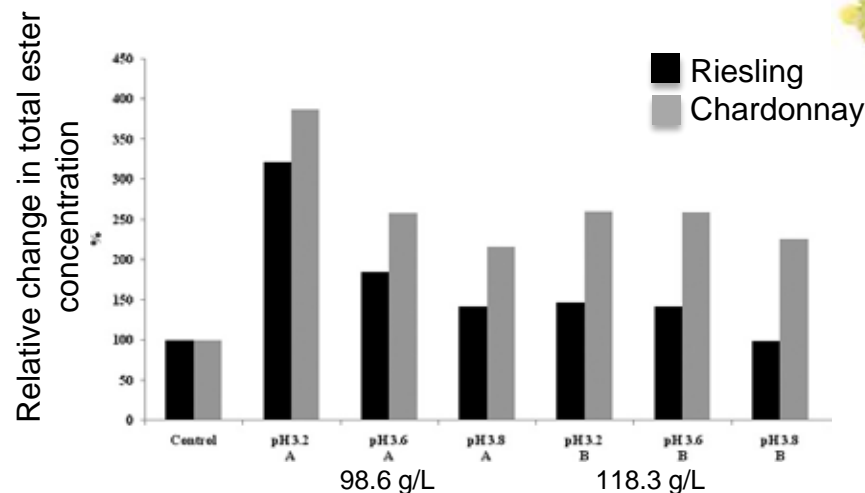
No MLF

MLF

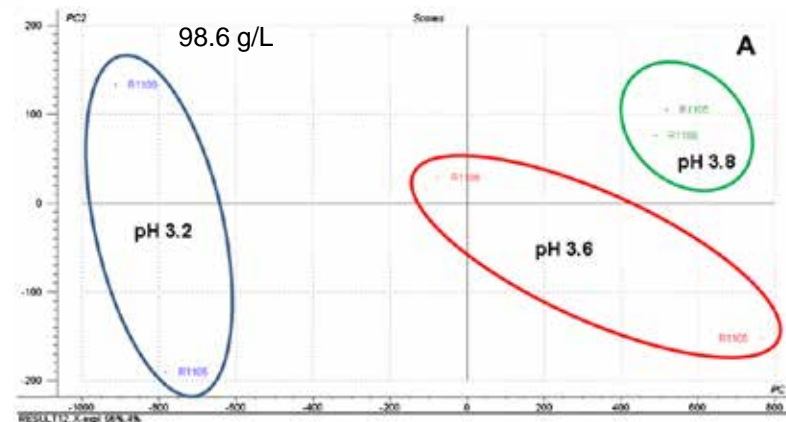


Cabernet Sauvignon,
Limestone Coast 2006

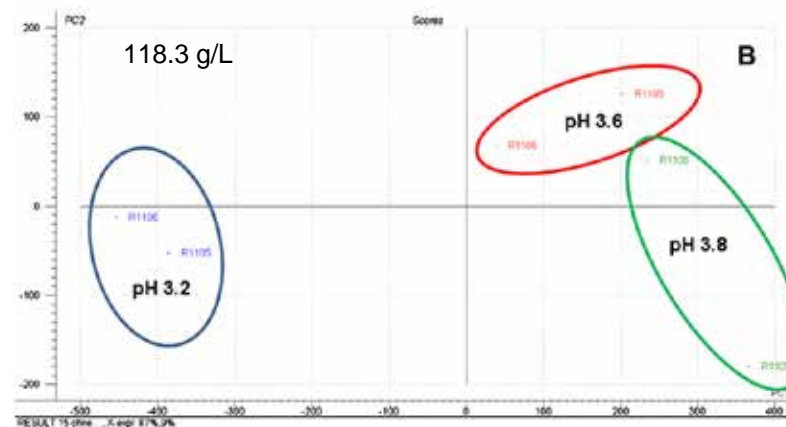
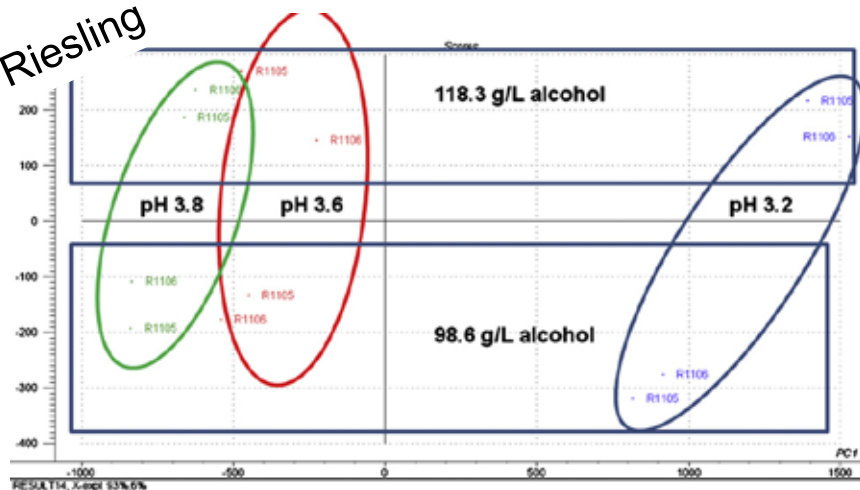
pH affects *O. oeni* metabolism



Chardonnay



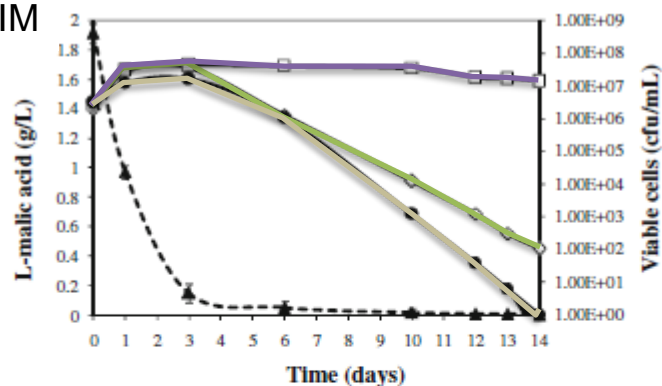
Riesling



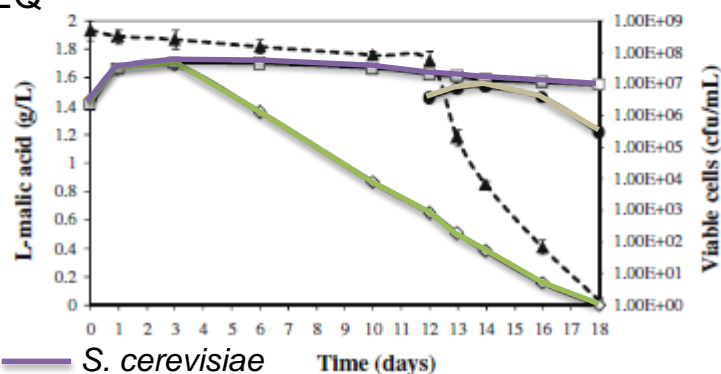


Yeast and bacteria

SIM



SEQ

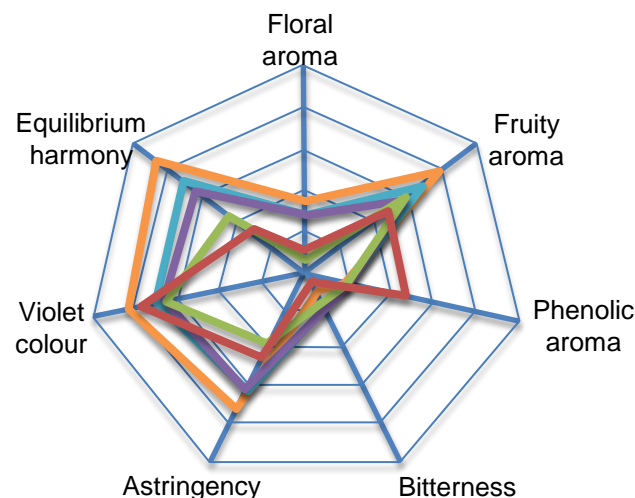


— *S. cerevisiae*
— *K. apiculata*
— *O. Oeni*
--- Malic acid

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

Malbec (2009)
Argentina

		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



— Mixed Ka & Sc
— *S. cerevisiae*
— *K. apiculata*
— Mixed yeast SIM MLF
— Mixed yeast SEQ MLF

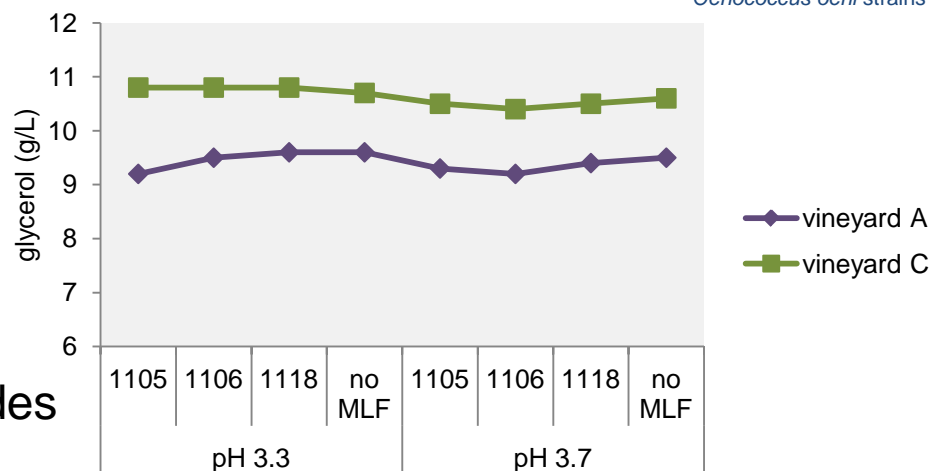


Palate and MLF

✓ MLF can influence the palate of wine

✓ Associated with volume and viscosity

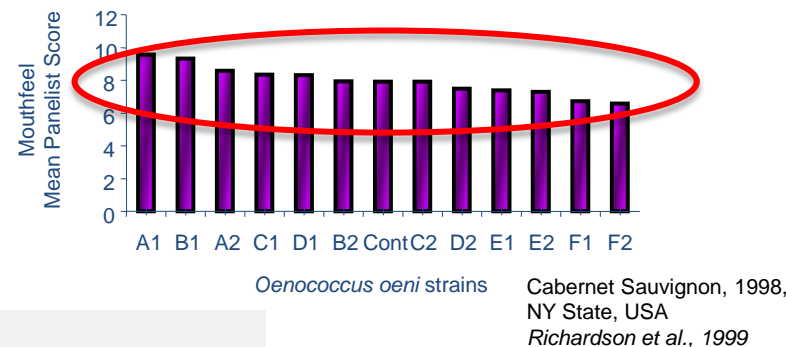
§ Glycerol



§ Exopolysaccharides

✓ Recent studies

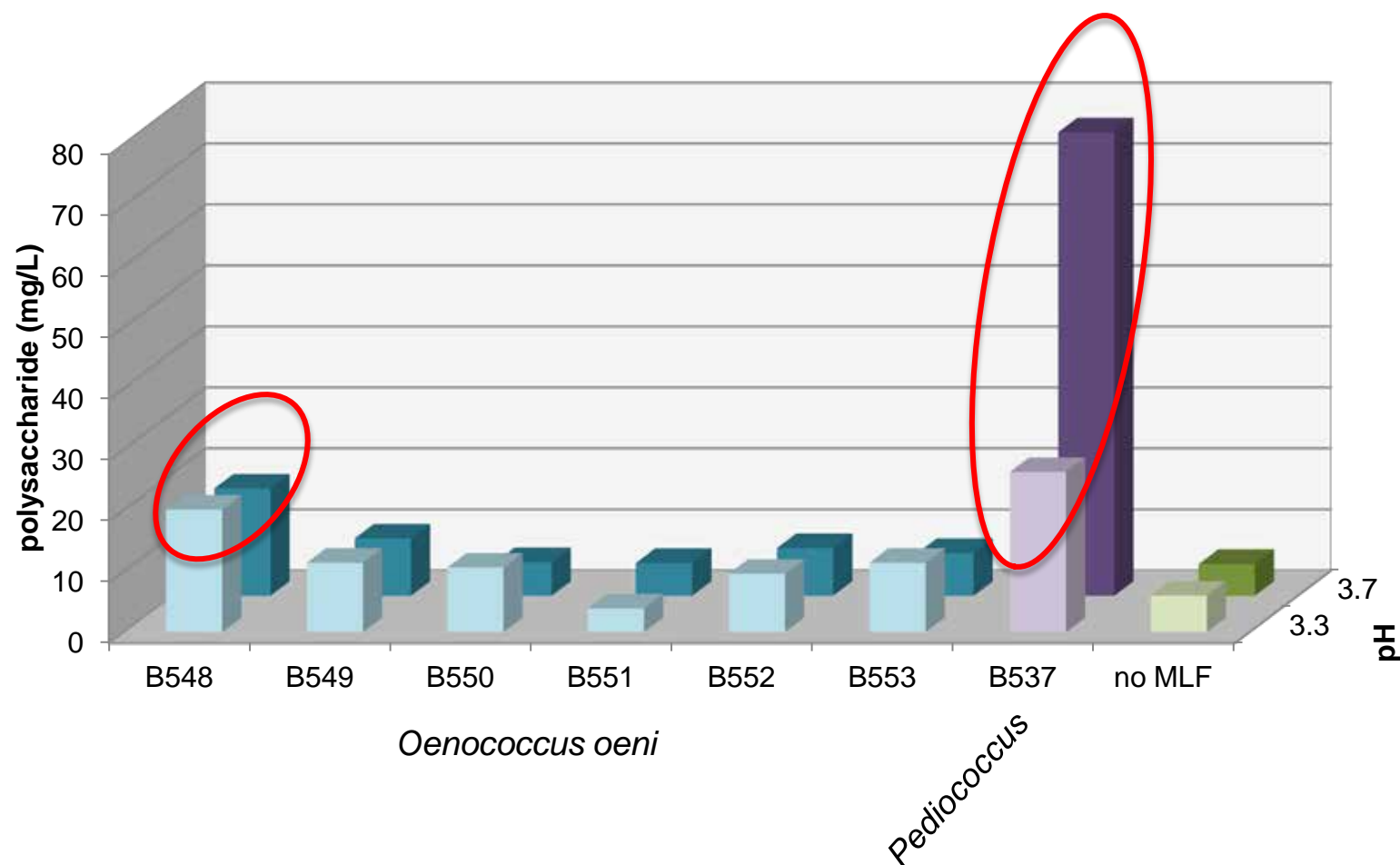
§ Some strains of *O. oeni* have the genes for exopolysaccharide production



Can *O. oeni* produce exopolysaccharides?



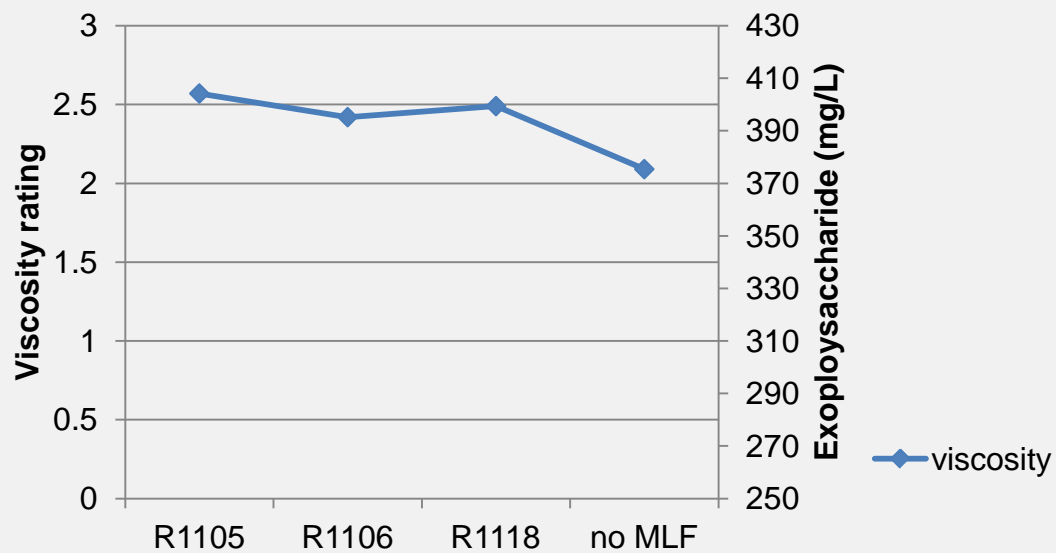
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Sensory & [exopolysaccharide]



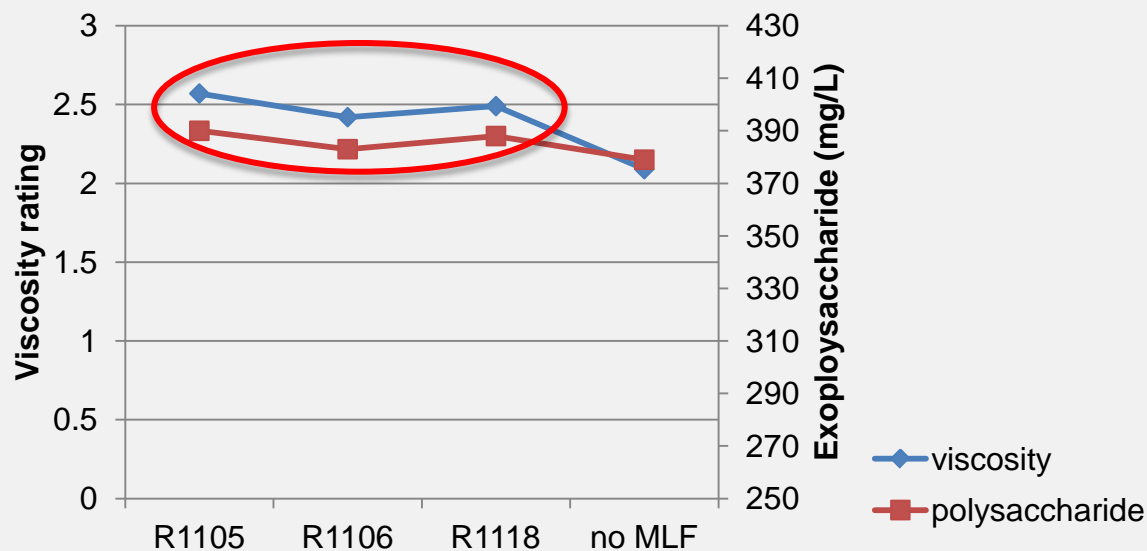
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Research Institute



Sensory & [exopolysaccharide]



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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?



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ü pH can influence bacterial metabolism

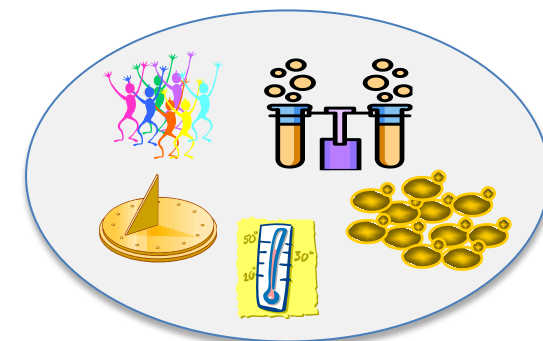
Ø affects wine aroma and flavour



ü Diacetyl – buttery attribute



§ Can be managed through winemaking practices



ü Berry & fruity characters in red wine

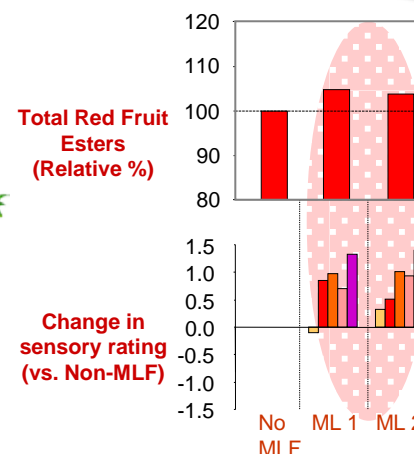
§ Strain

§ Lower pH



✓ Mouthfeel

§ Exopolysaccharide production



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

Acknowledgments



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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

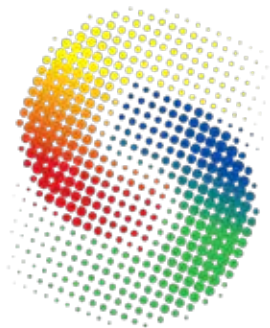
LALLEMAND

- ✓ 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

Cold Stability, CMCs and other crystallization inhibitors.

Dr Eric Wilkes

Group Manager Commercial Services



AWRI
COMMERCIAL SERVICES
SO MUCH MORE THAN A GREAT LAB



Tartrate instability



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“The deposit is harmless,
but the customers
reaction might not
be”potassium
hydrogen tartrate.....

Bryce Rankine, 1989



Cold stability, what is it?



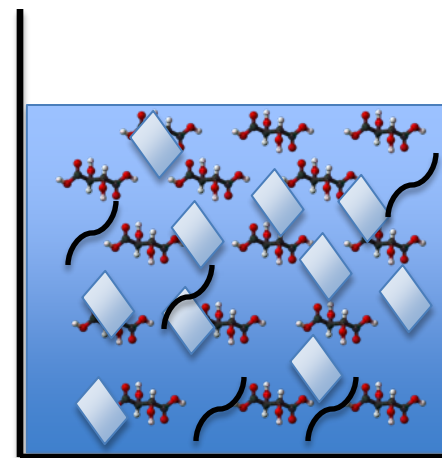
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§ **Cold stability** is essentially a wines ability to resist the precipitation of tartrates.

§ Components in wine (crystallization inhibitors) help prevent the tartrate from precipitating.

§ As the wine matures or undergoes winemaking processes the levels of these inhibition compounds can change, allowing tartrate to precipitate.

§ This can happen even after traditional cold stabilization.



Wine cold stabilisation methods



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✓ Elimination/reduction of precursor compounds (e.g. potassium, bitartrate):

§ Traditional slow cold stabilization

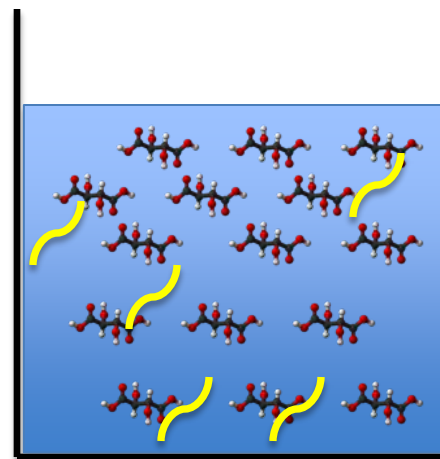
§ Rapid contact stabilization

- Batch
- Continuous

§ Ion-exchange

§ Membrane processes

- Electrodialysis



✓ Crystallisation inhibitors:

§ Metatartaric acid

§ Yeast mannoproteins

§ Carboxymethylcellulose (CMC)

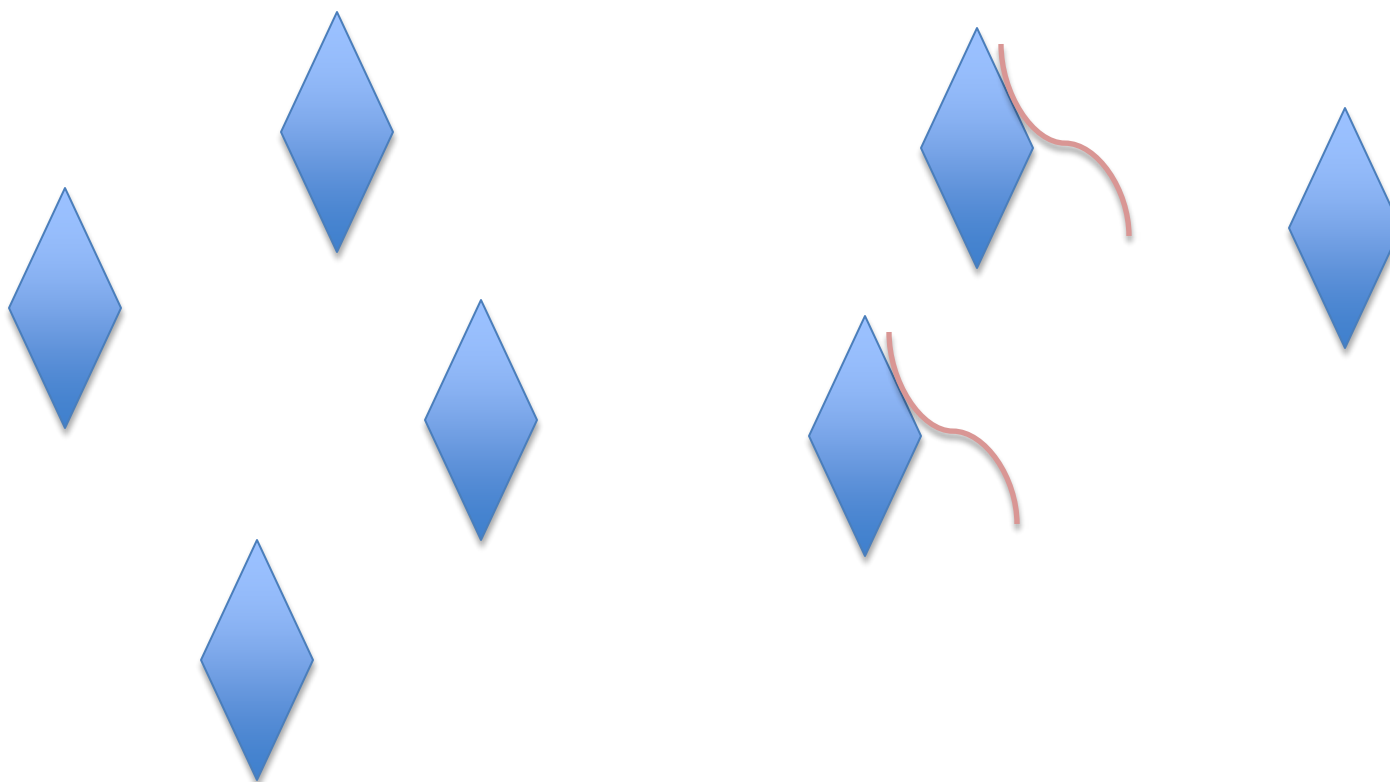
Crystallization inhibitors



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How do they work

- § They block potassium bitartrate crystal nucleation and growth by binding with one of the crystal faces preventing further growth and the appearance of visible crystals.



Why use crystallization inhibitors?

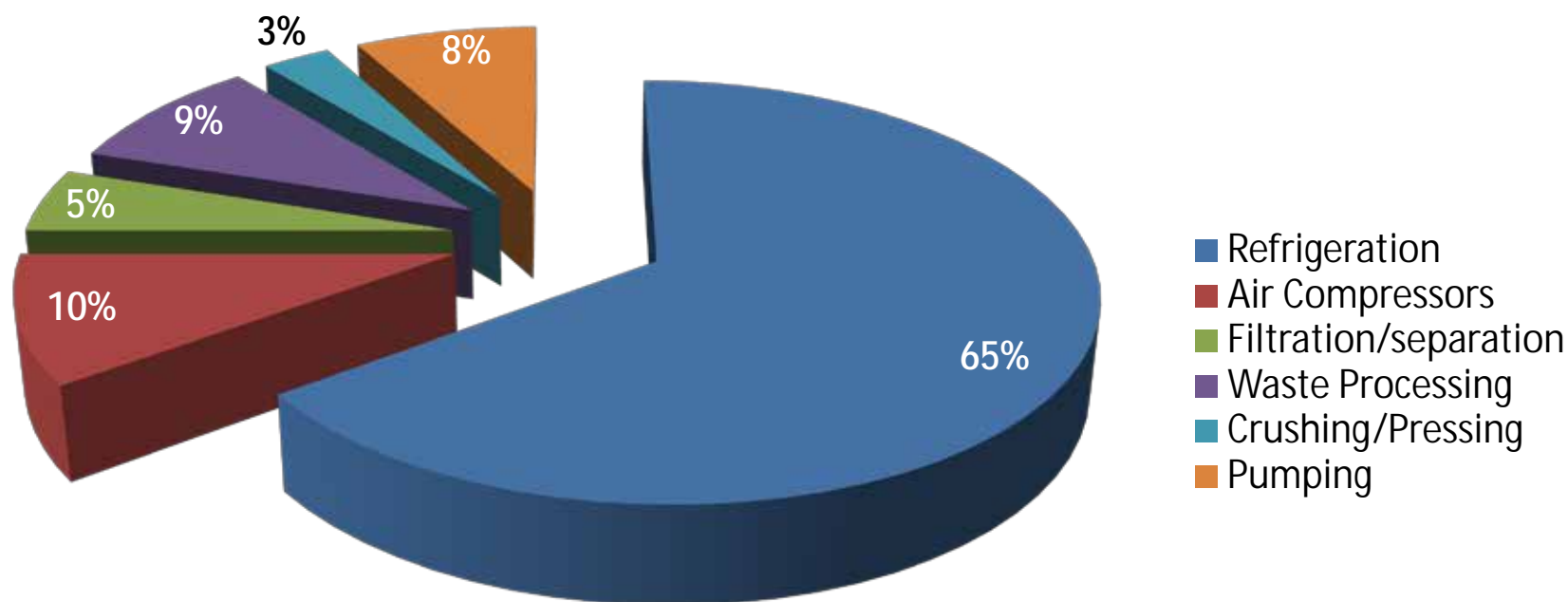


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Less impact on wine pH/TA than traditional stabilization by tartrate elimination.

Labour and time savings.

Lower energy impacts than traditional refrigeration.



Metatartaric acid



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✓ First used in Europe in 1950s.

Polymeric structure produced by heating of tartaric acid to 160 °C.

✓ Very effective at preventing tartrate precipitation (as long as it is there).

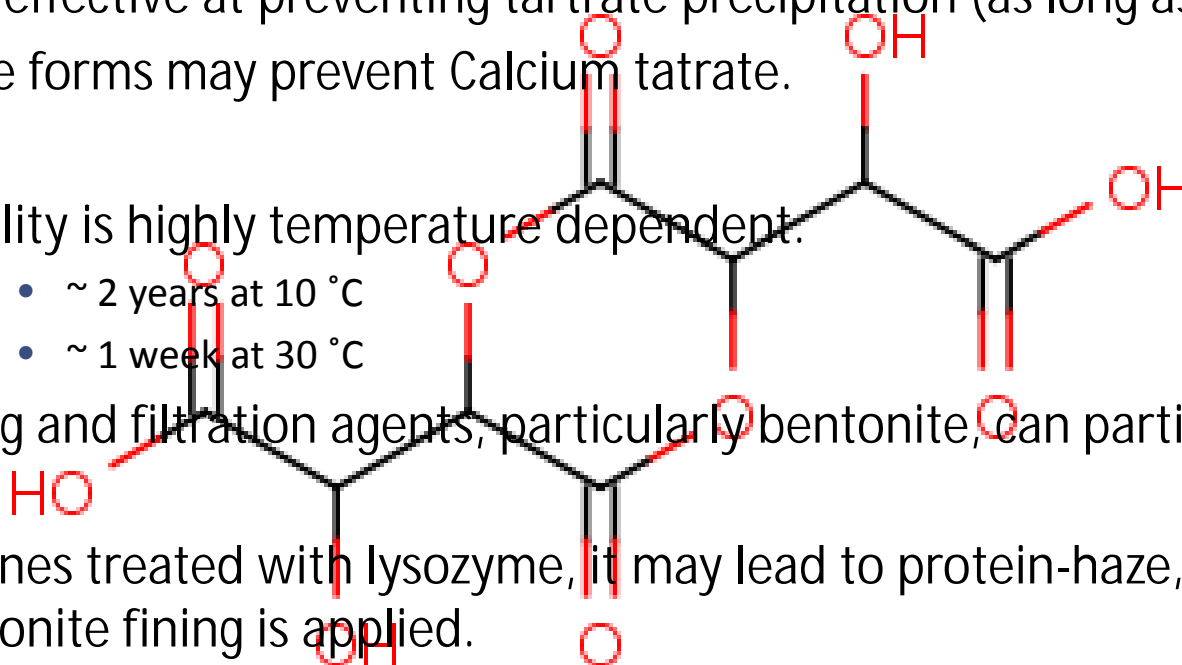
✓ Some forms may prevent Calcium tartrate.

✗ Stability is highly temperature dependent.

- ~ 2 years at 10 °C
- ~ 1 week at 30 °C

✗ Fining and filtration agents, particularly bentonite, can partially remove it.

✗ In wines treated with lysozyme, it may lead to protein-haze, even if bentonite fining is applied.



Yeast mannoproteins



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- ✓ Derived from wine - a specific fraction is extracted from yeast cell walls by enzymatic hydrolysis

✓ **Much more** stable than metatartaric acid to warm temperatures.

✓ Effective at preventing precipitation in many wines

✗ Important to perform bench trials first, as close to as bottling as possible.

✗ Need to add after fining and pre-filtration as fining and filtration using diatomaceous earth, perlite or cellulose fibres may remove them.

✗ May not be entirely suitable if a wine is supersaturated, if a high addition rate is needed (product may flocculate)

✗ Ineffective against calcium tartrate precipitation.

✗ *May interact with other wine components over time and become ineffective.*

← Mannoprotein

← β -Glucan

← β -Glucan + Chitin

← Mannoprotein

← Membrane

Carboxymethylcellulose



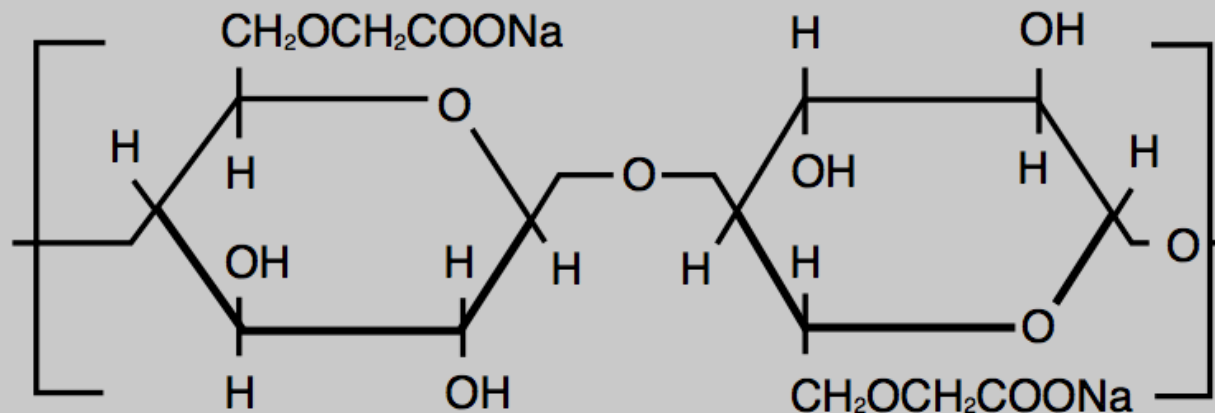
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What is it?

§ Polymer synthesized by the alkali-catalyzed reaction of cellulose.



E466



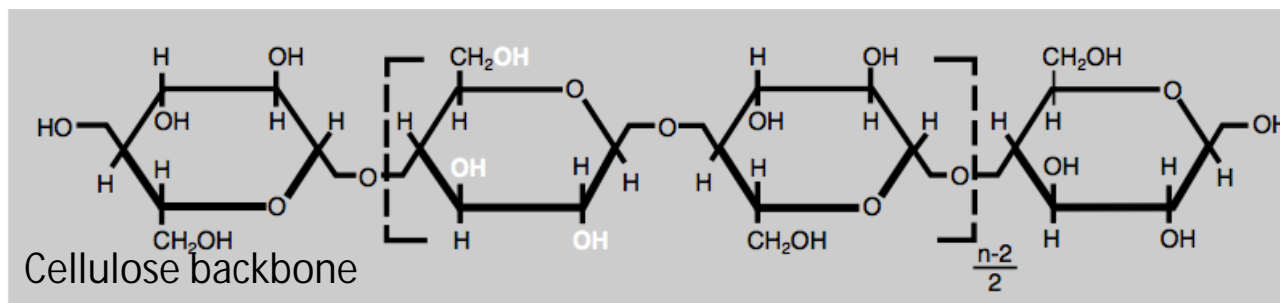
What differences are there?



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Substitution rates

- § Number of -OH groups substituted by carboxymethyl groups.
- § OIV specification is 0.6 to 0.95.



Polymer length

- § Impacts solubility and viscosity.
- § OIV between 17 and 300 kiloDaltons.

Filtration



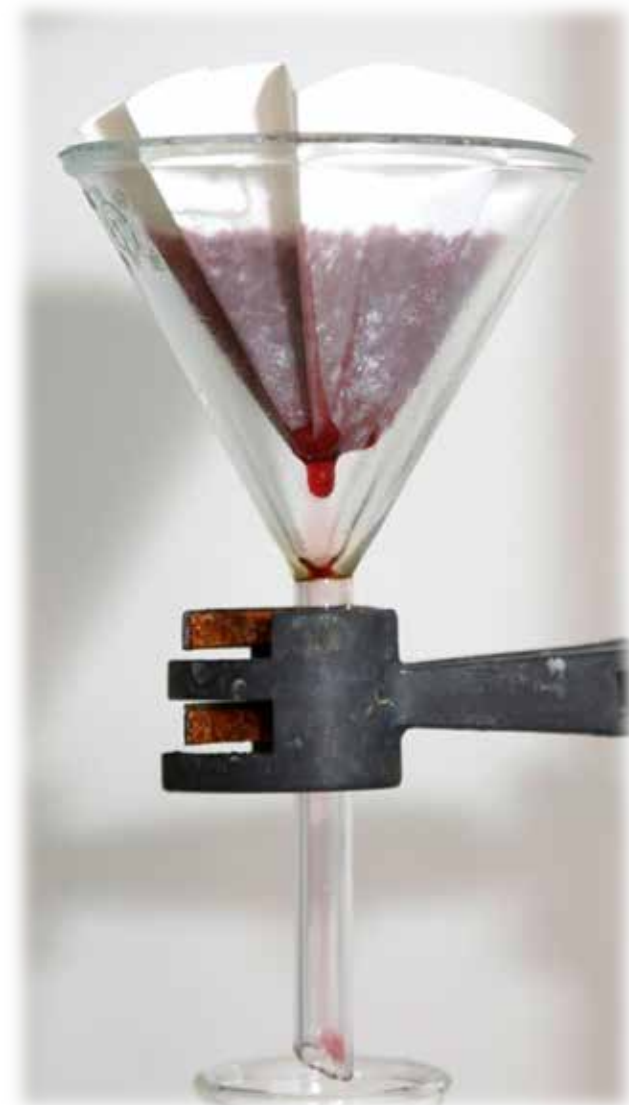
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CMC's don't instantly integrate with the wine!
They can take 2 to 7 days to fully integrate
(depending on temperature and CMC).

Most trials have suggested that if allowed to
fully integrate there is very little impact on
filterability.

Important to leave this time before cellar (and
tasting) operations.

A bigger issue may be actually getting them
to dissolve in the first place. Some real
advantages to using liquids!



Colour impacts



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Cellulose and its derivatives react with tannins (including pigmented tannins) and can precipitate out colour. As such CMC's are not recommended for reds.

Colour drop out not always apparent immediately, often only occurring after time at low temperature.

Even without significant colour dropout can get hazes.

Can be used with some **rose** but need to do trials first.



Haze formation



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Protein can crosslink with CMC's to form hazes.

This can happen post filtration and be temperature linked.

Wines to be treated with CMC ***must*** be protein stable.

Some literature has raised issues with certain metal ions also leading to cross linking and hazes. Only an issue for di and tri-valent species (eg, copper and iron) but no confirmed reports of this in wine.



Wine style and age

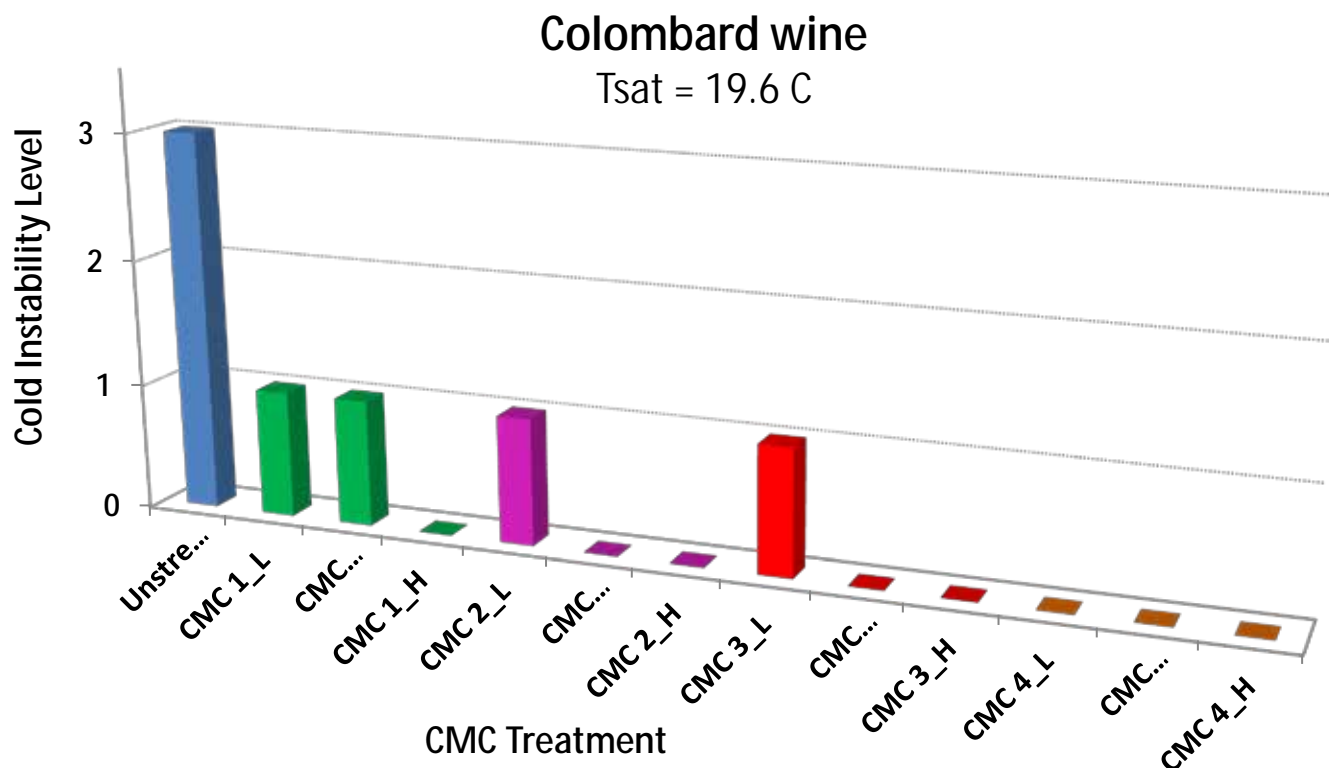


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Trials to date have given positive results for all wine styles tested.

Some question with younger wines with very high T-sat.

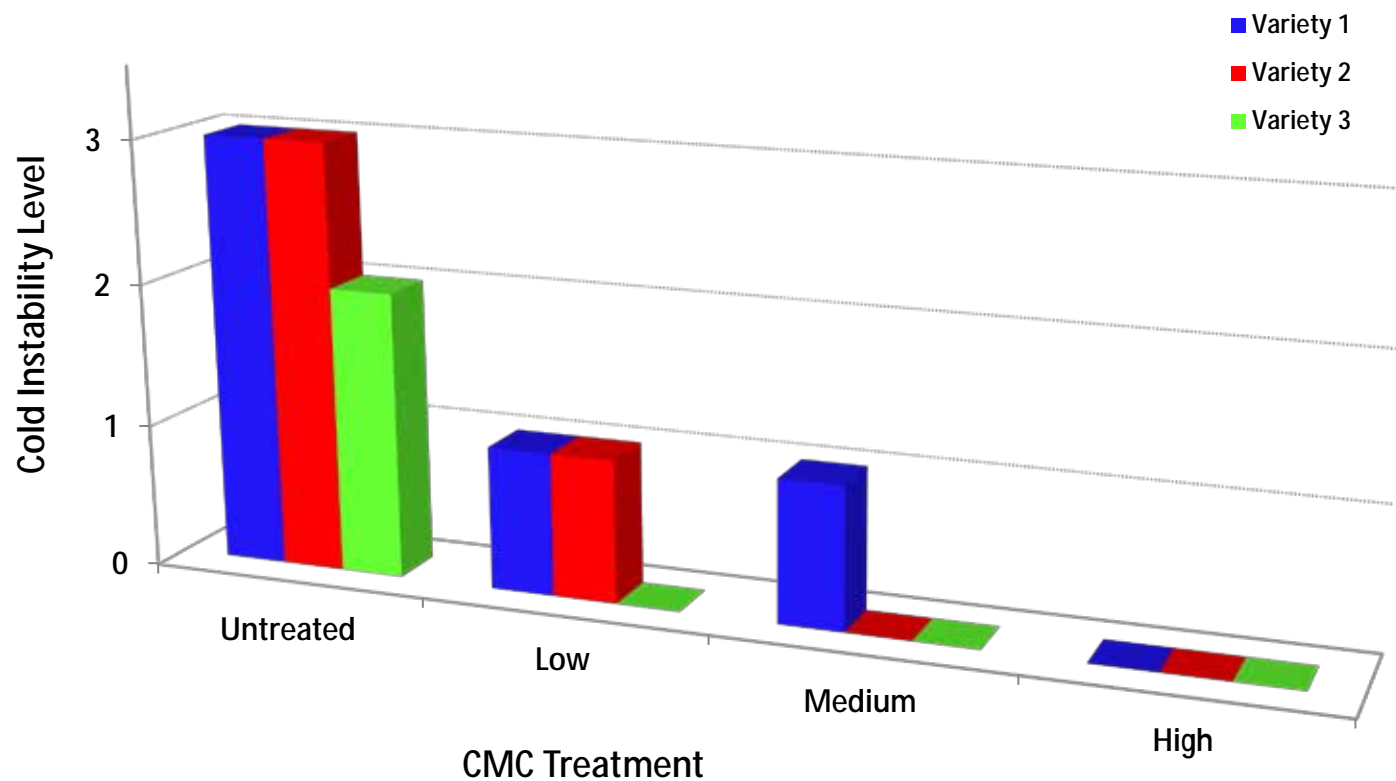
Also some evidence that in very young wines other compounds may be interacting with CMC's removing them from the equation.



Variety impact on CMC effectiveness

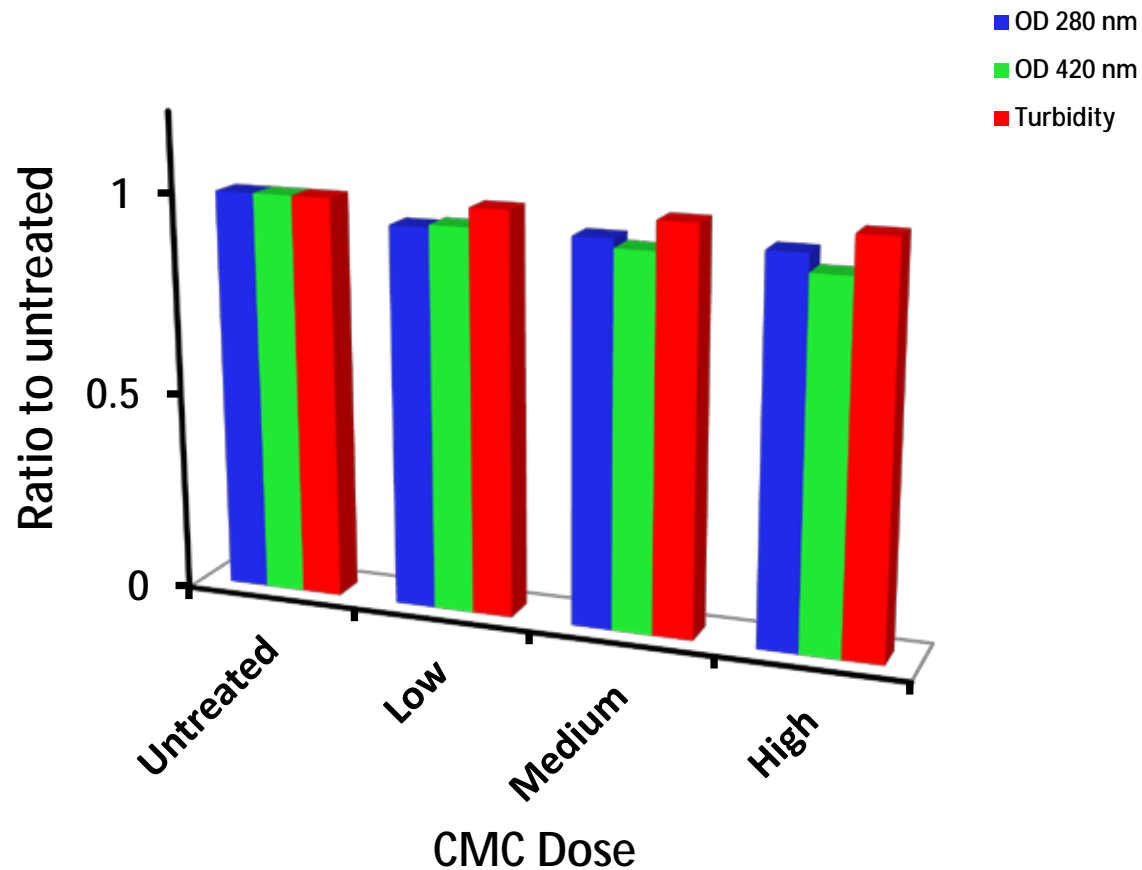


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Effects of CMC on white wine colour and turbidity



What else?



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Calcium stability

- § Trials published to date suggest that CMC's don't appear to be particularly effective in the prevention of calcium tartrate instability.
- § This may be because of the different crystal structure of CaT.

Flavour impacts

- § There are reports that CMC's can impact wine mouth feel.
- § Highly wine and CMC dependent.
- § Make sure trials give time for full integration.

Testing

- § Saturation temp gives no indication of CMC induced stability but does give an indication of suitability.
- § Brine test does work.
- § Mini contact can be impacted depending on seeding rates.



1. They can work!
2. Best used when packaging ready (cellar bright and protein stable).
3. Need 2 to 7 days post treatment before filtration.
4. Wines should have a Sat-Temp in the region of <20 .
5. Some wine styles may have other components that could reduce the effectiveness of a given dose of CMC.
6. Not a great idea for reds that you want to keep colour stable and haze free.
7. Must choose a CMC that is workable for your cellar operations.
8. Need to adjust testing regimes.

Questions?



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COMMERCIAL
SERVICES



VESDA- Developing tools to assist land managers and industry.

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

Department of
Environment and
Primary Industries



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-

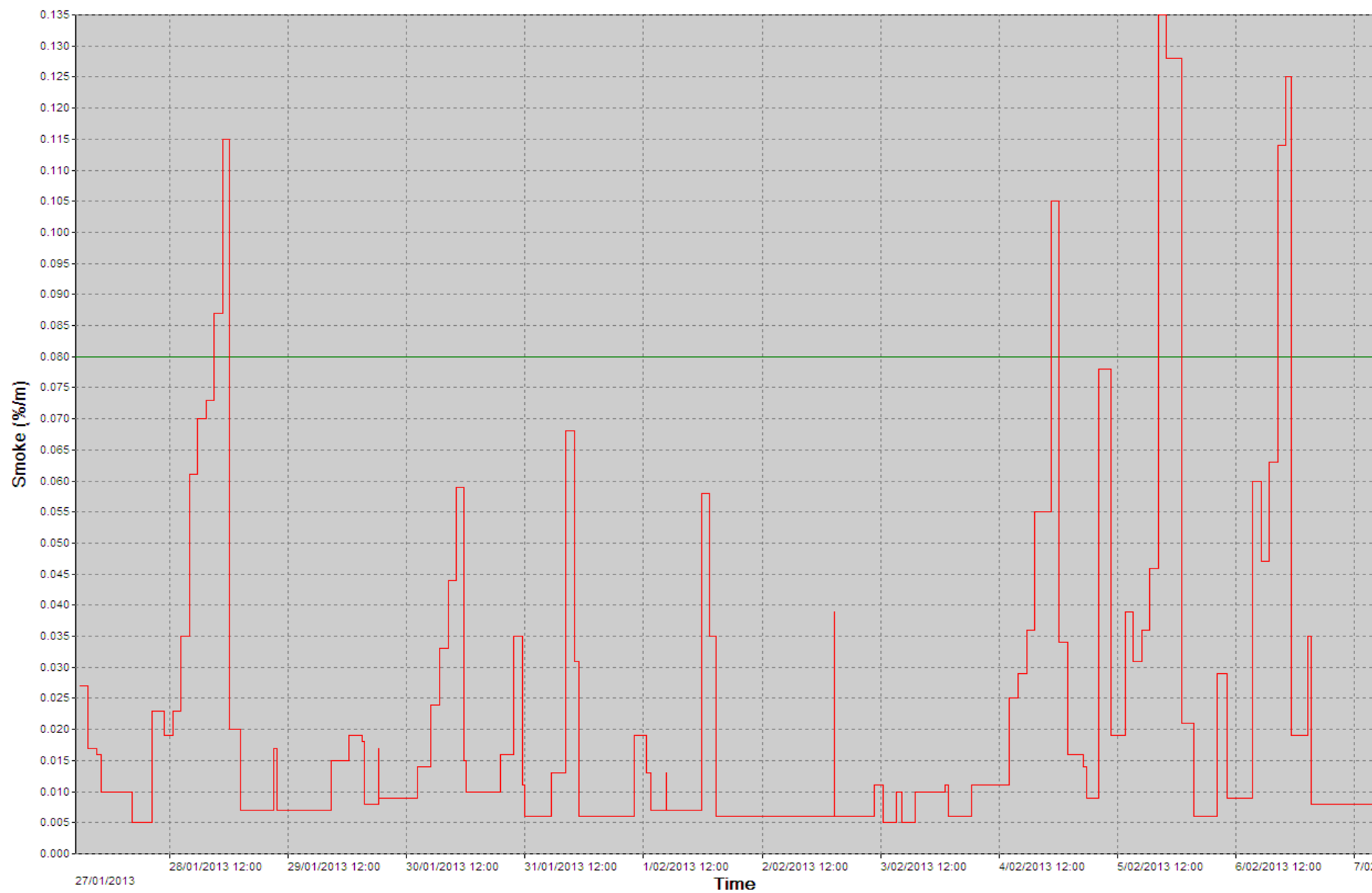
- Intensity of smoke- just like wine, very subjective, need to put a number on it.
- Duration of exposure-how long has the smoke been in the vineyard?

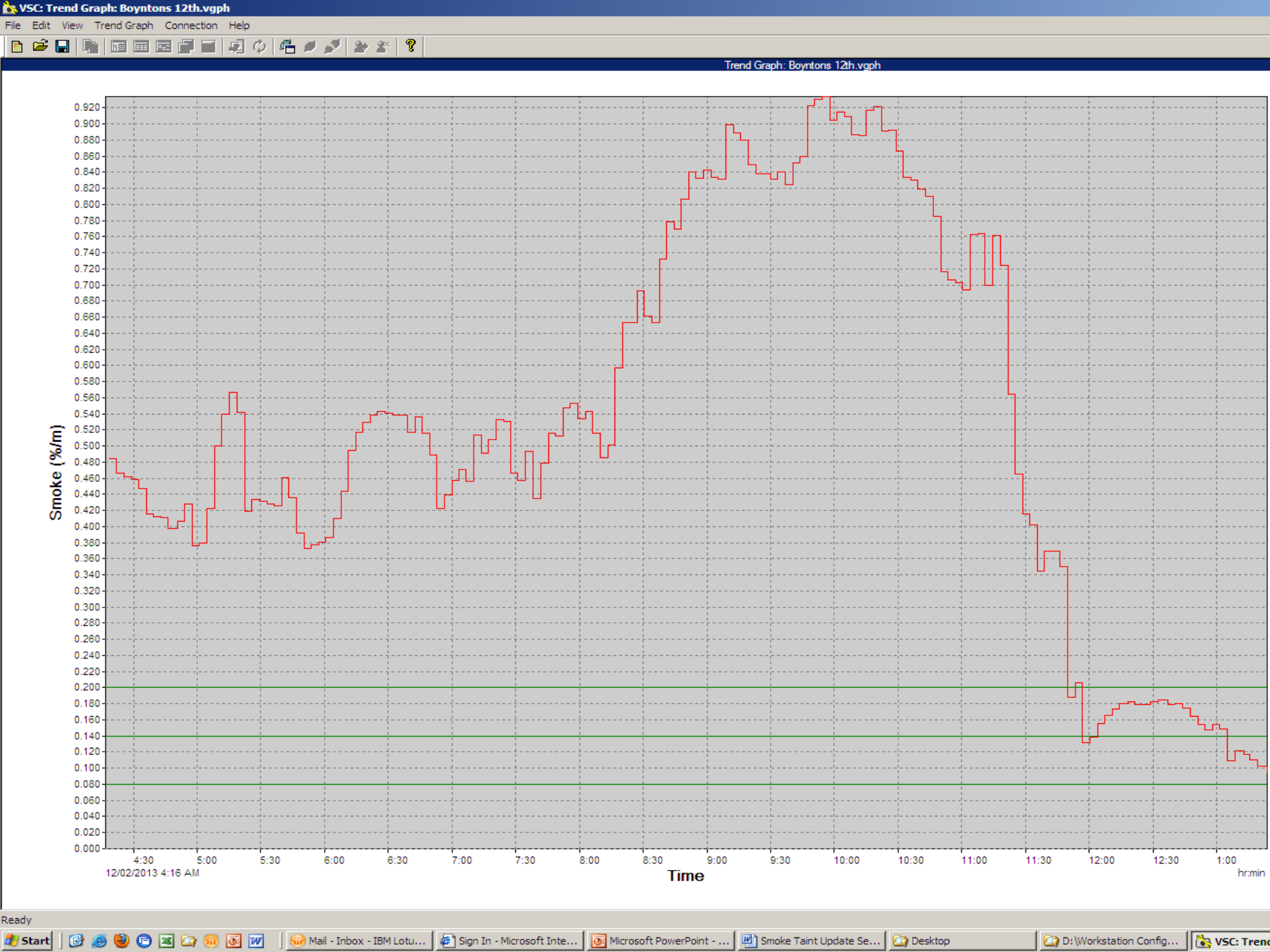
VESDA

- 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.



Trend Graph: Boyntons Feb 7th Graph.vgph





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.

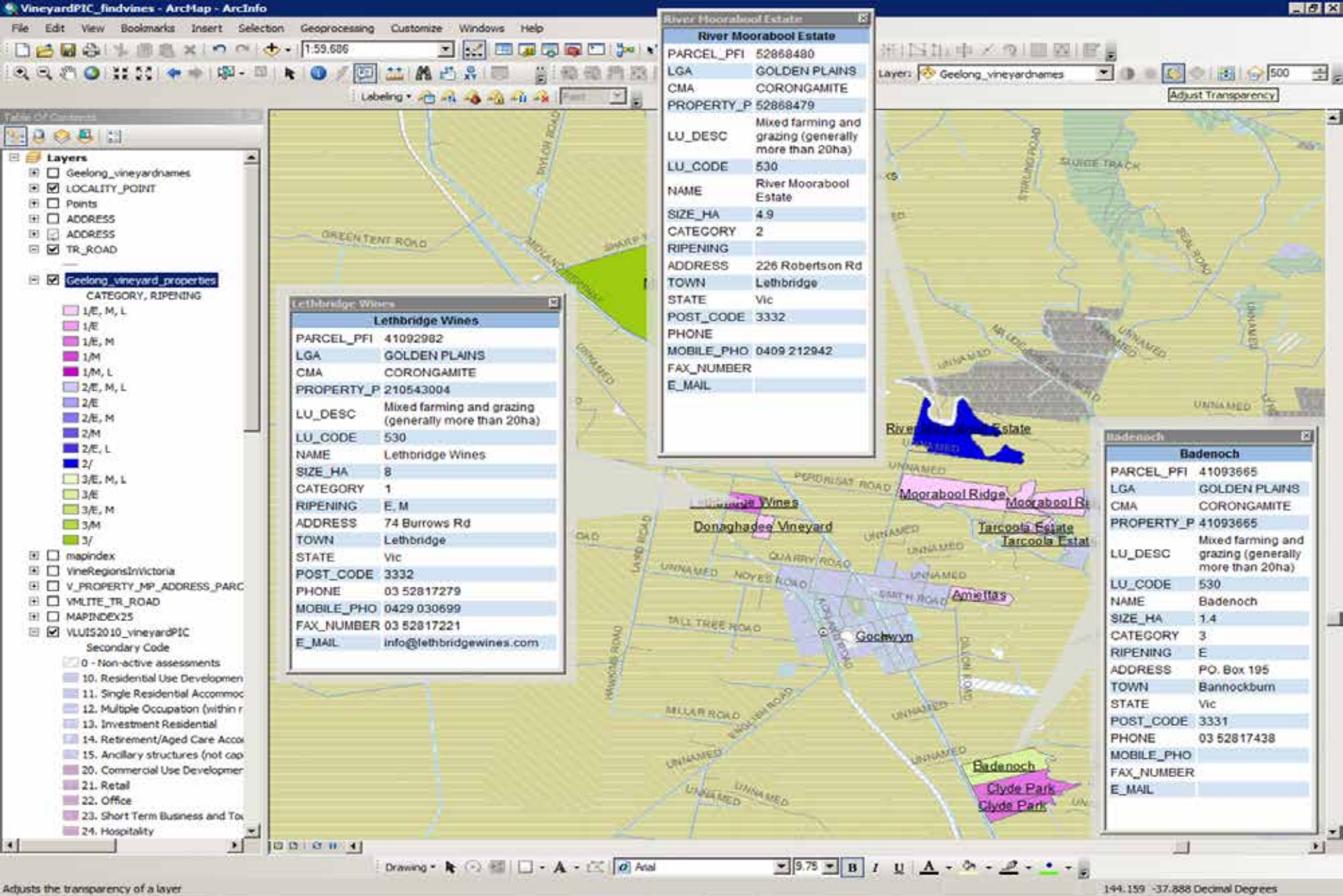
Who, What and Where??

Project Management Plan- Objective 6

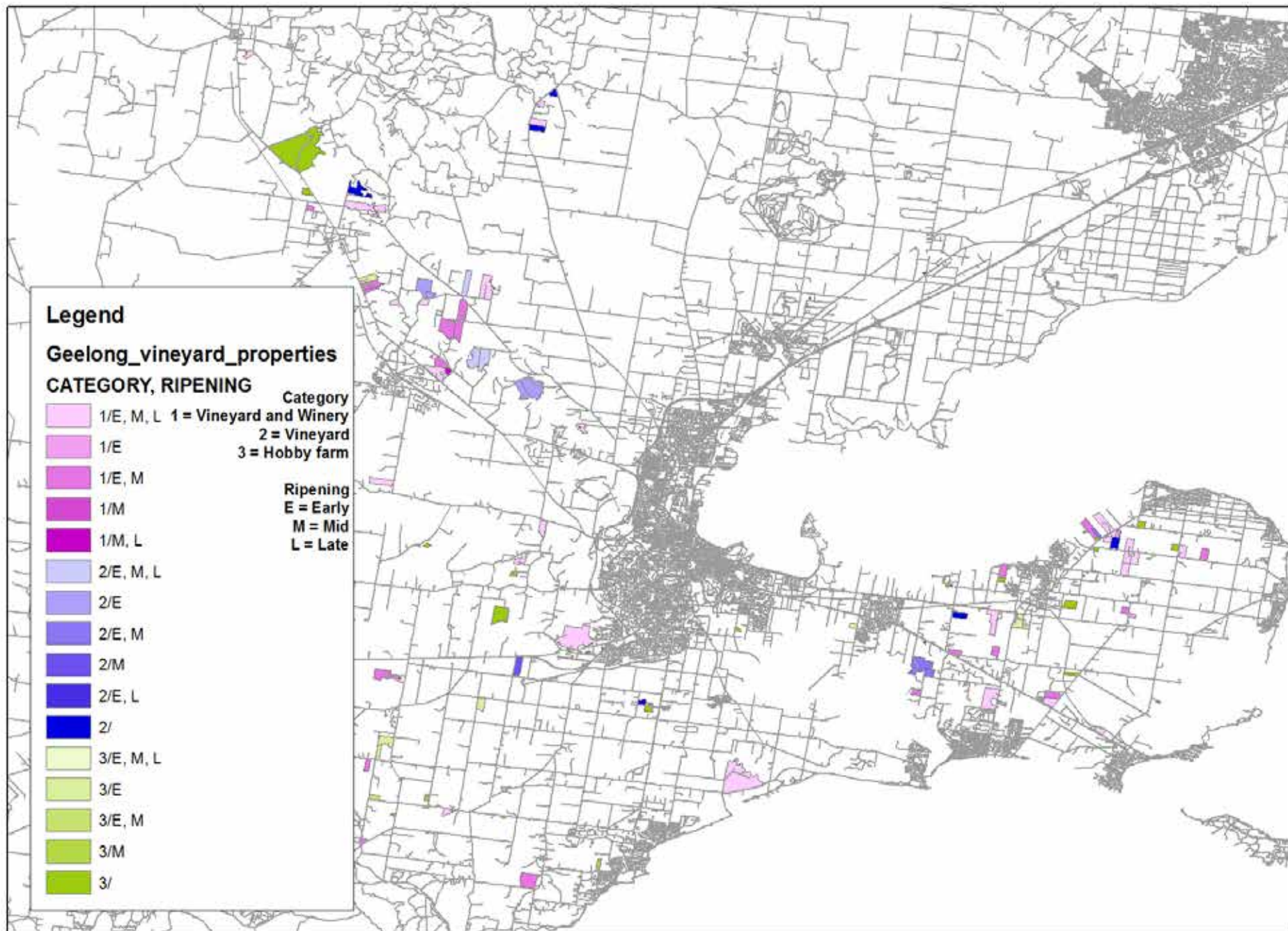
Develop and evaluate a risk assessment tool to enable industry and land managers to determine suitable burning periods based on varietal sensitivity and grapevine development.

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



Adjusts the transparency of a layer



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.

Control



Heated



23/12/11

29/12/11

4/1/12

20/1/12

Questions???



Using the timing of MLF inoculation to optimise your winemaking

(Will I get a quicker MLF?)

(How can I use MLF on sensory attributes?)

Eveline Bartowsky

Senior Research Microbiologist

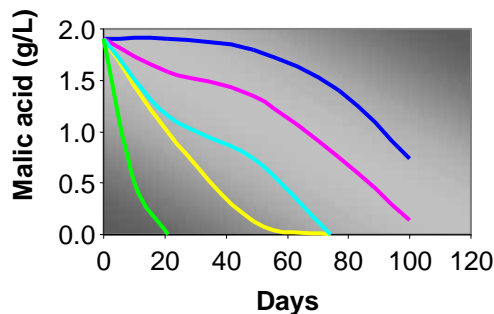
What is a successful MLF?

✓ 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

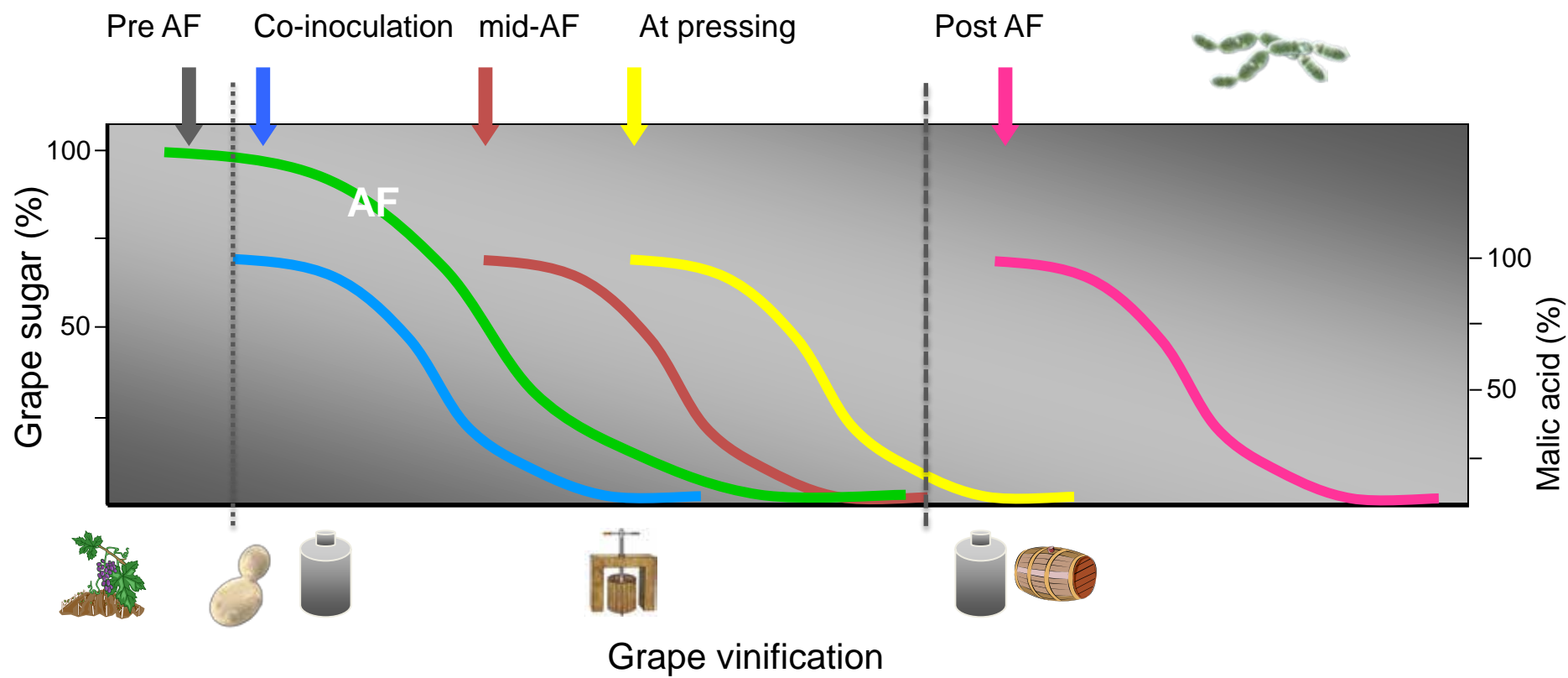


MLF is generally more difficult to manage than AF

What are my options for MLF inoculⁿ ?



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What are my options? *Considerations*



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With AF (co-inoculation/simultaneous)

More nutrients available

EtOH & SO₂ at lower concentration

Could be more rapid

At pressing (co-inoculation)

EtOH still lower concentration

Ferment is still warm

After AF (sequential inoculation)

Yeast lees interactions

Less risk of VA

Less risk of sugar metabolism by bacteria

Sensory modifications

Better temperature control

Concerns

Acetic acid production

Antagonistic problems with yeast & bacteria

May lead to yeast arrest

Antagonistic problems with yeast & bacteria

Nutrients depleted

It all comes down to ...

- § What do you want from MLF
- § Wine Style

✓ Concerns

§ VA production during AF by the bacteria

§ The yeast will stop fermenting

§ Wine colour affected

✓ What are some sensory consequences?

Co-inoculation concerns



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- ✓ VA production by the bacteria during AF

- § Very much linked to metabolism and grape must pH



pH & *O. oeni* metabolism

✓ *O. oeni*

§ Heterolactic fermentation = lactate + CO₂, acetate & ethanol

✓ Acetate formation from glucose metabolism

§ Reductive conditions

- Produce EtOH in preference to acetate (NAD⁺ balance)

§ Oxidative conditions

- Acetate produced (- ATP)

✓ Glucose & fructose metabolism in *O. oeni* pH dependent

§ Higher pH

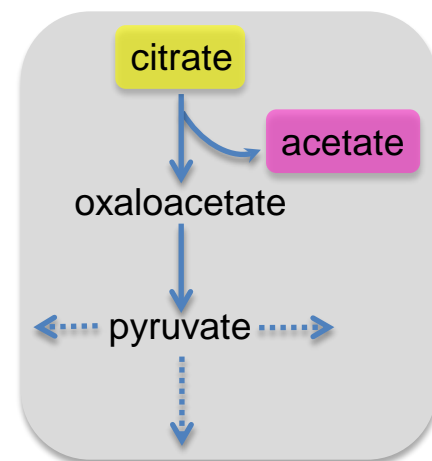
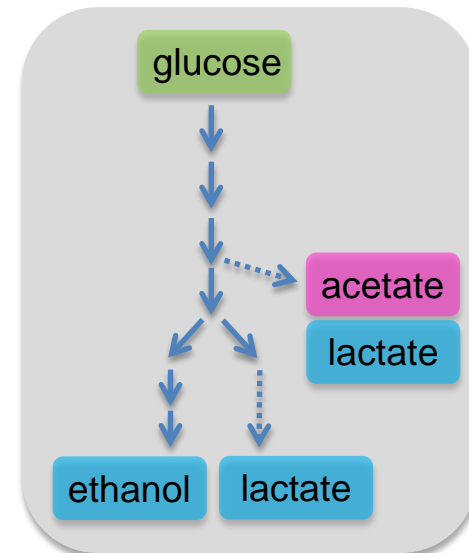
- Utilise sugars after organic acids

§ Lower pH

- Preferentially use organic acids (ATP gain)

✓ Citric acid metabolism

§ Always gives rise to acetic acid (VA)





Co-inoculation concerns

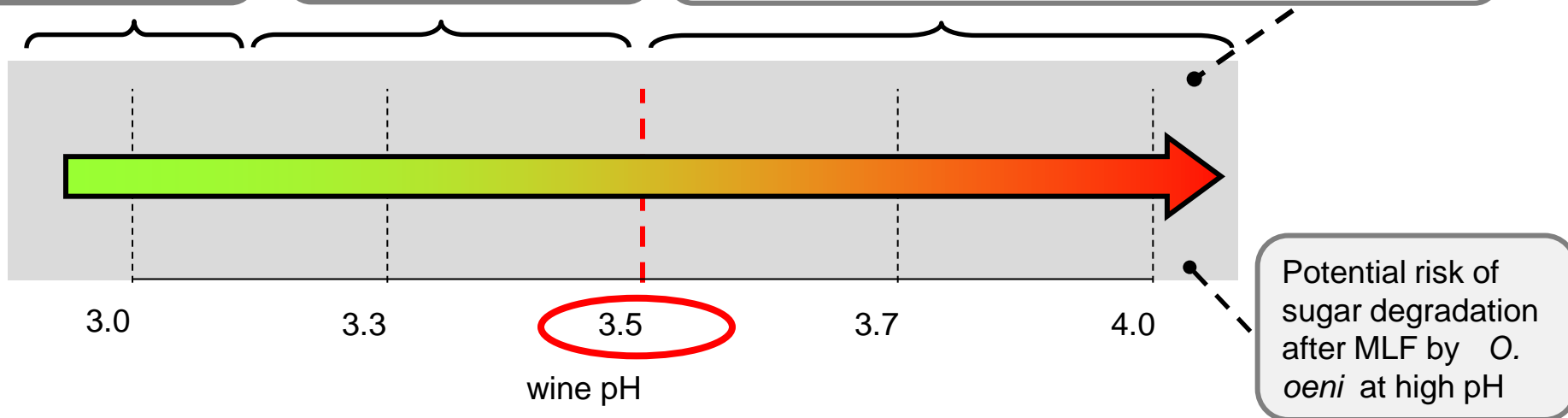
✓ VA production by the bacteria during AF

§ Very much linked to metabolism and grape must pH

Risk, that
MLF does not
occur

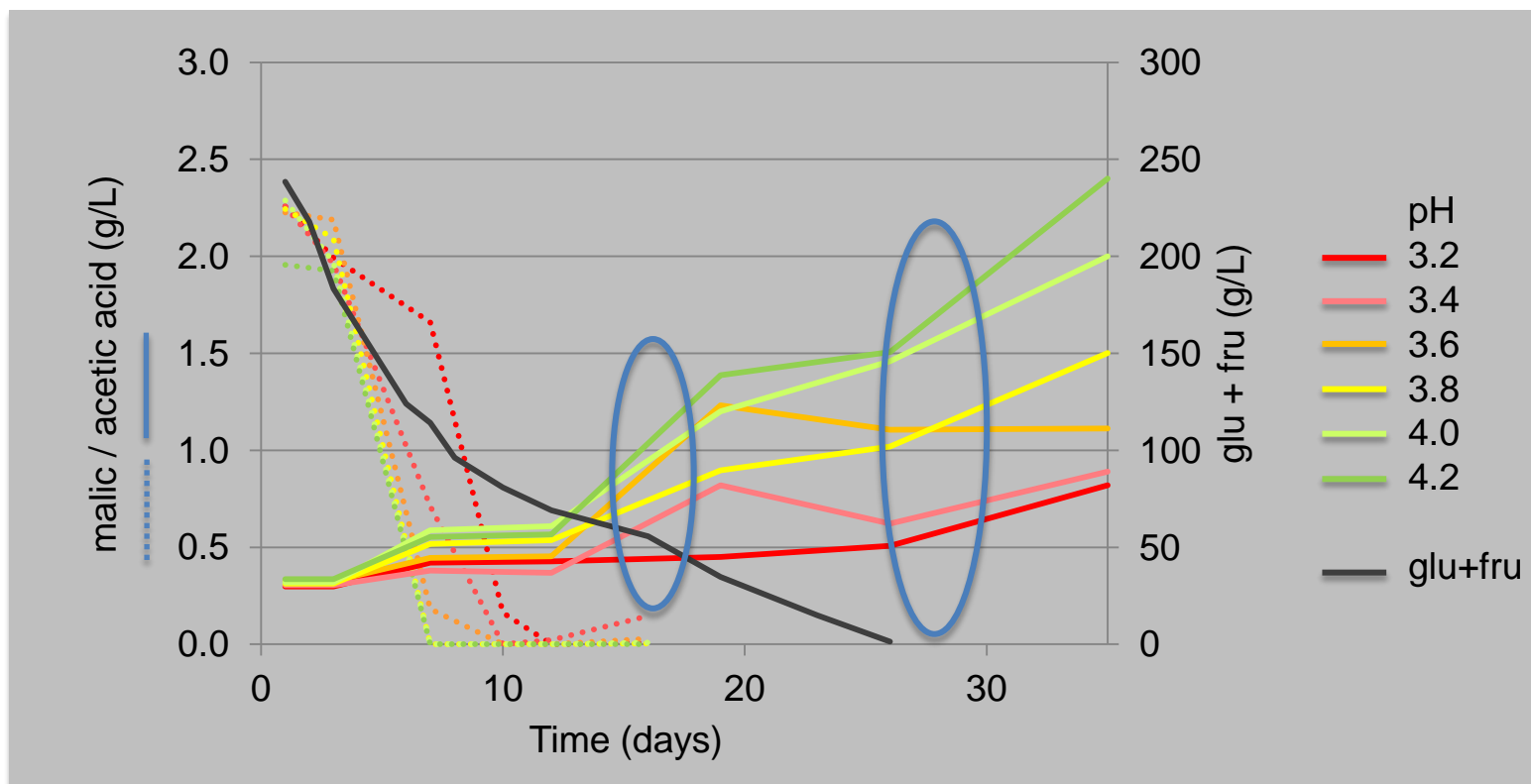
Difficulties to induce a
spontaneous MLF

Increasing risk of spontaneous MLF
induced by *Lactobacillus* & *Pediococcus* sp.
OFF-FLAVORS





Acetic acid & co-inoculation



Chardonnay, 2008
Eden Valley
230 g/L glu+fru

✓ Concerns

§ VA production during AF by the bacteria

§ The yeast will stop fermenting

§ Wine colour affected

✓ What are some sensory consequences

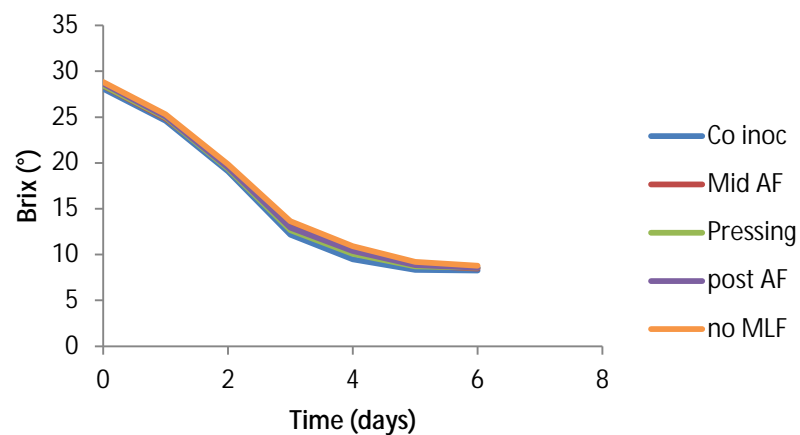
Ø *Three examples examining these questions*

Ø Cabernet Sauvignon, Shiraz, and Chardonnay

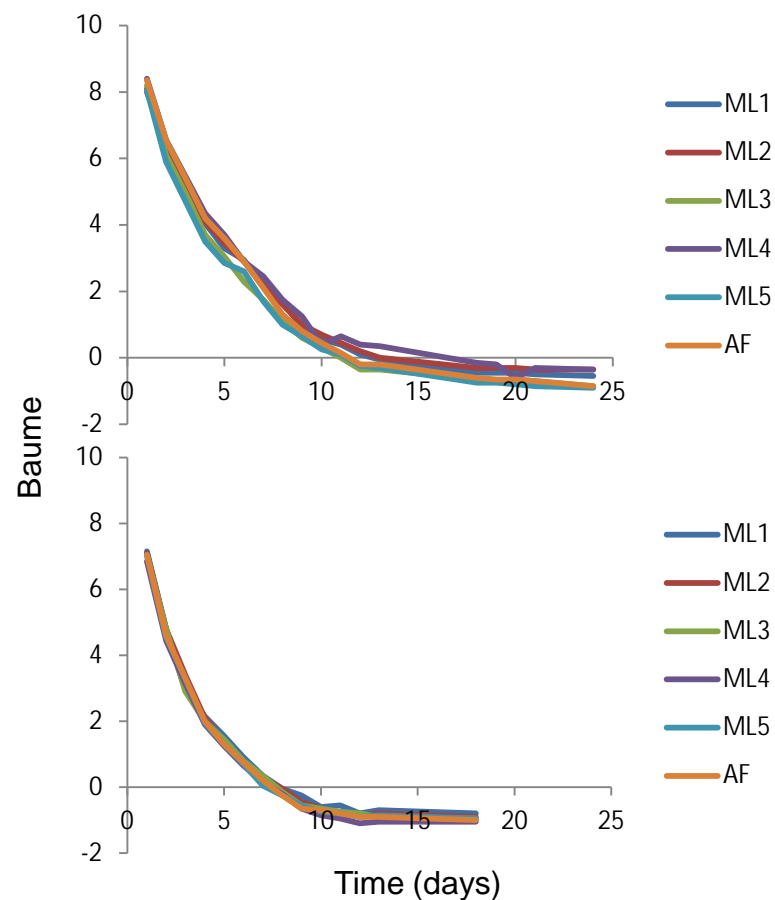


Co-inoculation

✓ The yeast will stop fermenting



Shiraz (2008)
Clare Valley

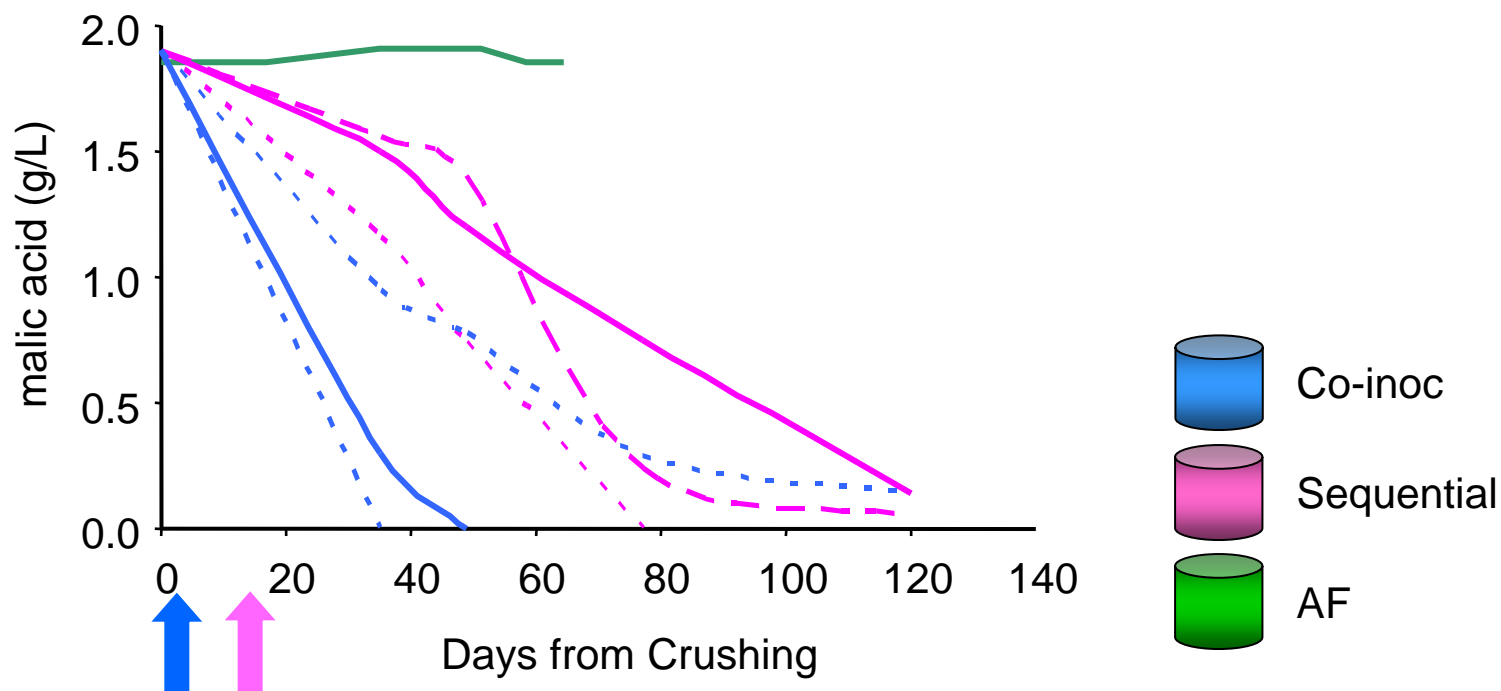


Chardonnay (2011)
Barossa Valley





Example: Cabernet Sauvignon

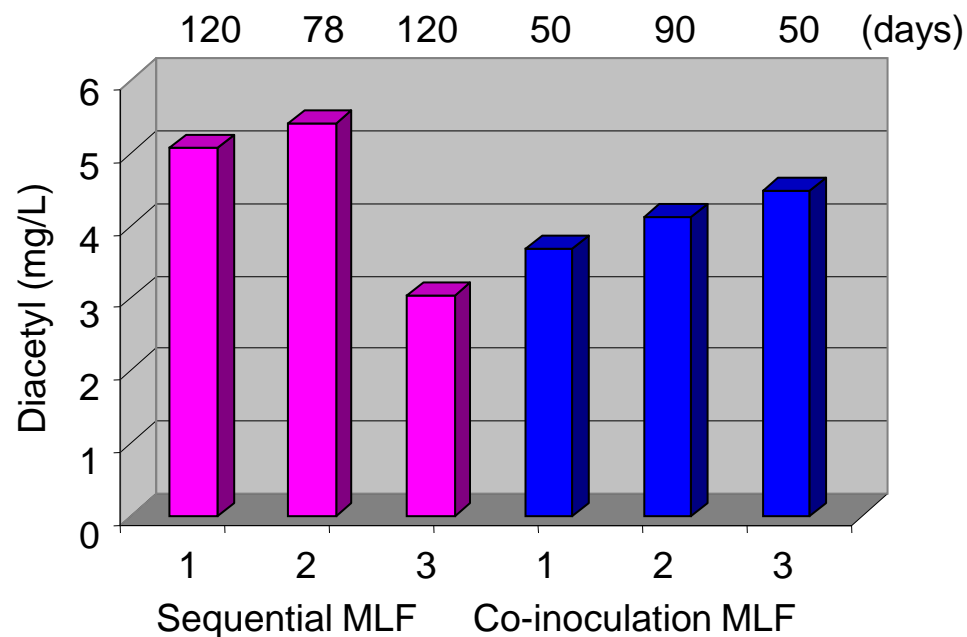
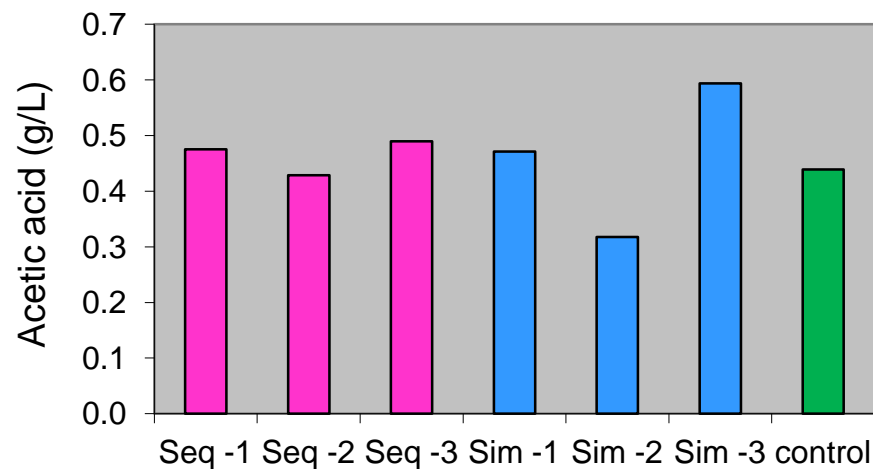


Cabernet Sauvignon
2006
Bordertown

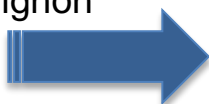


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Cab Sauv: Acetic acid & diacetyl



Cabernet Sauvignon
2006
Bordertown

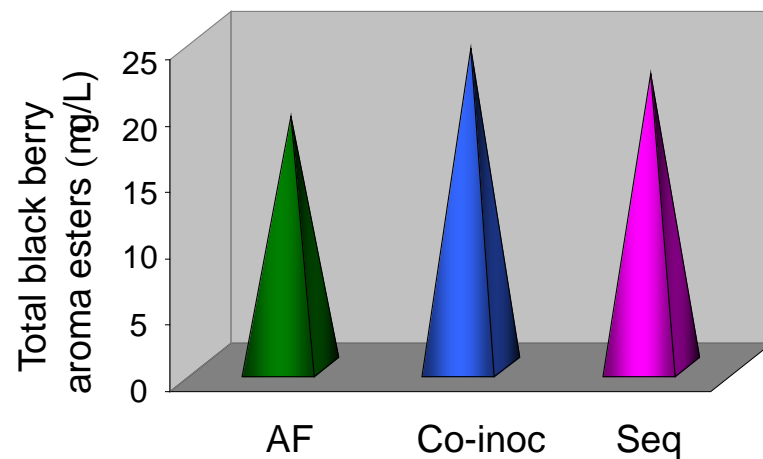
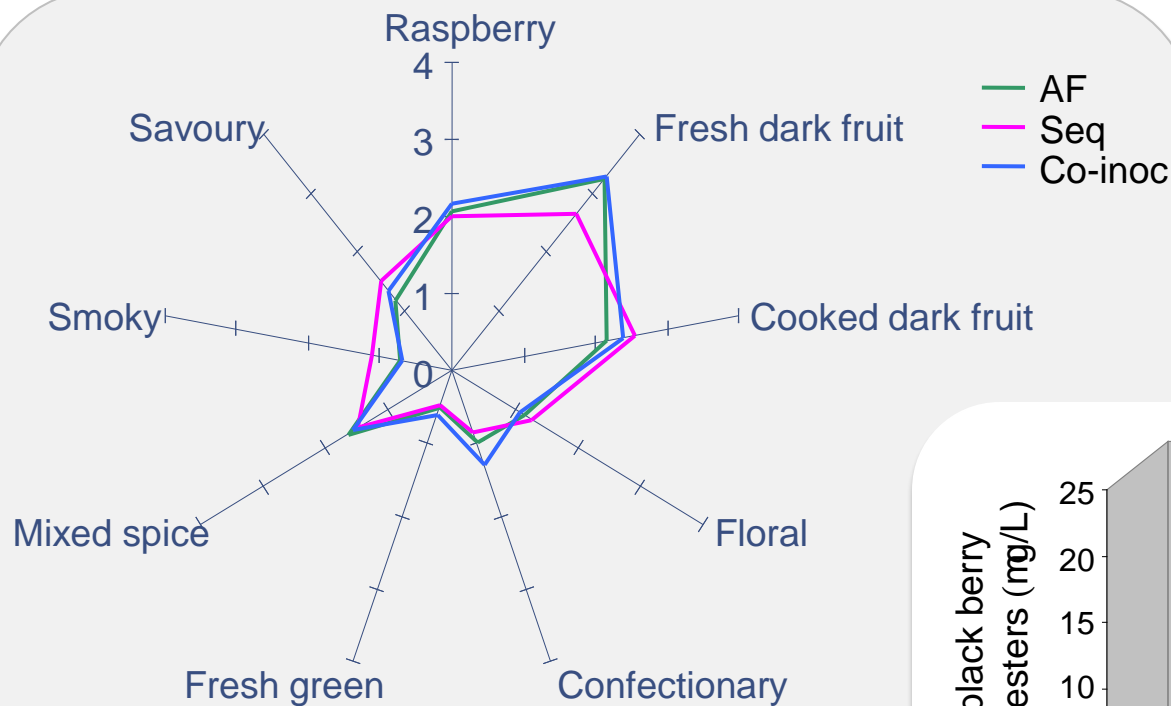


Tend to have higher diacetyl concentrations with a slower MLF



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Cab Sauv: Aroma profile



Cabernet Sauvignon
2006
Bordertown

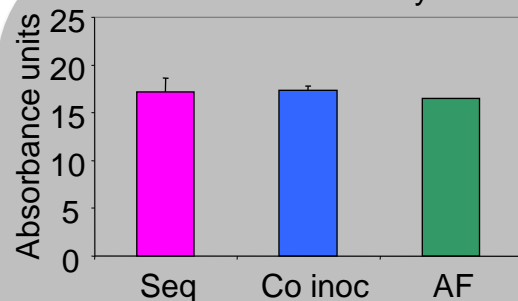


Co-inoculation increased fruity characters

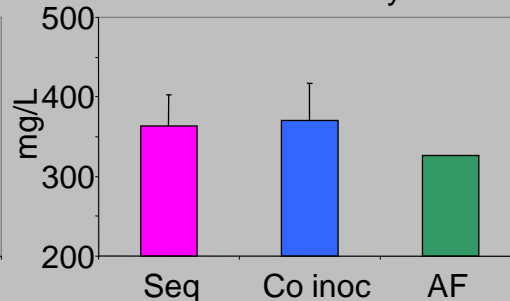


Cab Sauv: Wine colour

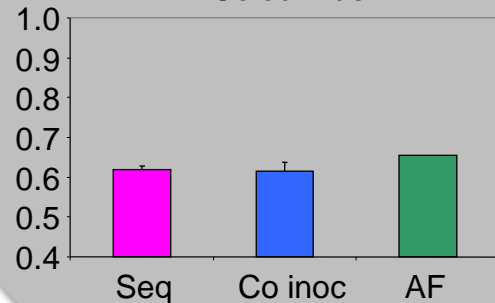
Colour density



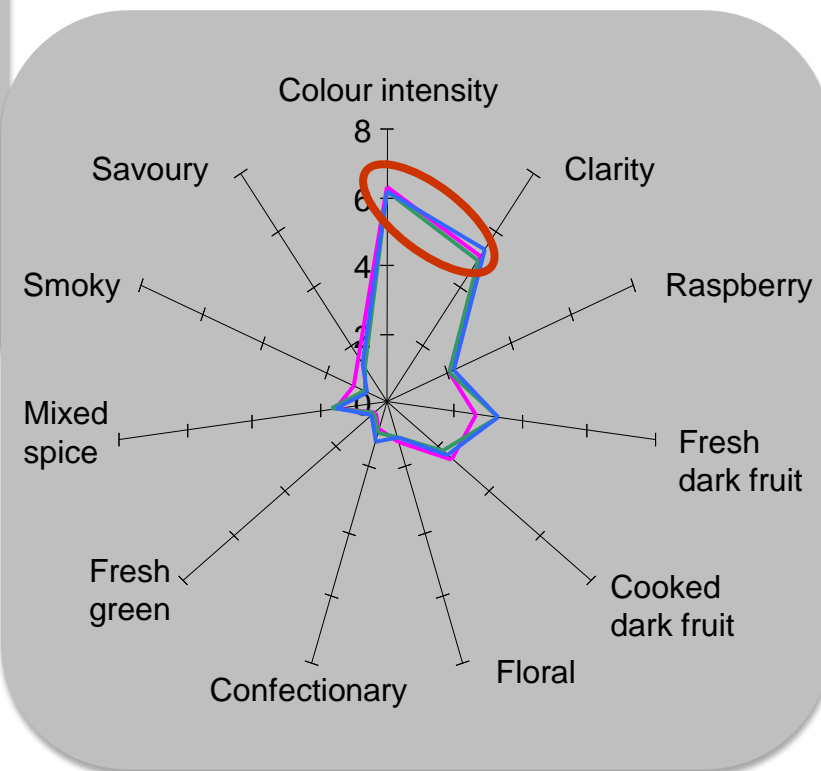
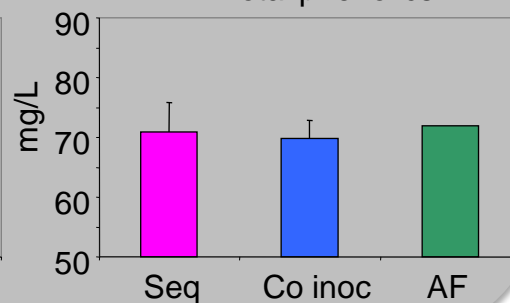
Total anthocyanins



Colour hue



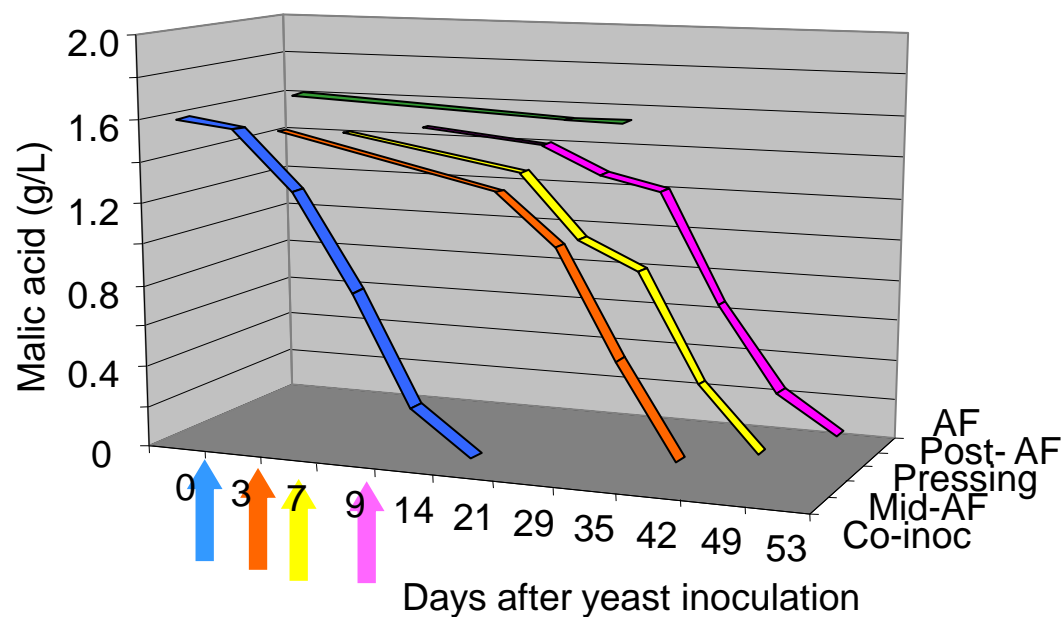
Total phenolics





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Example: Shiraz



Shiraz
2008
Clare Valley



Reduced fermentation time with co-inoculation



Shiraz: Wine composition

	RS (g/L)	EtOH (%)	Malic (g/L)	Lactic (g/L)	Citric (g/L)	Acetic (g/L)	pH	TA (g/L)
Juice	189.6	0.0	1.8	0.0	0.1	0.1	3.5	6.45
AF	0.0	14.8	1.8	0.3	0.1	0.5	3.62	6.45
Co-inoc	0.7	14.0	0.2	1.3	0.0	1.0	3.8	6.0
Mid AF	0.7	14.3	0.3	1.3	0.0	0.9	3.8	6.0
At Pressing	0.7	14.1	0.3	1.3	0.0	0.9	3.8	6.0
Post AF	0.7	14.4	0.3	1.3	0.0	0.9	3.82	6.0

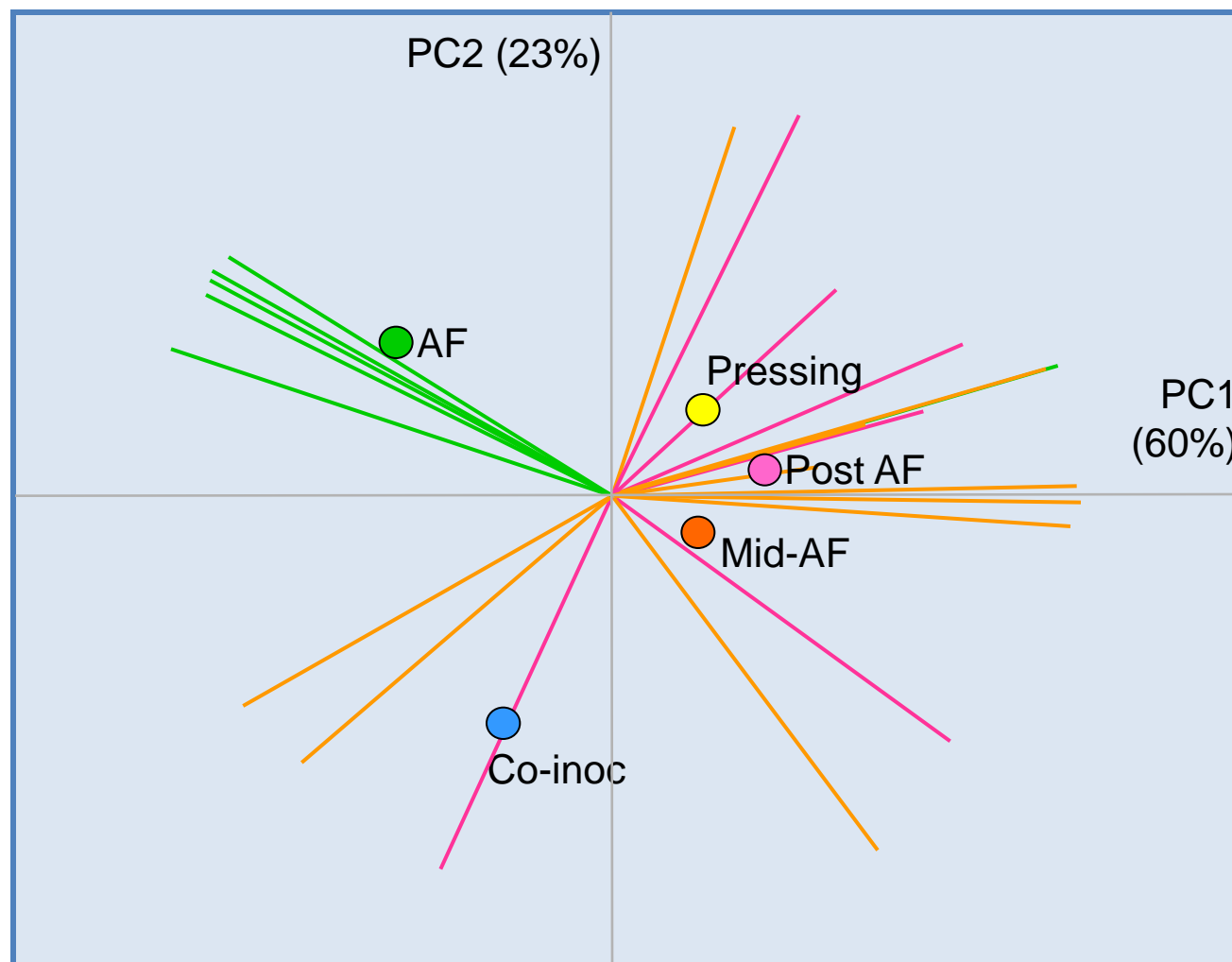


Shiraz
2008
Clare Valley

Shiraz: Volatile fermentaⁿ metabolites



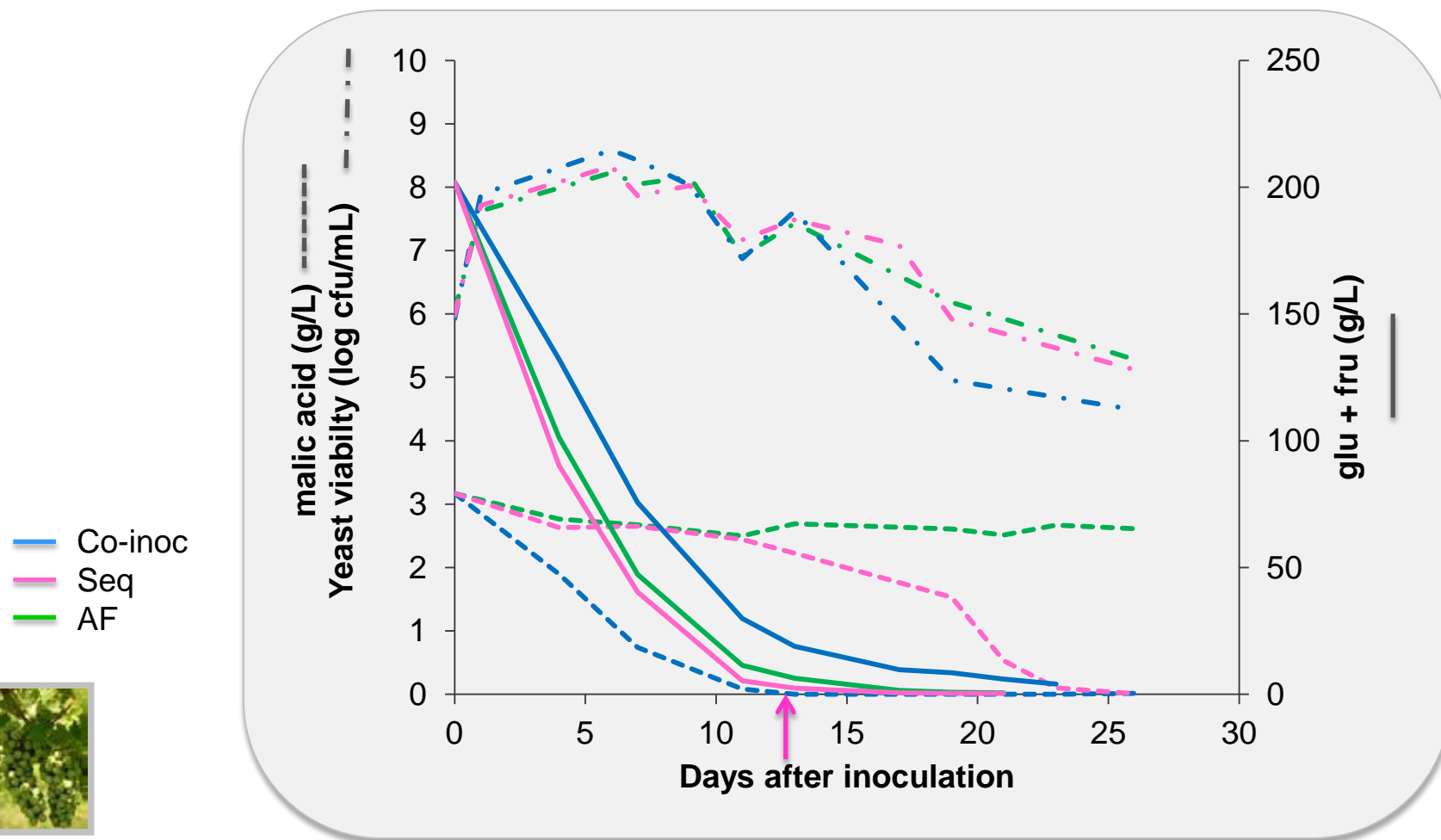
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Shiraz
2008
Clare Valley



Example: Chardonnay



Chardonnay
2001
Langhorne Creek



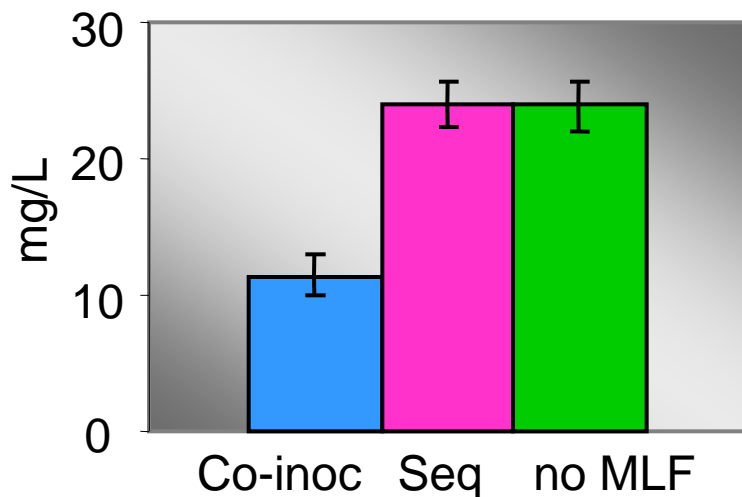
***Co-inoculation – Reduced overall fermentation time
– Yeast performance unaffected***

Chard: Volatile fermentaⁿ metabolites

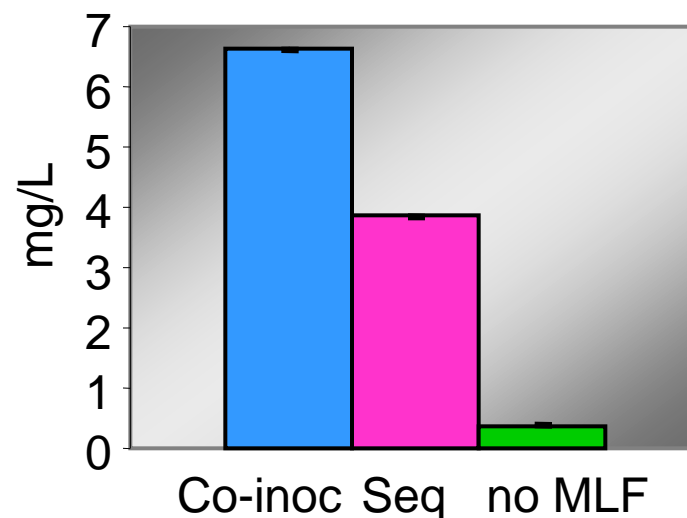


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Total higher alcohols



Total ethyl fatty esters



Chardonnay
2001
Langhorne Creek

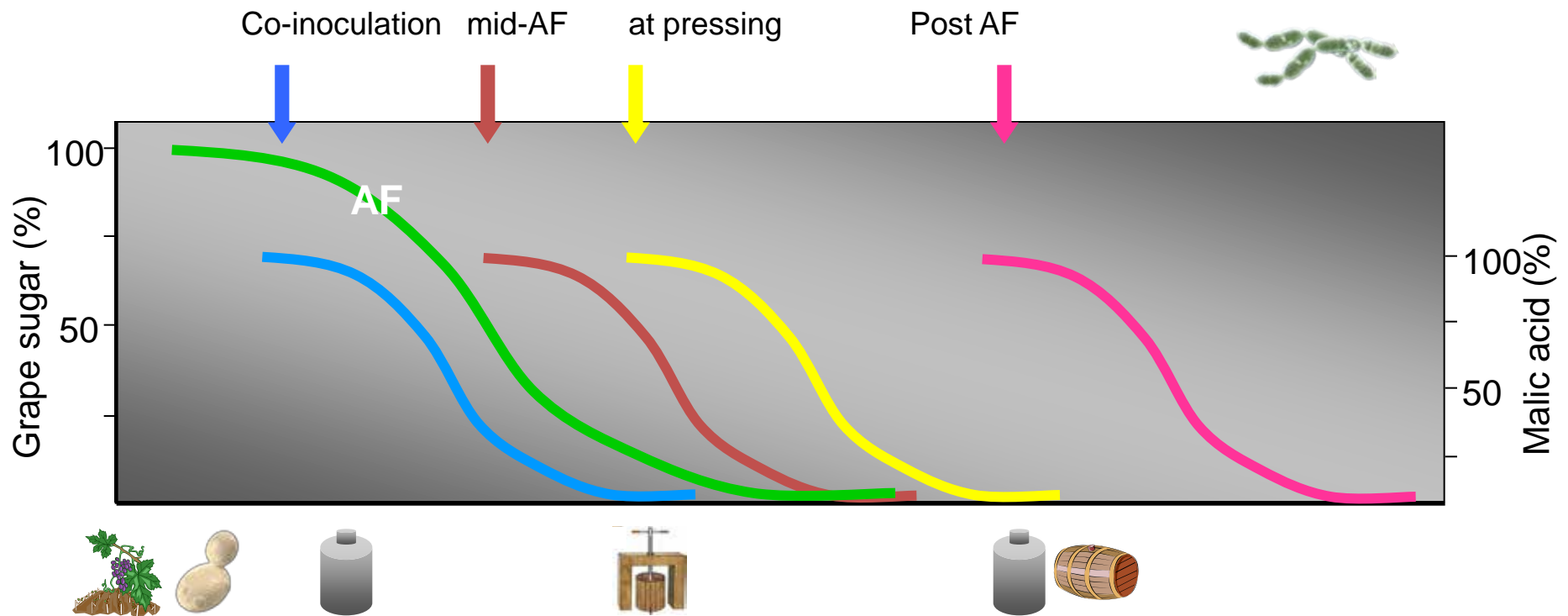


Co-inoculation increased fruity characters

Options for MLF inoculation



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Choice of inoculation point affects the relative difficulty of MLF induction and competition by indigenous strains, which are often better adapted to the wine

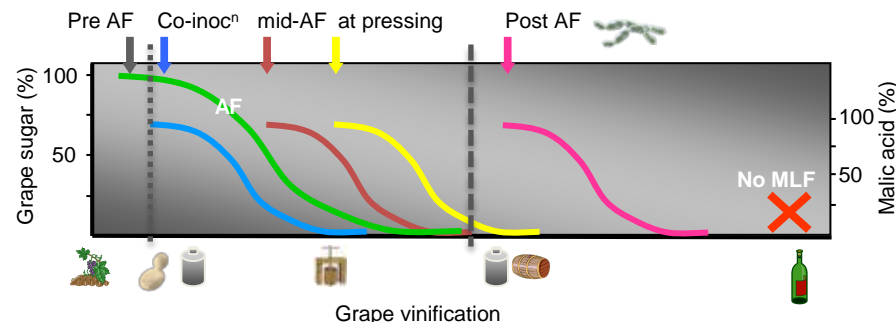
Using timing of inoculation to optimise MLF



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✓ What are my options?

- § Co-inoculation
- § Sequential inoculation



✓ Co-inoculation:

- § Can be used in red & white wine
- § Reduces overall fermentation time
- § No affect on wine colour
- § Does not affect AF or yeast performance
- § Sometimes slightly higher acetic acid/VA



✓ Sensory

- § Fruity compounds tend to be higher with co-inoculation and correlate with wine sensory characters

Ø ***MLF inoculation regime can be used to influence wine style***

Acknowledgments



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✓ AWRI Wine Biosciences

MLF Team

- § Caroline Abrahamse
- § Peter Costello
- § Jane McCarthy



✓ Wineries: kind donation of grapes & wine for 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

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



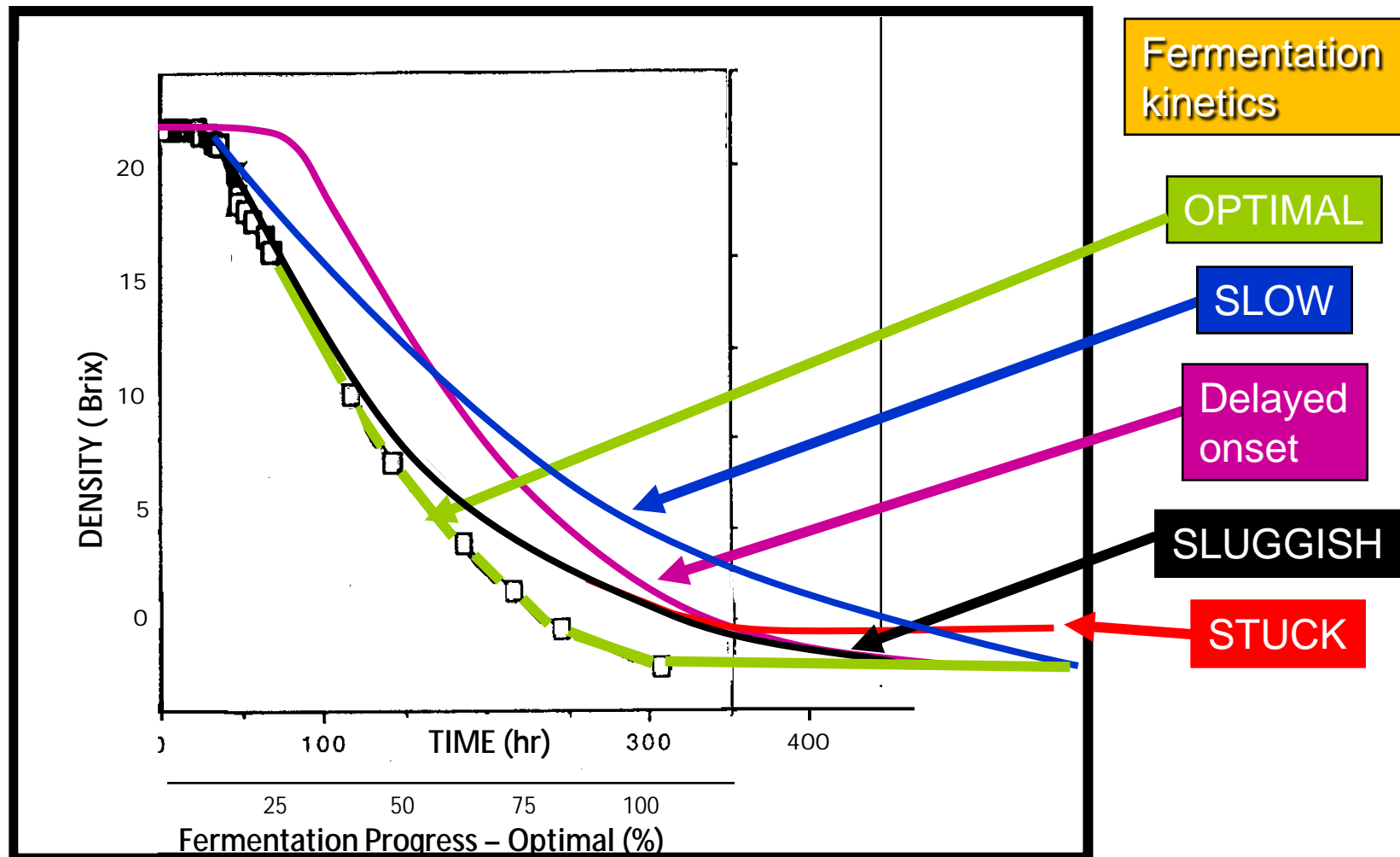
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- ✓ A common seasonal problem, but exacerbated by hot weather
 - ✓ Affects most wineries at some stage, both in Australia and overseas
 - ✓ White, red & sparkling wines, in tanks & barrels
 - ✓ Multifactorial problem, including yeast, nutrients, toxic 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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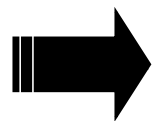
Environmental changes during fermentation

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



✓ 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:

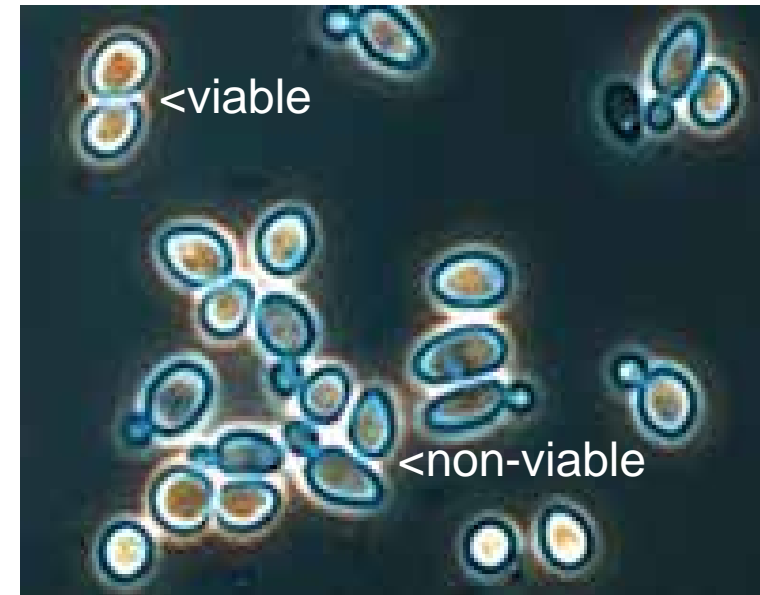
- § Perform a microscopic cell count before & after treating the sample with vital stain, eg methylene blue (see Iland et al. 2007)
- § Viability $<75\%$ indicates poor yeast culture or must toxicity, eg SO_2
- § Measure must/juice SO_2 ; should be $<10\text{-}15$ mg/L free SO_2

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



✓ Slow (continuously) fermentation

Causes:

- § Low yeast biomass or cell number
- § Low budding index
- § Low level of key nutrient, typically YAN, O₂ or lipids

Diagnosis:

- § Confirm by microscopic cell count:
 - 0% FP (Fermentation Progress) count should be $>1-5 \times 10^6$ cells/mL;
 - 35% FP should exceed 50×10^6 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



✓ 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)
- SO₂
- toxic (killer) proteins/other organisms
- residues (pesticides, cleaning agents)

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



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



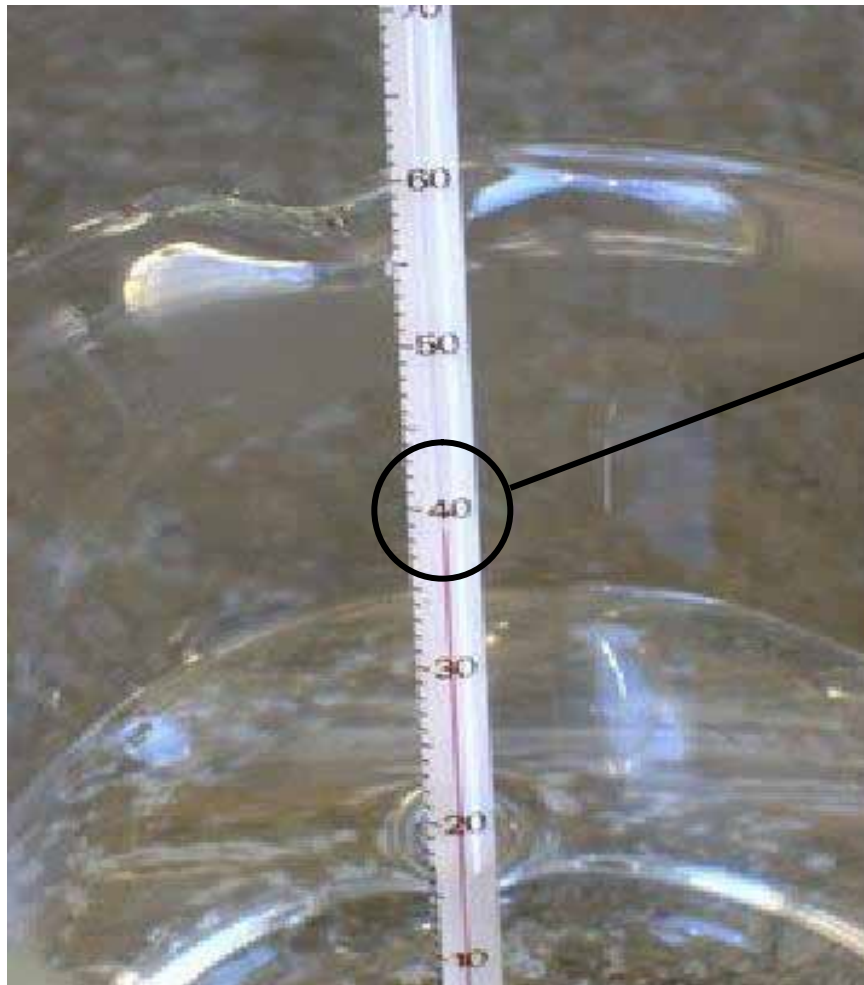
Active Dried Yeast - rehydration/reactivation (2)

- 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 rehydration 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



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Correct



Incorrect



Hydration final step – temperature amelioration



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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



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Active Dried Yeast - rehydration/reactivation (3)

- Add rehydrated yeast to pre-warmed juice (ie after cold settling or cold soak, preferably $>15^{\circ}\text{C}$)
- For cold ($<15^{\circ}\text{C}$), highly clarified, high sugar juice ferments, step-wise cool reactivated yeast in $5\text{-}10^{\circ}\text{C}$ 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



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 70×10^6 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



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 $3-4 \times 10^8$ /ml
 - ADWY viable cell population should exceed 2×10^{10} cfu/g
- ✓ Recommended Inoculation rates
 - whites: 5×10^6 cells/ml (typically 250 g ADWY per kL juice);
 - reds: 4×10^6 cells/ml ; lower rates can compromise yeast implantation

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 dO₂ & contact with O₂ (stimulates oxidative microbes)
- **Fermentation conditions**
 - Temperature (18-27 ° C favours *S. cerevisiae* (Sc) over non-Sacch. sp)
 - pH (<3.5 favours *S. cerevisiae*)
 - SO₂ (favours *S. cerevisiae*, generally tolerant to 10 ppm free)
 - O₂ at 30-40% Ferm Progress prolongs survival of Sc yeast

Nutritional deficiency risk factors



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✓ 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 (dO₂)

- § dO₂ 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



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✓ Vitamins

- § Vitamin status of Australian musts/juices is unknown
- § **Thiamine** - essential for ethanol production by yeast
 - losses caused by high SO₂ 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

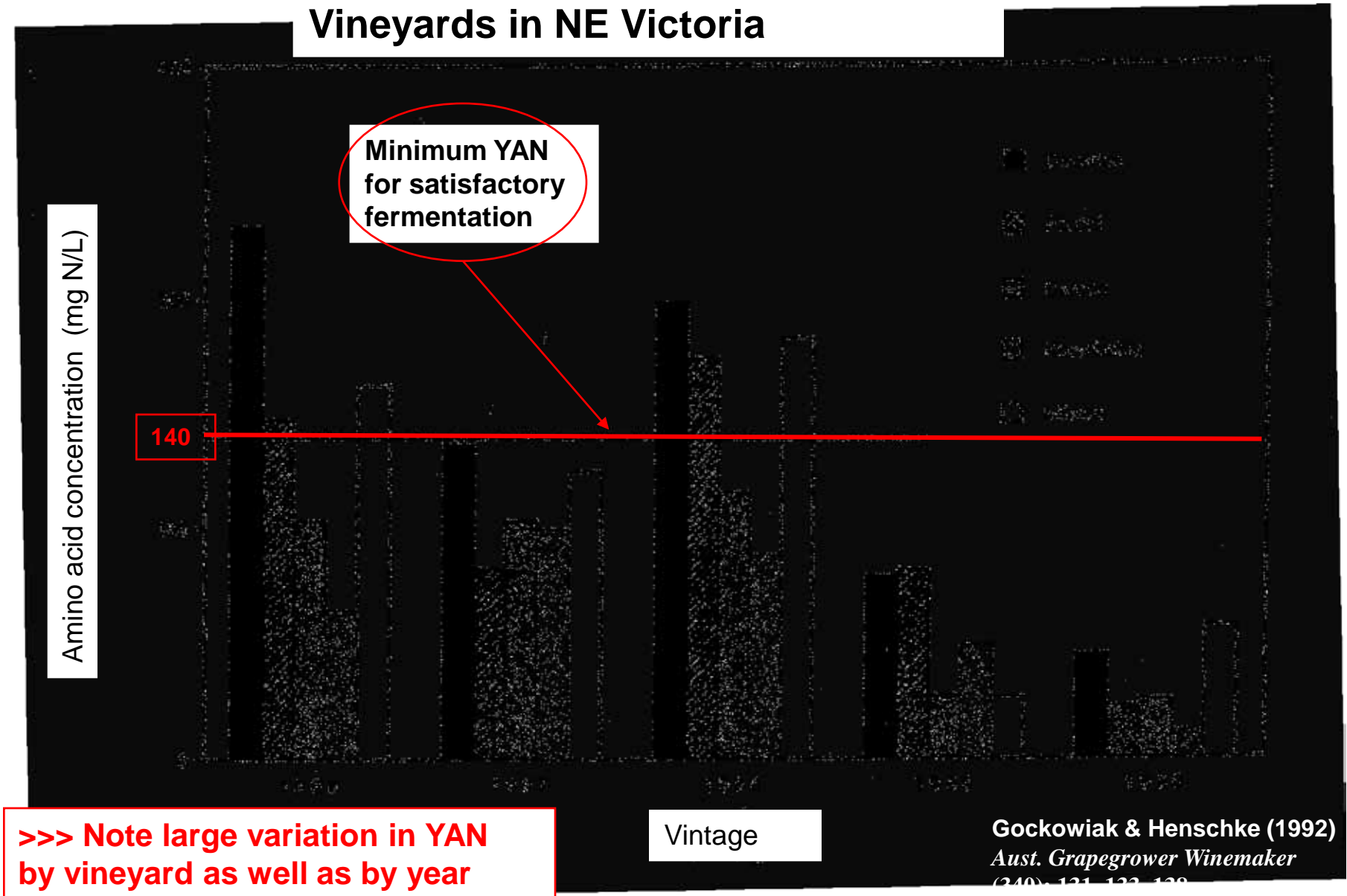
- § Mineral status of Australian musts/juices is unknown
- § **Phosphate – normally considered adequate; can be added with DAP**
- § **Low K⁺/Low pH** can lead to stuck ferms with some yeast strains (sparkling/tirage)
- § **Magnesium, zinc, manganese, which are enzyme co-factors are thought to be sub-optimal** (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**

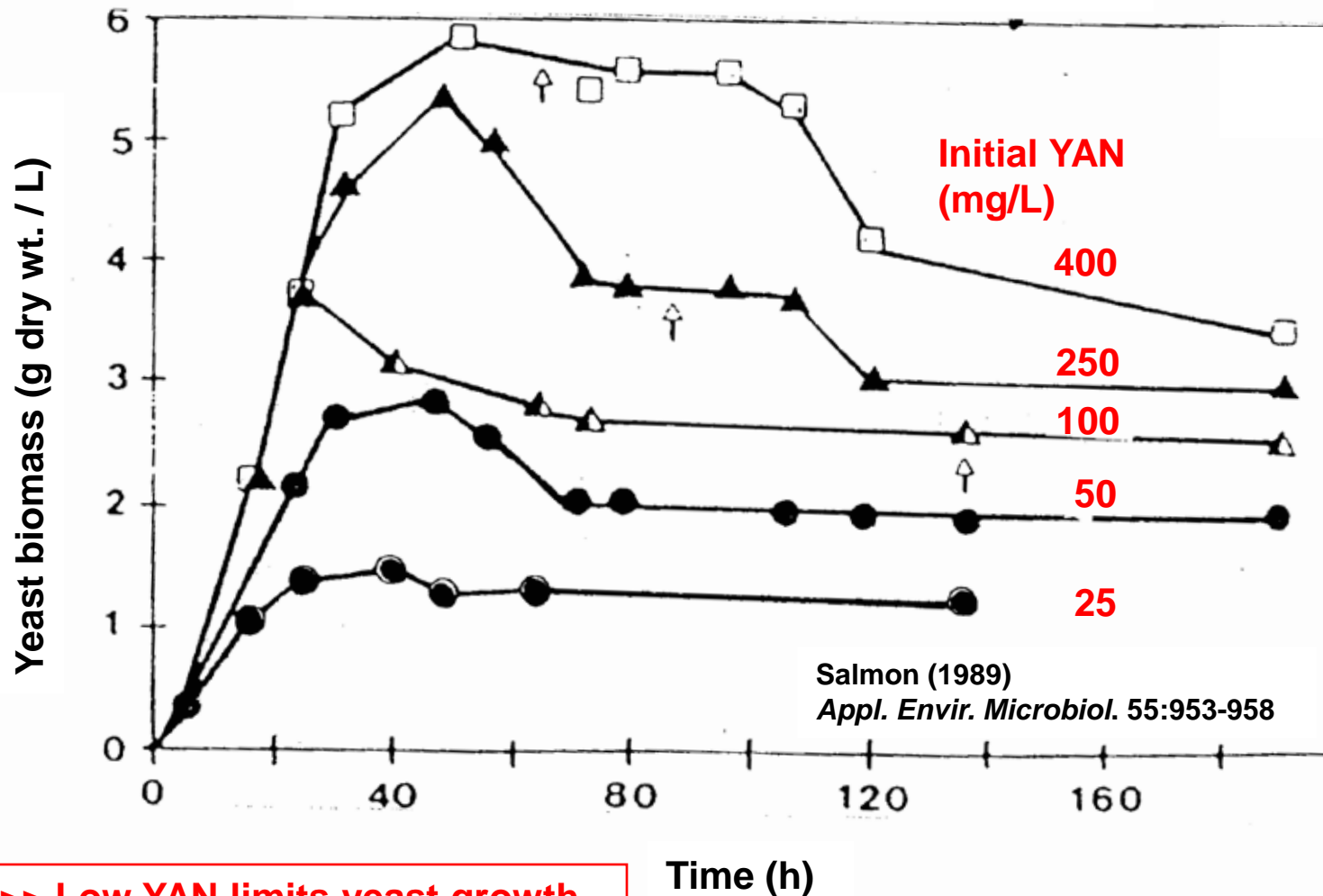
Vineyard & Year effect on juice YAN

Vineyards in NE Victoria



Yeast growth response to YAN

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

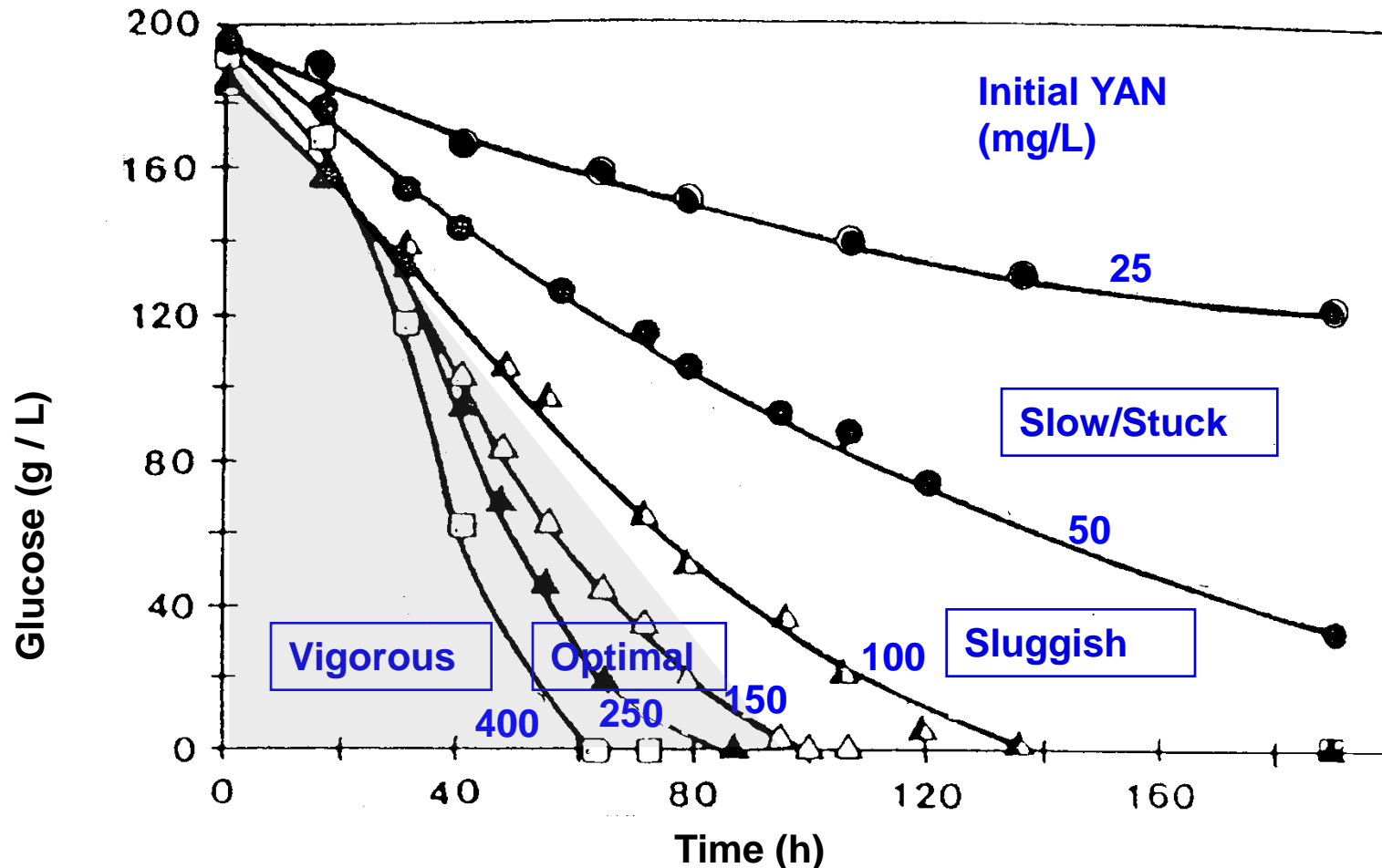


>>> Low YAN limits yeast growth
Excessive YAN has no benefit

Fermentation response to YAN

Synthetic juice \equiv '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

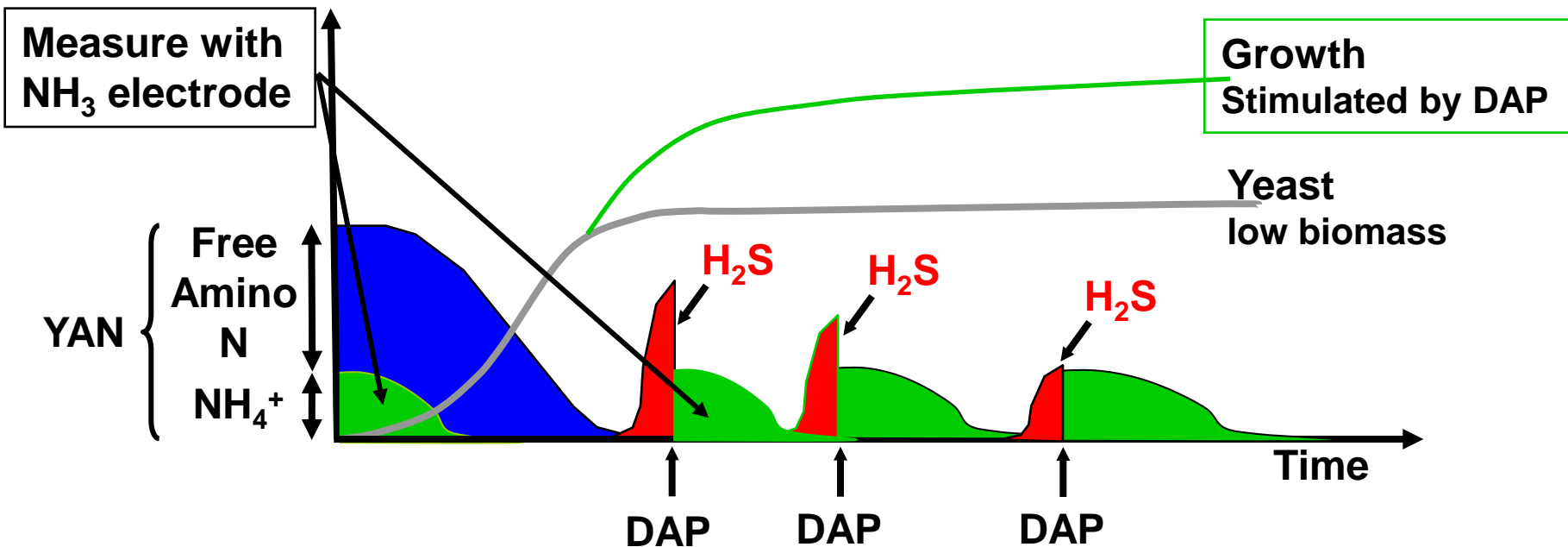
Nitrogen utilisation during fermentation



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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)



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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 H₂S

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 Lallemend range



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

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

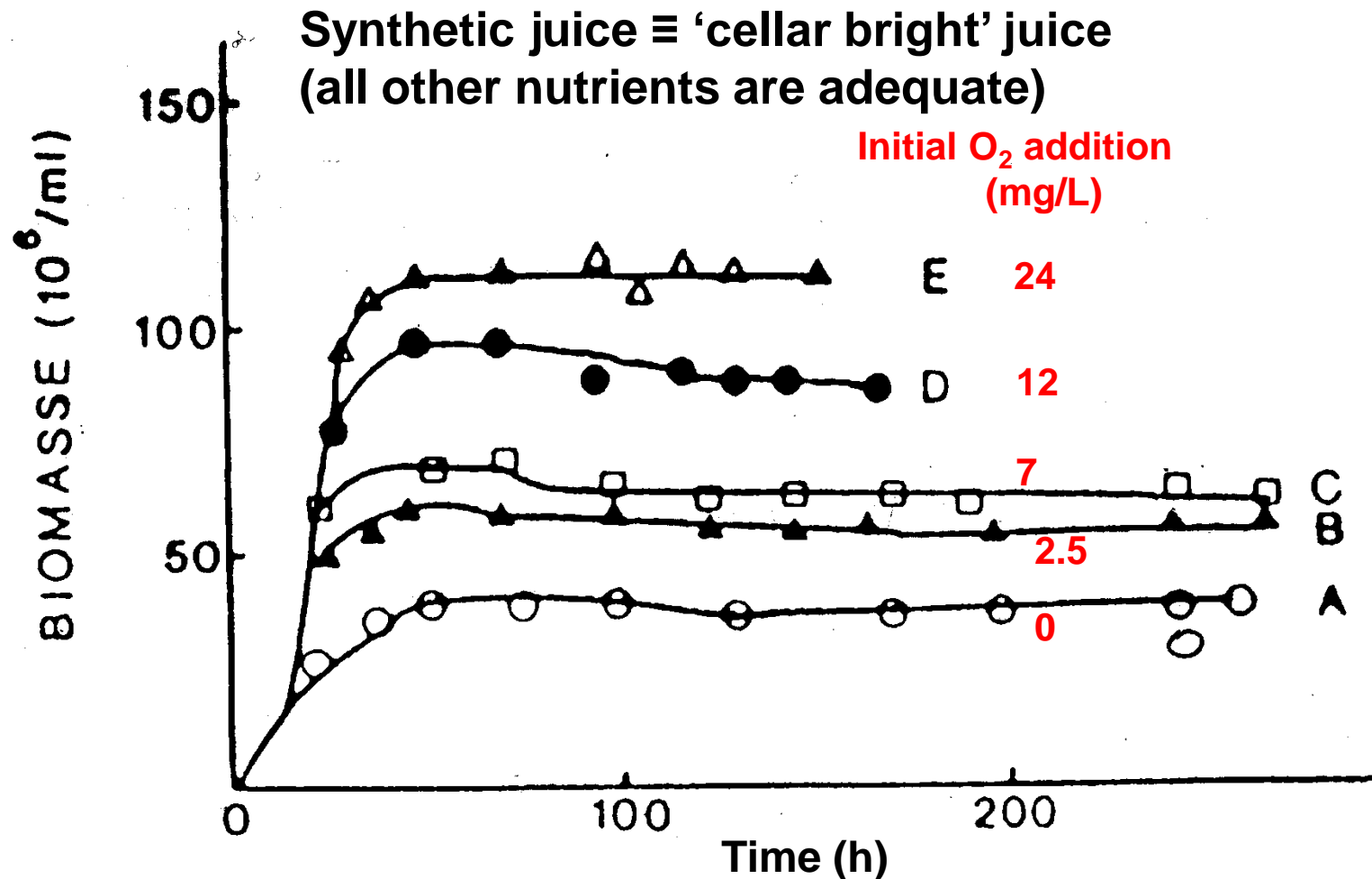
Defined as Nitrogen needed to maintain constant fermentation rate in synthetic medium with initial YAN = 100 mg/L; sugar = 200 g/L

Adapted from Lallemend 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

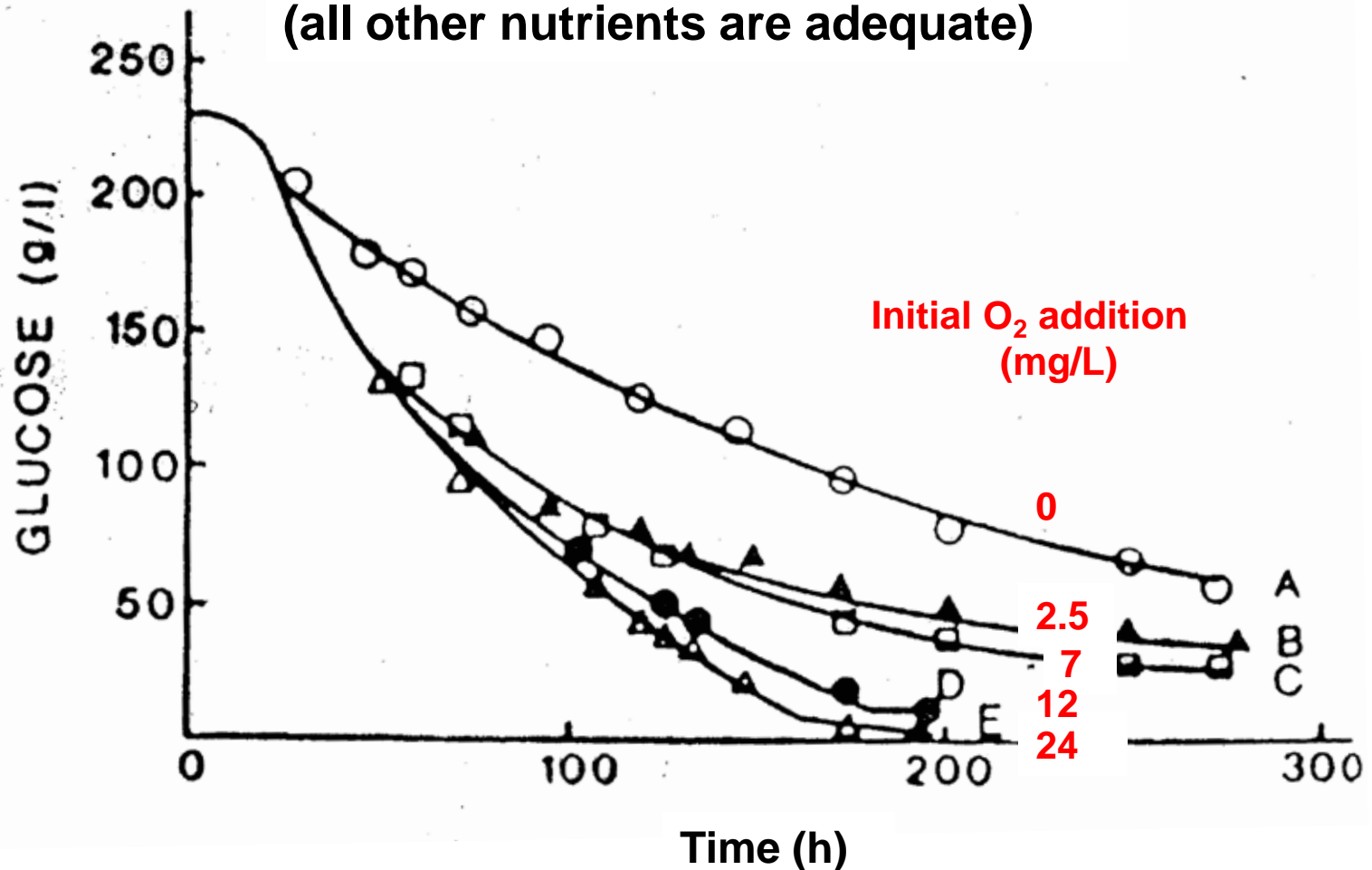
Sablayrolles & Barre (1986)
Sciences des Aliments 6:177-185

Fermentation response to O₂



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**Synthetic juice \equiv 'cellar bright' juice
(all other nutrients are adequate)**



>>> Oxygen stimulates fermentation rate

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

Effect of O₂ on fermentation rate



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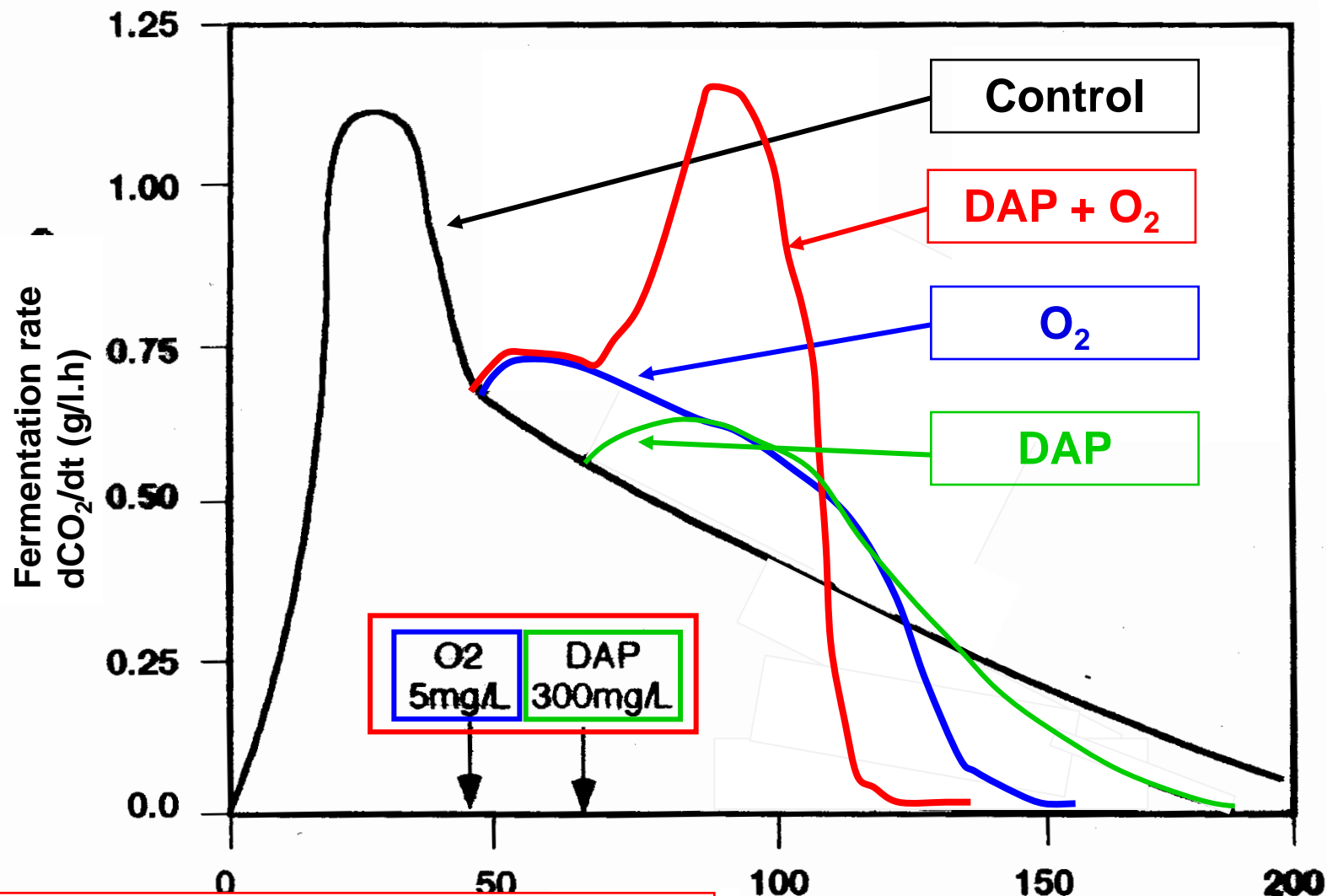
Rate Anaerobic (Max rate of CO ₂ production)	Yeast	%Rate gain with O ₂ added at 1/3 fermentation progress	Yeast
Low <0.5	S6U CEG EC1118 DV10 QA23	Low (16%)	CSM K1M V1116 CY3079 R2
Medium 0.6-0.7	K1M VL3 L2323 L2226 VL1 71B R2	Medium (30%)	71B/Actiflore S6U VL1 D47 DV10 QA23
High >0.7	D47 CSM V1116 CY3079	High (45%)	EC1118 IOC182007

Combined effect of DAP + O₂ on fermentation

Nutrient strategy for stimulating fermentation



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

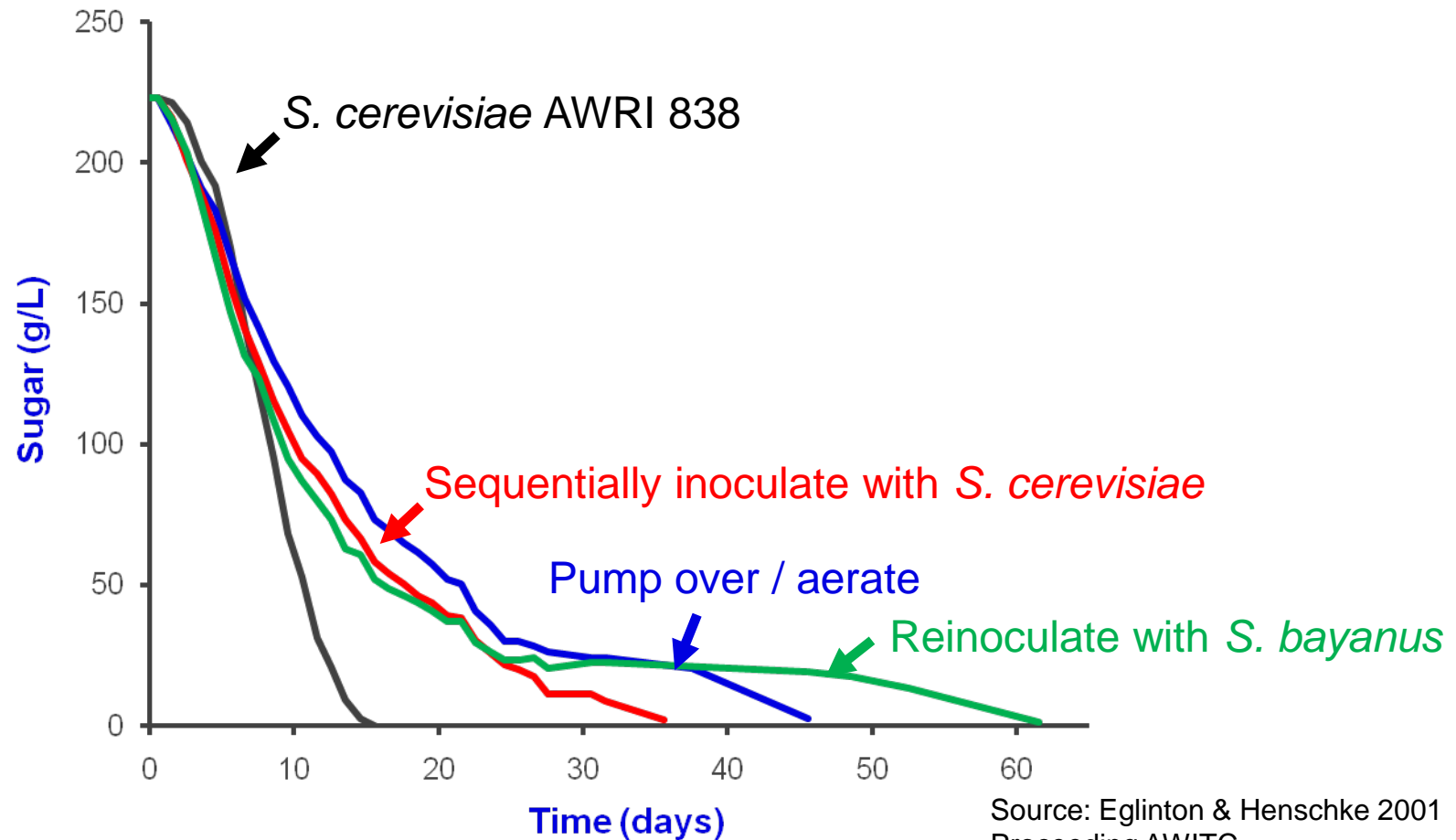
Jablayrolles, Dubois, Manginot & Barre (1986)
J. Ferment. Bioeng. 82:377-381

Practical strategies for ensuring a complete fermentation with low vigour yeasts

eg *S. bayanus* AWRI 1375



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Source: Eglinton & Henschke 2001
Proceeding AWITC

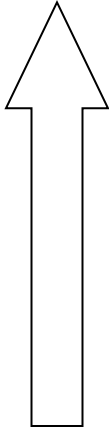


All treatments tested
promoted refermentation

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

Juice Clarification affects Fermentation Rate and Wine Residual Sugar



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

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

Inhibitory substances – risk factors



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- ✓ Ethanol – probably largest cause of stuck ferments
 - strain dependent: growth at 8-12%, fermentation >12 %
 - **determined by grape maturity at harvest**
- ✓ SO₂
 - strain dependent, typ. >10 mg/L free SO₂ at pH 3.5
 - cell death at 45 mg SO₂/L, pH 3.5 (0.8 mg/L mol. SO₂)
- ✓ 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 O₂

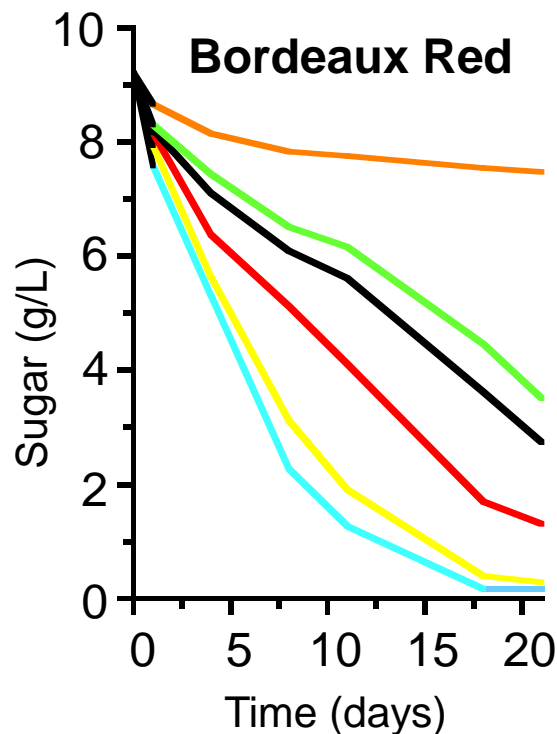
Effect of acetic acid on refermentation

Fermentation rate








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



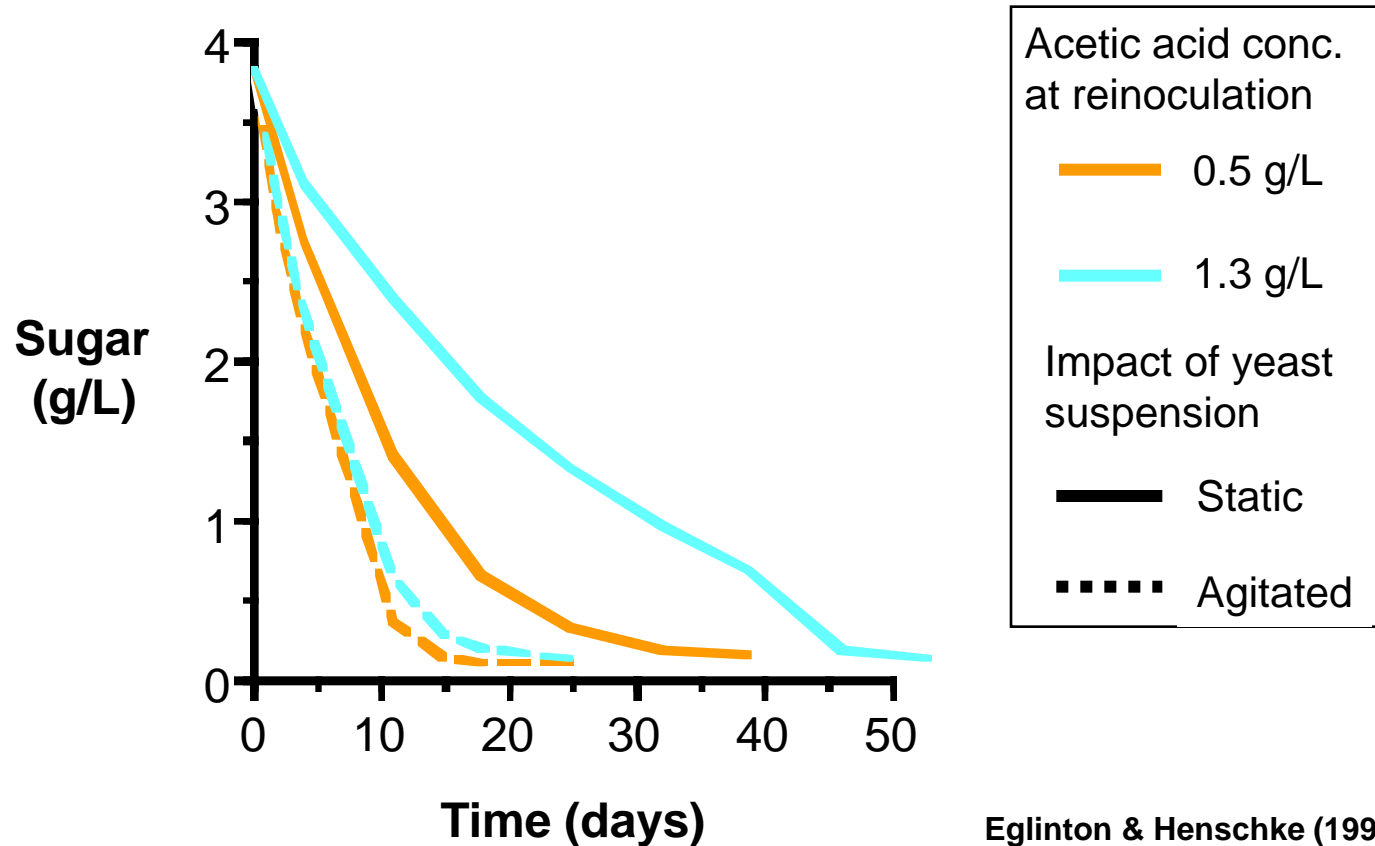
Acetic acid concentration

	0.5 g/L		1.7 g/L
	0.9 g/L		2.0 g/L
	1.3 g/L		4.0 g/L

Agitation aids refermentation



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

>>> When restarting fermentation, important to keep yeast in suspension by physical means until CO₂ 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



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✓ Temperature stress

Do not commence cooling until 10% sugar fermented

Excessive temperature (32-35 °C depend on [EtOH])

Over-cooling for particular yeast (non-cryogenic) / may need to use methods to maintain yeast in suspension if <13-15 °C

Excess heating or cooling (transition exceeding 5 °C)

Cooling preferably should be <3 °C per day

✓ Vigour and sedimentation (flocculation)

Yeast sediments in low vigour ferments (CO₂ 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

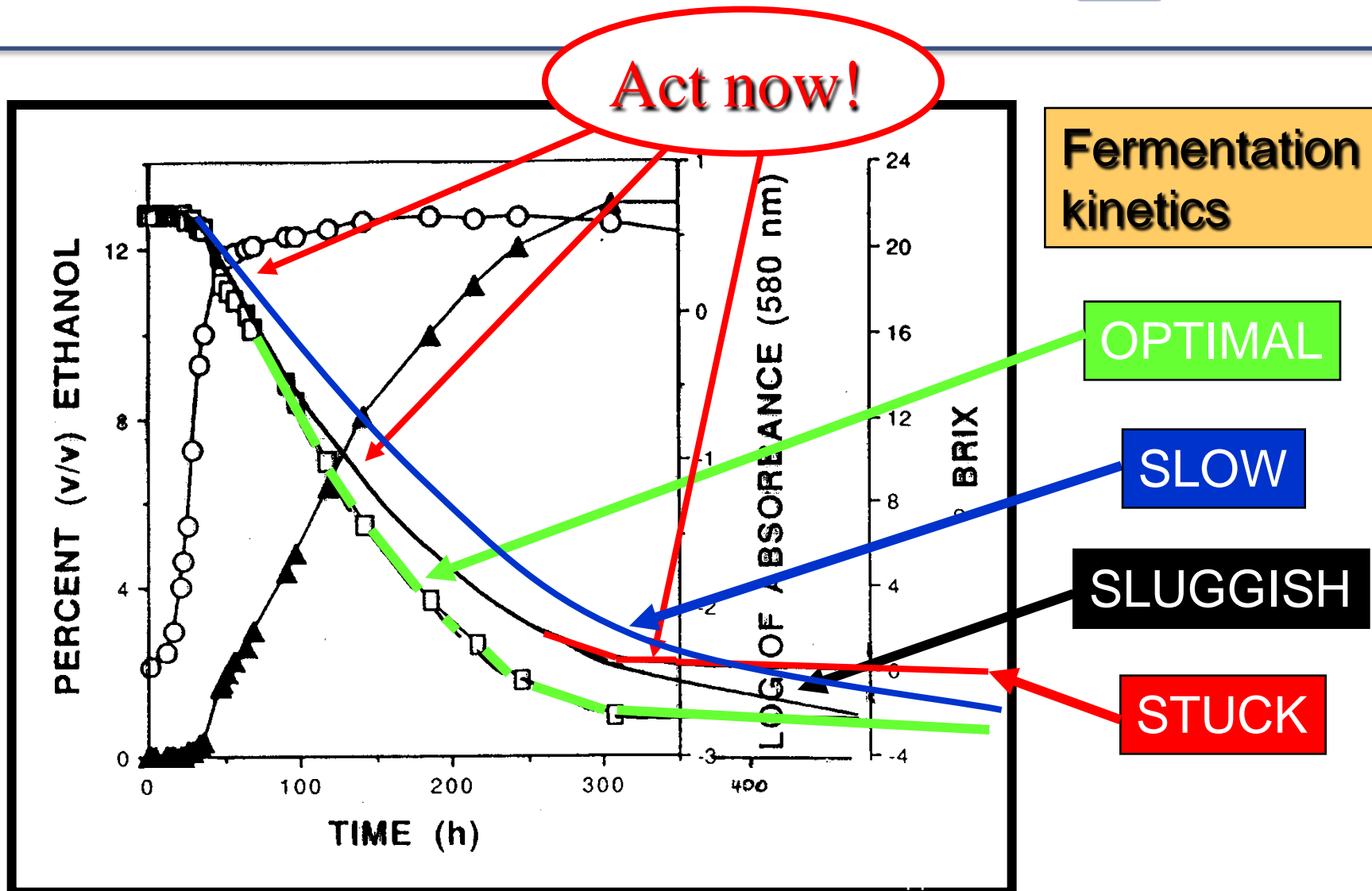
✓ Nutrients

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

Problem fermentations



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Take corrective action early



- ✓ 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 SO₂ 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



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)



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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%
	2	75%
	3	88%
	4	94%
3	Inoculate problem ferment	1020 L



- 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



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- **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

Peter Leske (former AWRI team leader)/Peter Godden (IA Team)

Wine Microbiology team:

Simon Schmidt, Paul Chambers (former members: Jeff Eglinton, Holger Gockowiak, Nancy Davis and Lisa Buckingham)

Research at The AWRI 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.





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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)

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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	MAXIMUM
Alcohol strength at 20°C		
wines ^a	7.0 % v/v	—
Total sugar (glucose) ^a		
Still	—	—
Dry wines ^a	—	4.0 g/L
Semi-dry ^a	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 ^a	—	12.0 g/L
Extra-dry ^a	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



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- [Acid addition](#)
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Number of standard drinks

Suggestions / questions / comments? [email the calculator services staff](#)

Approximate standard drinks

Container volume	<input type="text" value="750"/>	mL
Alcohol content	<input type="text" value="14.5"/>	% v/v
Calculate number of standard drinks	<input type="text" value="8.6"/>	standard drinks

Clear

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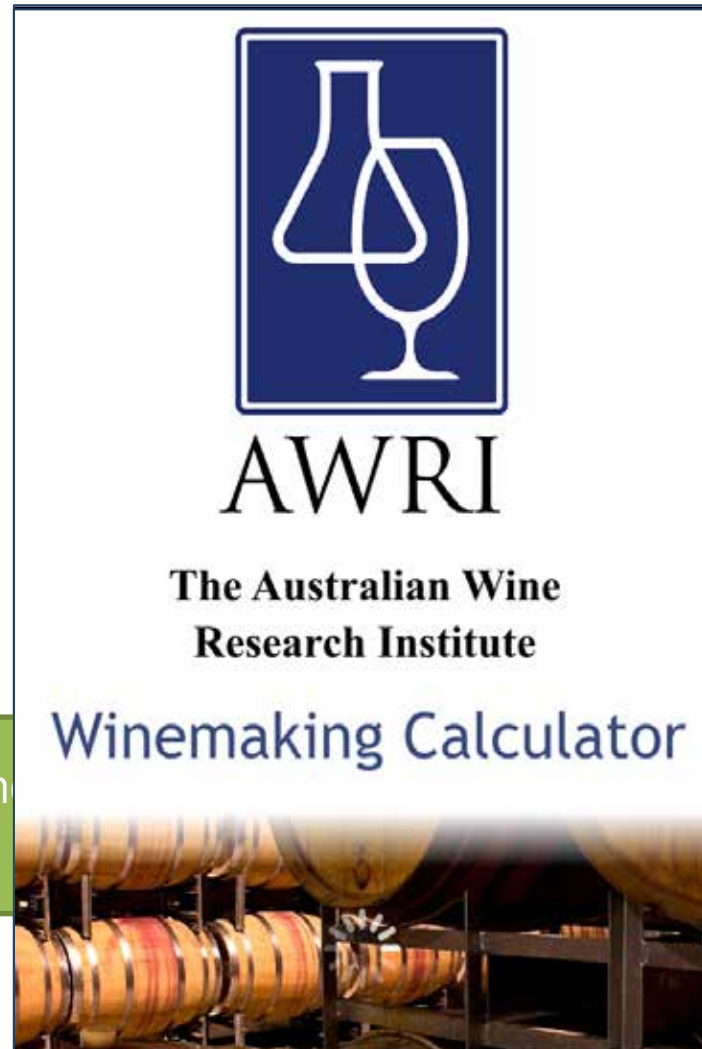


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2013 webinar program

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

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