Malolactic fermentation seminar

Webinar
with the Northern Grapes project

January 8, 2012

Sigrid Gertsen-Schibbye
Lallemand & Scott Labs

Overview

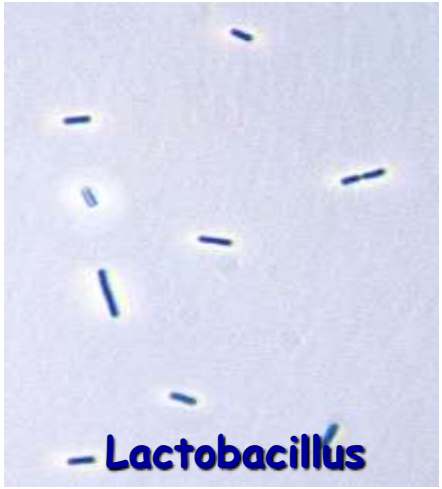
- Intro to MLF & MLF without borders
- Biogenic amines
- pH and SO_2
- Timing of MLF (co-inoculation)
- Nutrition
- Sensory contribution

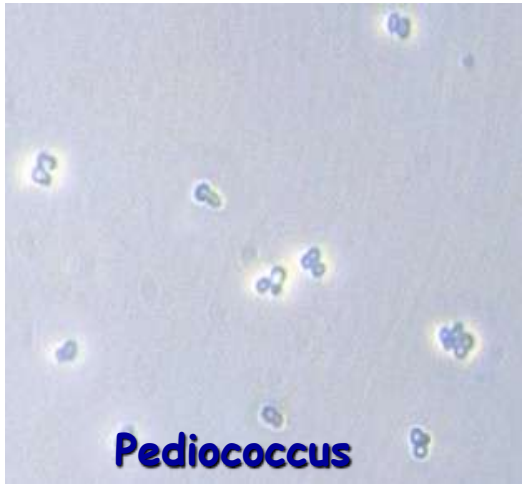
- (up my sleeve: Brett management, phenolic compound in grapes and ML)

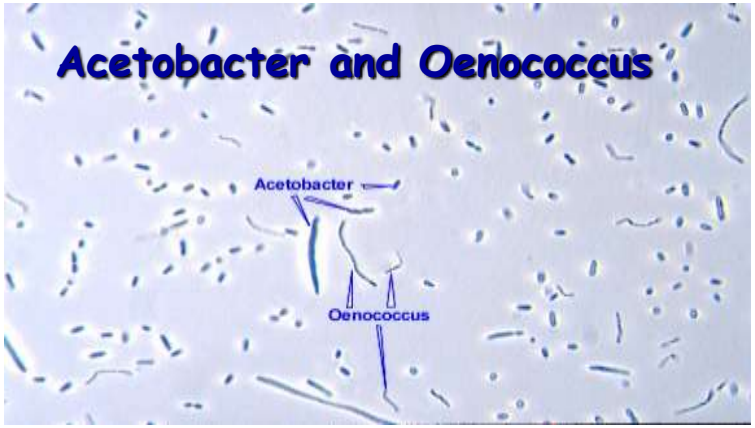
Role of microbes in winemaking

- Yeast
  - Conventional
    - *Saccharomyces spp.*
      - Primary function
      - Secondary function
  - Non-Conventional
    - Native (un-inoculated)
    - *Torulaspora delbrueckii, Kluyveromyces marxianus*
- Bacteria
  - *O. oeni* only if you are planning on a MLF
  - *Lactobacillus plantarum*?
- Mold
  - Botrytis, only if making a Botrytis style wine

Microflora in must and wine







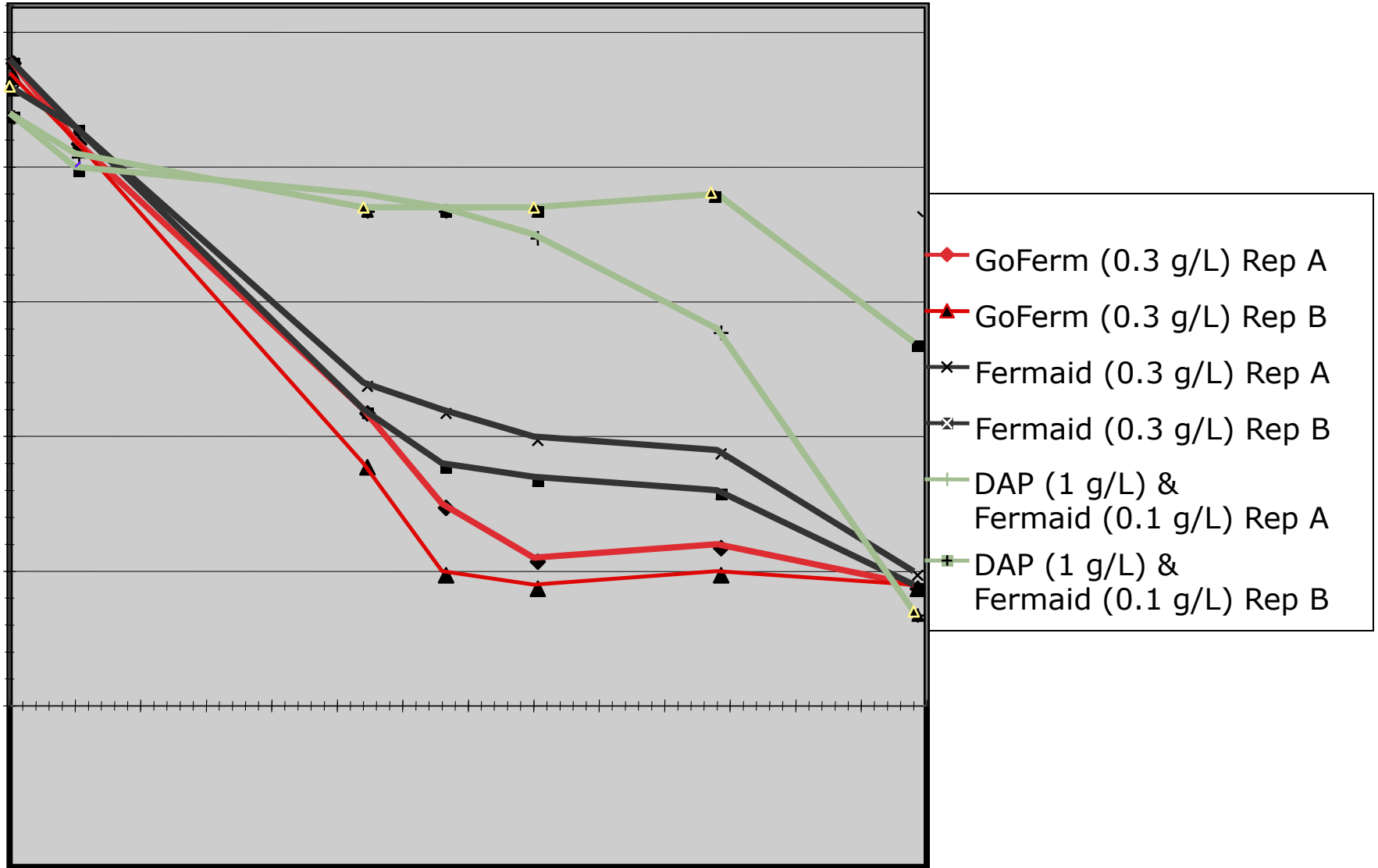
2 small comments to set the stage

- ML is not a separate entity
- ML is influenced by winery AND vineyard practices!

Yeast nutrition impact on MLF – 2006 Chardonnay (NY State)

Yeast: Lalvin ICV D254 - MBR® starter culture

(Thomas Henick-Kling, Cornell University)



Pesticide Residue Inhibition of Malolactic Bacteria

Plate Diffusion Test

Acid MRS-Agar (yellow: 10ppm, green: 100ppm, rosé: 1000ppm).

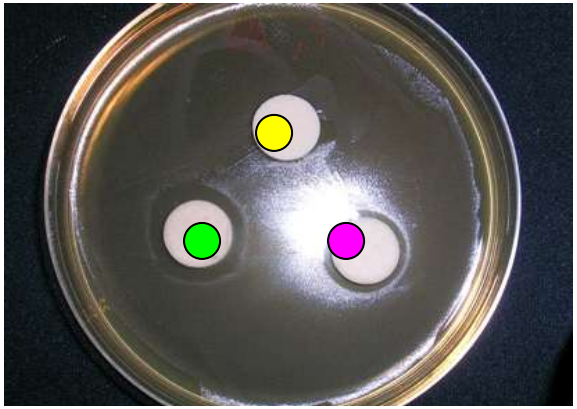


Fig. 1 **cymoxanil + dithianon**

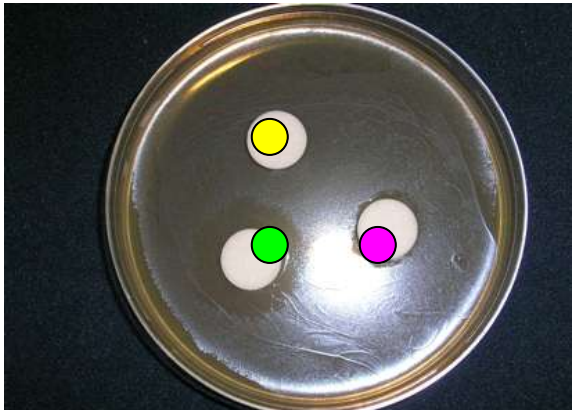


Fig. 2 **Cyprodinil**

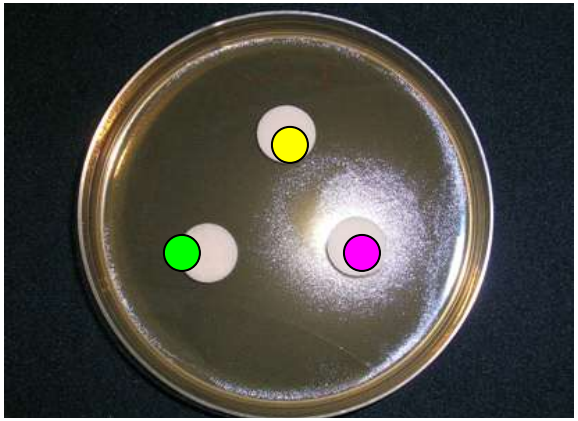
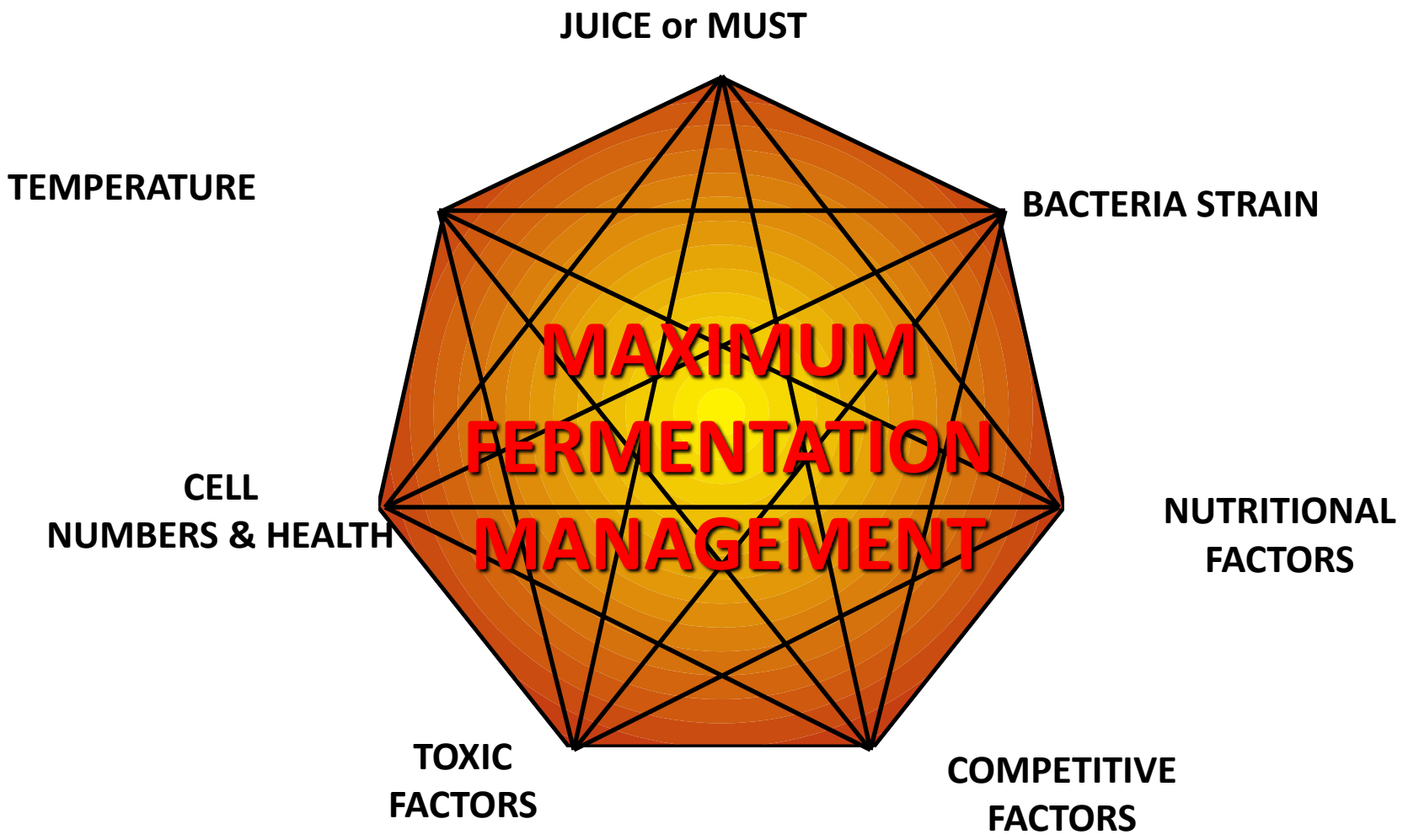


Fig. 3 **fludioxonil**

Pesticide Residue Inhibition of Malolactic Bacteria..

Pesticide	Active Ingredient	Inhibition
AKTUAN	cymoxanil+ dithianon	High
SCALA	pyrimethanil	Low
SWITCH	Cyprodinil or fludioxonil	Moderate
FRUCPICA	mepanipyrim	None

Factors affecting Fermentation Management - Key Interrelationships

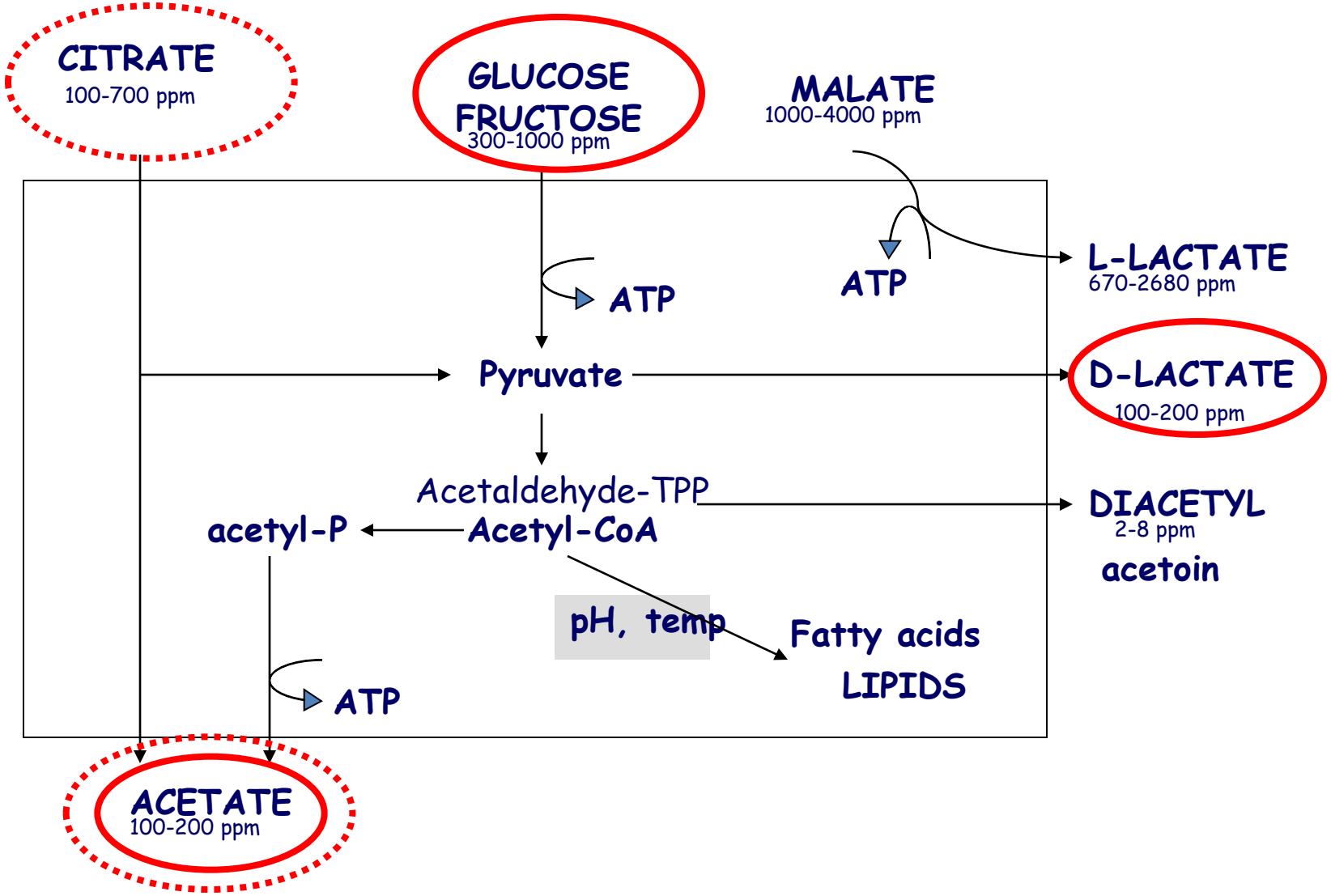


The joy of malolactic fermentation!

THE CHEMISTRY

- L (-) malic acid converted into L(+) lactic acid (commercial additions of D(+) will remain untouched).
- Not really a ‘fermentation’ as no energy is produced
- Reduction of acidity by 1-3 g/L
- Production of diacetyl: good or bad?

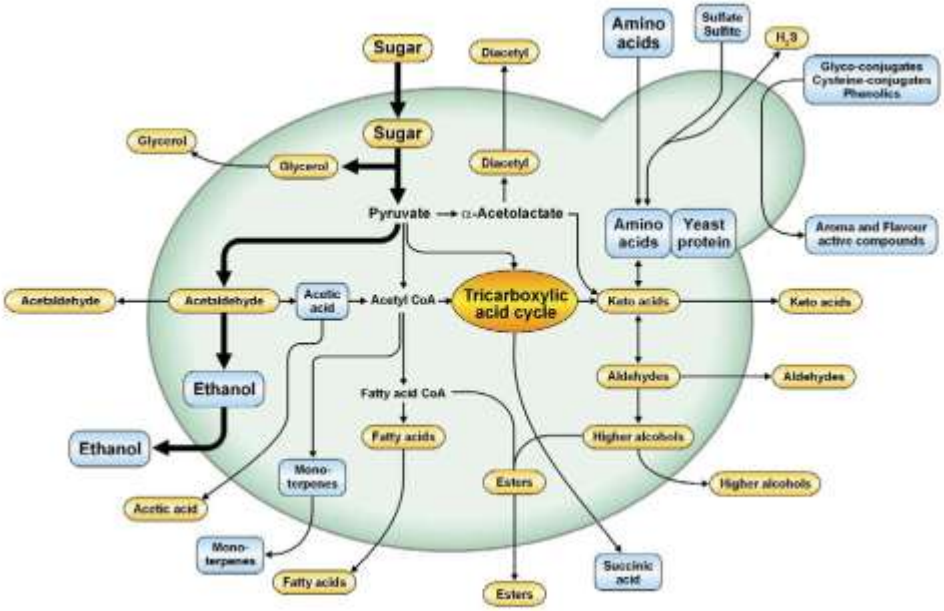
Metabolism in heterofermentative Lactic Acid Bacteria

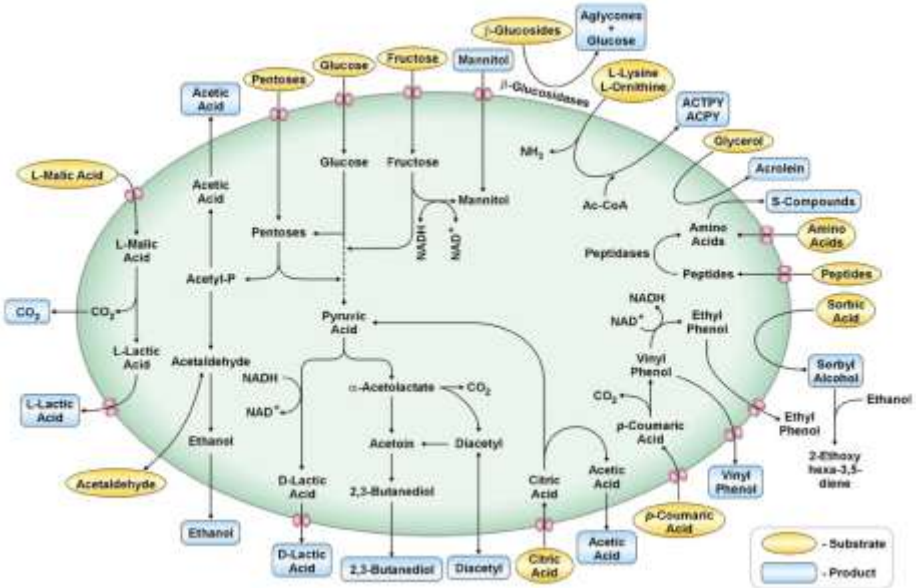


The more you know…

…the better!

Microbial metabolism flavor-active compounds

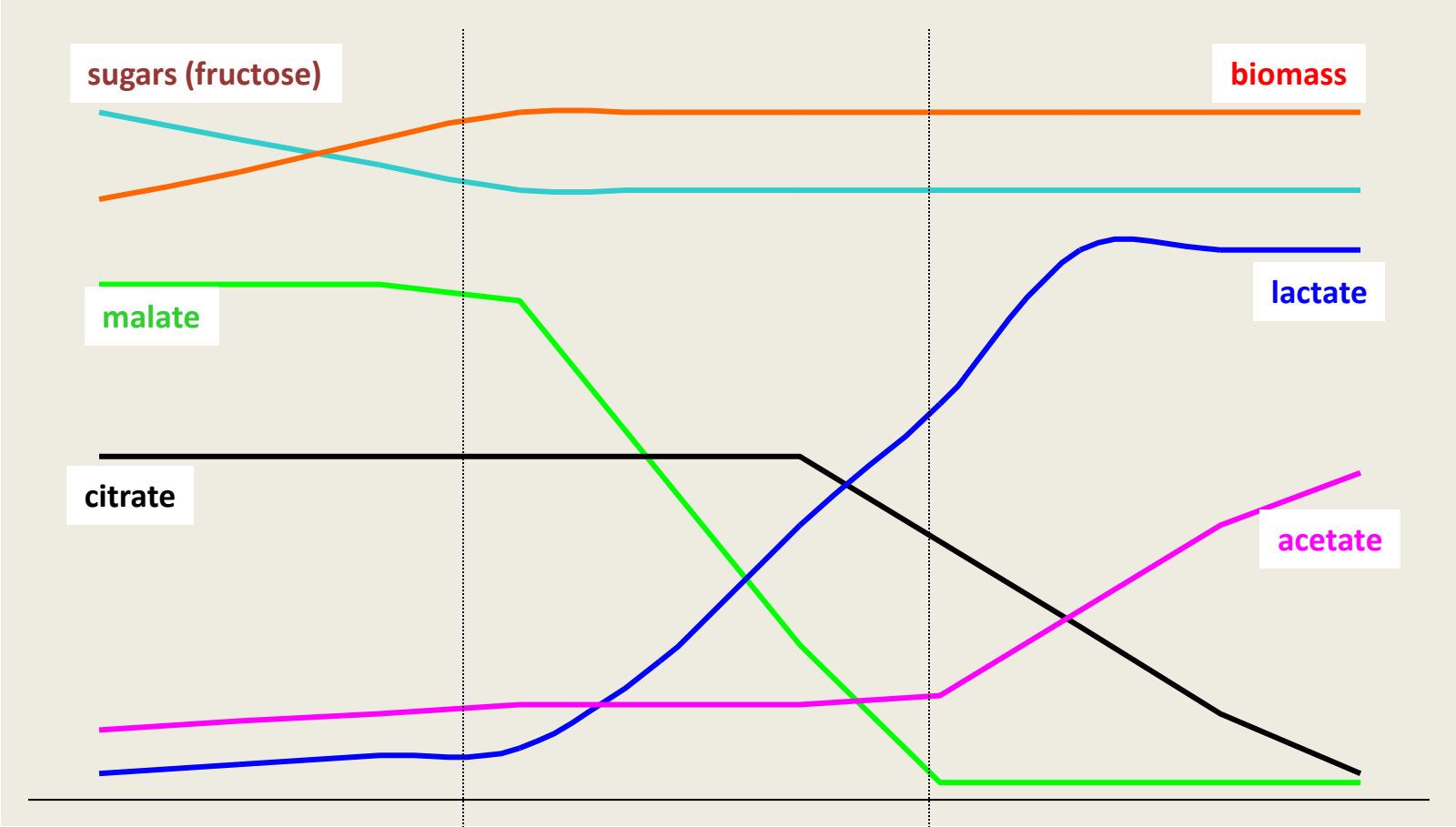




From Swiegers, Bartowsky, Henschke & Pretorius, 2005

Eveline Bartowski, AWRI, 2009

Metabolism of sugars and organic acids during MLF



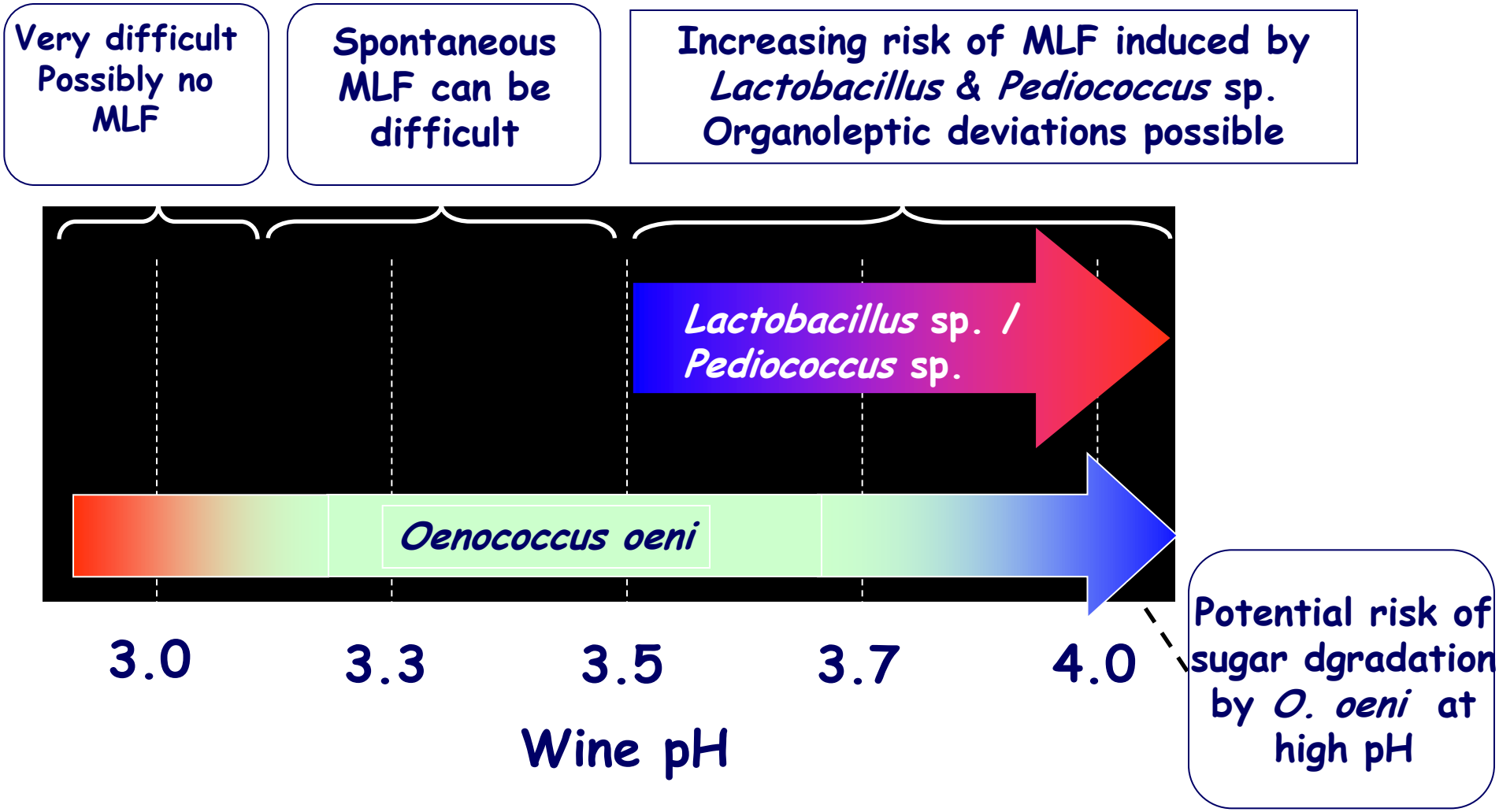
CELL GROWTH
sugar catabolism
no malate catabolism
no citrate catabolism
slight acetate production
slight lactate production

STATIONARY PHASE I
no sugar catabolism
malate catabolism
no citrate catabolism
no acetate production
lactate production

STATIONARY PHASE II
no sugar catabolism
no malate catabolism
citrate catabolism
acetate production
no lactate production

A **sugar metabolism** could **only be detected after consumption of the organic acids** (malic and citric)**!**

Potential risk of spontaneous MLF
- pH influence



Degradation of Biogenic Amines

Either by
Monoamine Oxidases (Monoamines) or
Diamine Oxidases (Diamines)

Inhibited by Alcohol

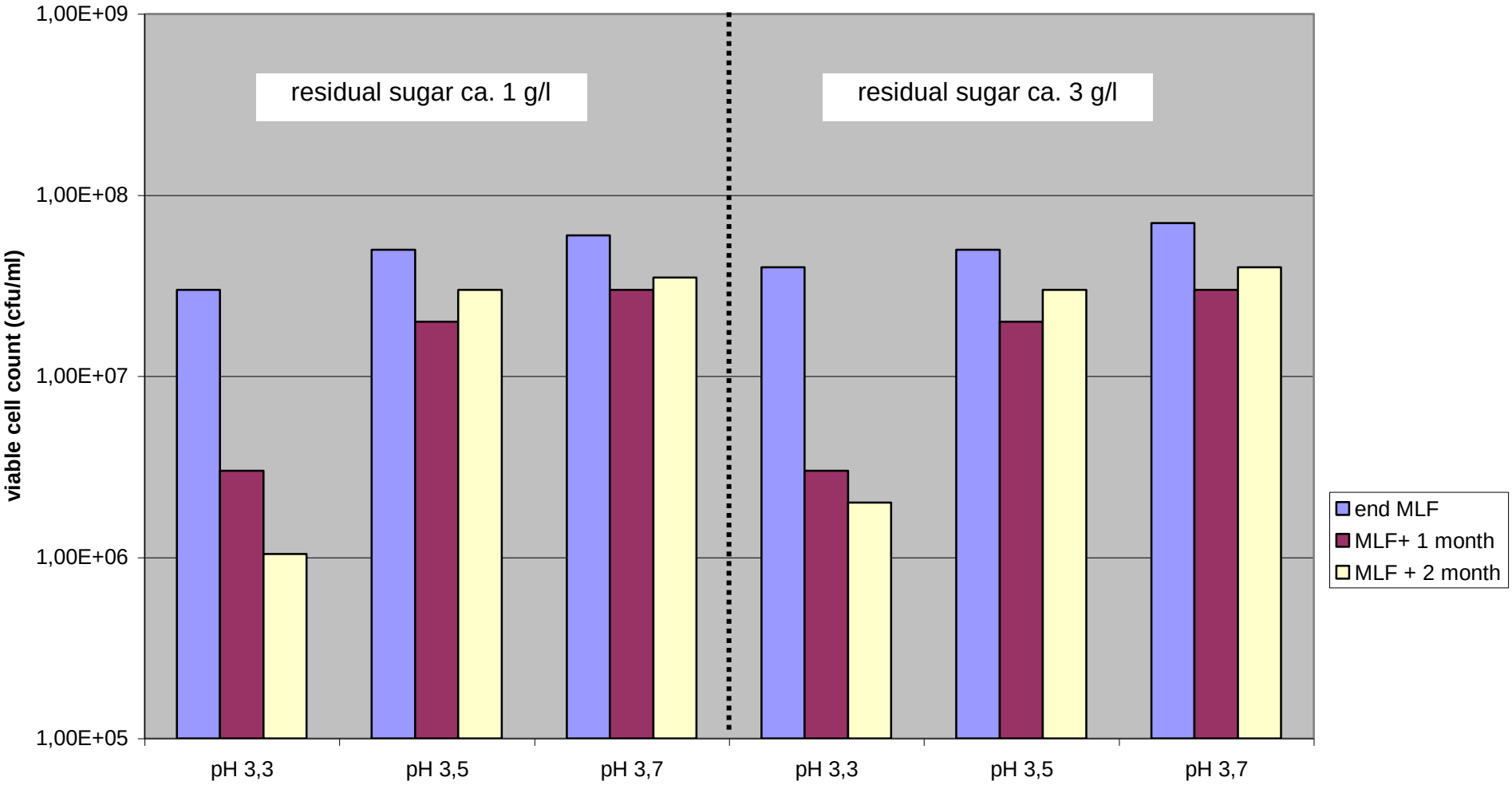
Have Vitamine B6 as Cofactor

Selected strains do not produce biogenic amines

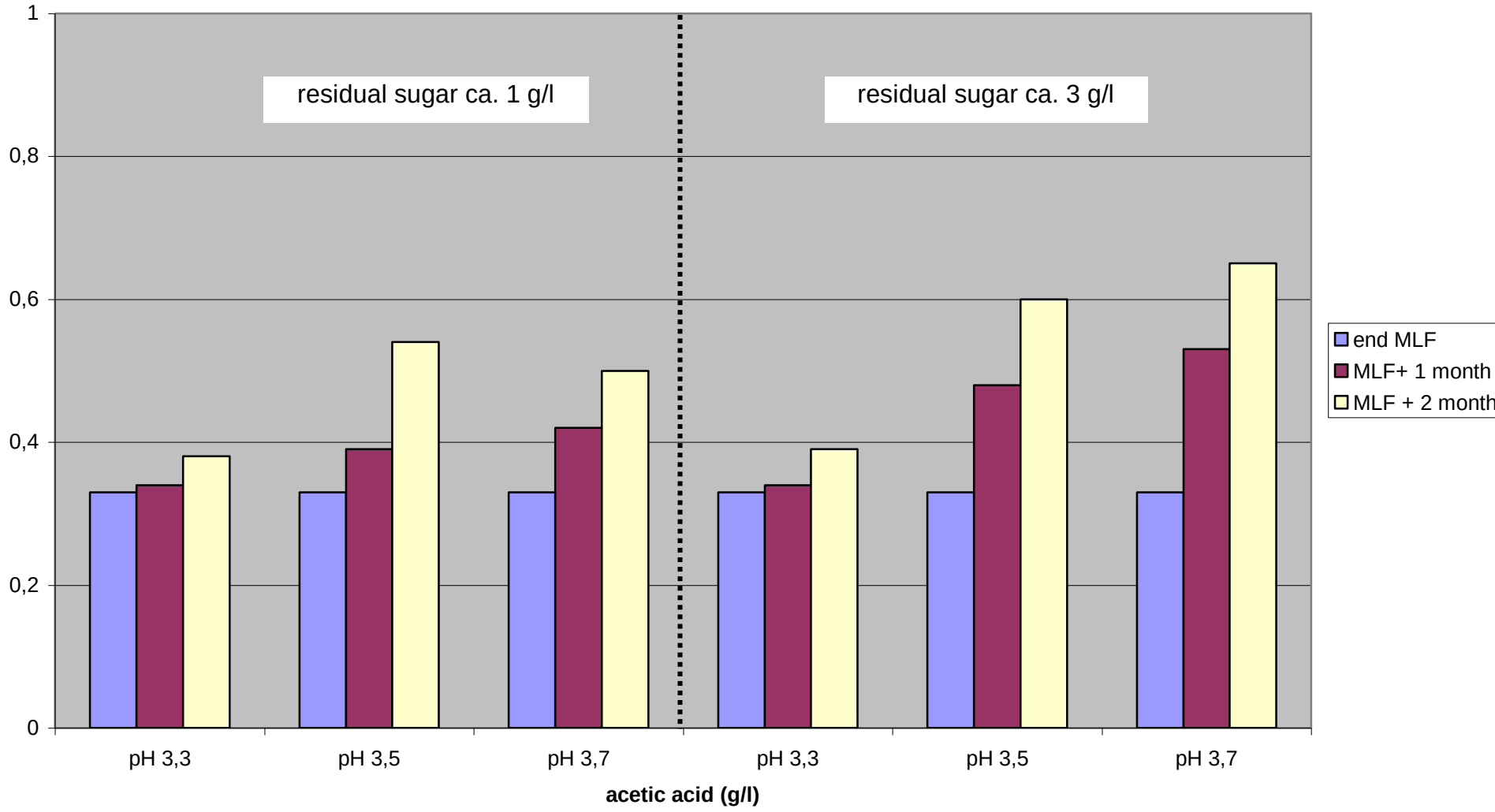
Spontaneous bacteria???

= It all depends on which strain took over to do the malolactic conversion??

Survial and growth of a complex O. oen population after MLF at different pH and res. glucose levels



Evolution of acetic acid in a Pinot Noir after MLF:
- influence of pH and residual sugar levels



Scorecard for determining the ease of malolactic fermentation

	My wine	Evaluation				Score
		1 point each	2 points each	8 points each	10 points each	
Alcohol (% vol.)		< 13	13 - 15	15 - 17	> 17	
pH		> 3.4	3.1 - 3.4	2.9 - 3.1	< 2.9	
Free SO_2 (mg/L)		< 8	8 - 12	12 - 15	> 15	
Total SO_2 (mg/L)		< 30	30 - 40	40 - 60	> 60	
Temperature (°C)		18 - 22	14 - 18 or 22 - 24	10 - 14 or 24 - 29	< 10 or > 29	
Yeast's nutritional needs		Low	Medium	High	Very high	
Ease of Alcoholic Fermentation		No problems	Transient yeast stress	Sluggish / stuck AF	Prolonged yeast contact	
Initial level of malic acid (g/L)		2 - 4	4 - 5 or 1 - 2	5 - 7 or 0.5 - 1	> 7 or < 0.5	
Maximum AF rate (maximum loss of brix/day)		< 2	2 - 4	4 - 6	> 6	

NOTE: Other, currently less well-known factors that are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.

TOTAL

< 13 points	13 - 22 points	23 - 40 points	> 40 points
Favorable	Not so favorable	Difficult	Extreme

Total score for the ease of malolactic fermentation:

Other, currently less understood factors that may impact Malolactic Fermentation and are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.

Effective SO_2 and pH

Molecular SO_2 (%) = 100 / [10 $^{pH-1,81}$ + 1]

pH	Free SO_2 mg/l	% molecular SO_2 mg/l	Total molecular SO_2 mg/l
3	25	6,06 %	1,54
3	8,2	6,06 %	0,5
3,6	25	1,6 %	0,4
3,6	31	1,6 %	0,5
4	25	0,64 %	0,16
4	78	0,64 %	0,5

bactericide concentration

bactericide concentration

bactericide concentration

At pH = 3, mol. SO_2 = 6,06 % $\Rightarrow$ 8,2 mg/l of free SO_2 is sufficient

At pH = 4; mol. SO_2 = 0,64 %, $\Rightarrow$ 78 mg/l of free SO_2 is sufficient

INTERMISSION

Playing with the timing of MLF

Co-fermentation/ Co-inoculation
vs. Sequential

Yeast & ML

Pros and Cons of Early Inoculation

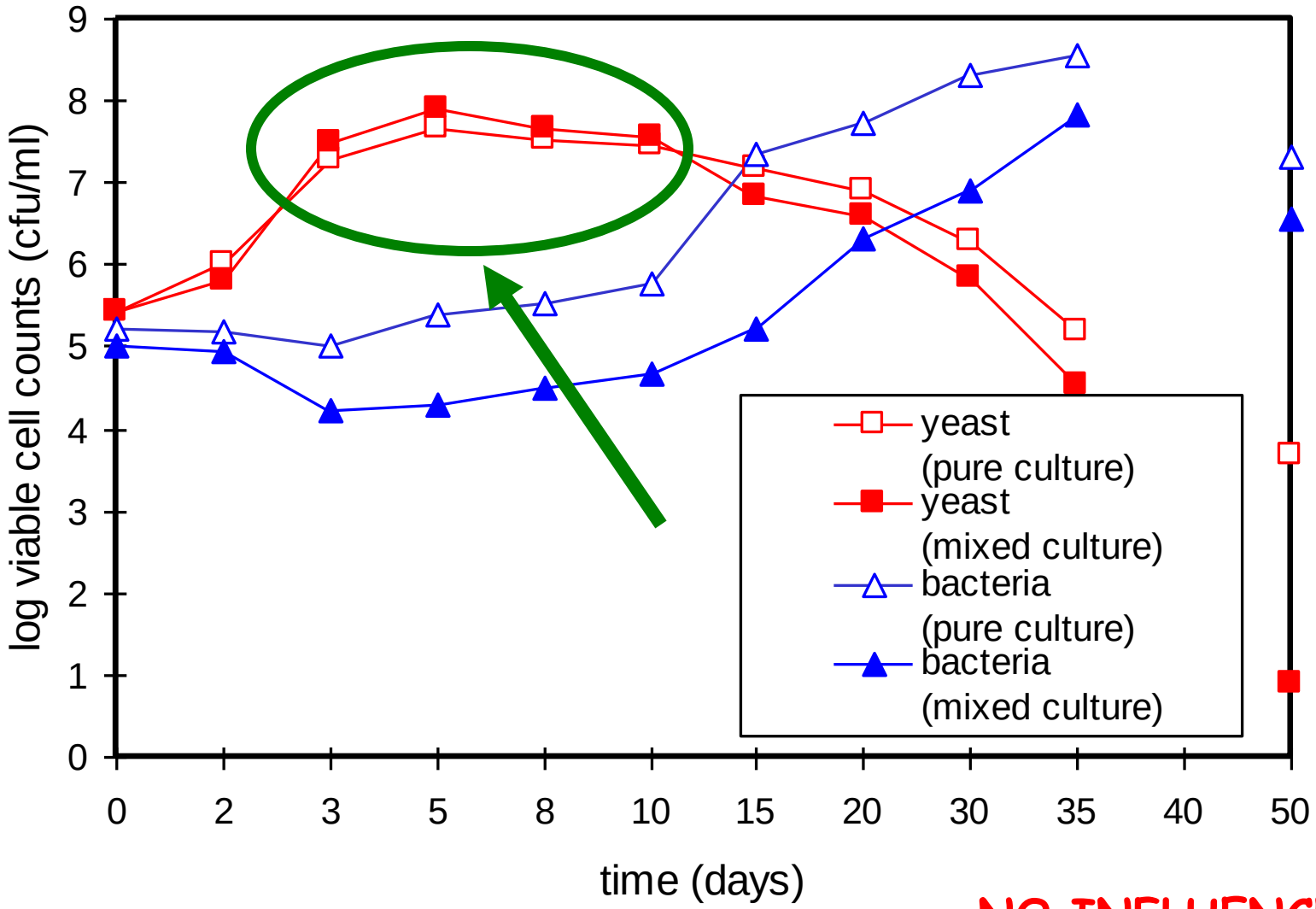
PROS

- Time Savings
- No Alcohol inhibition
- Temperature from AF
- Flavor Implications

CONS

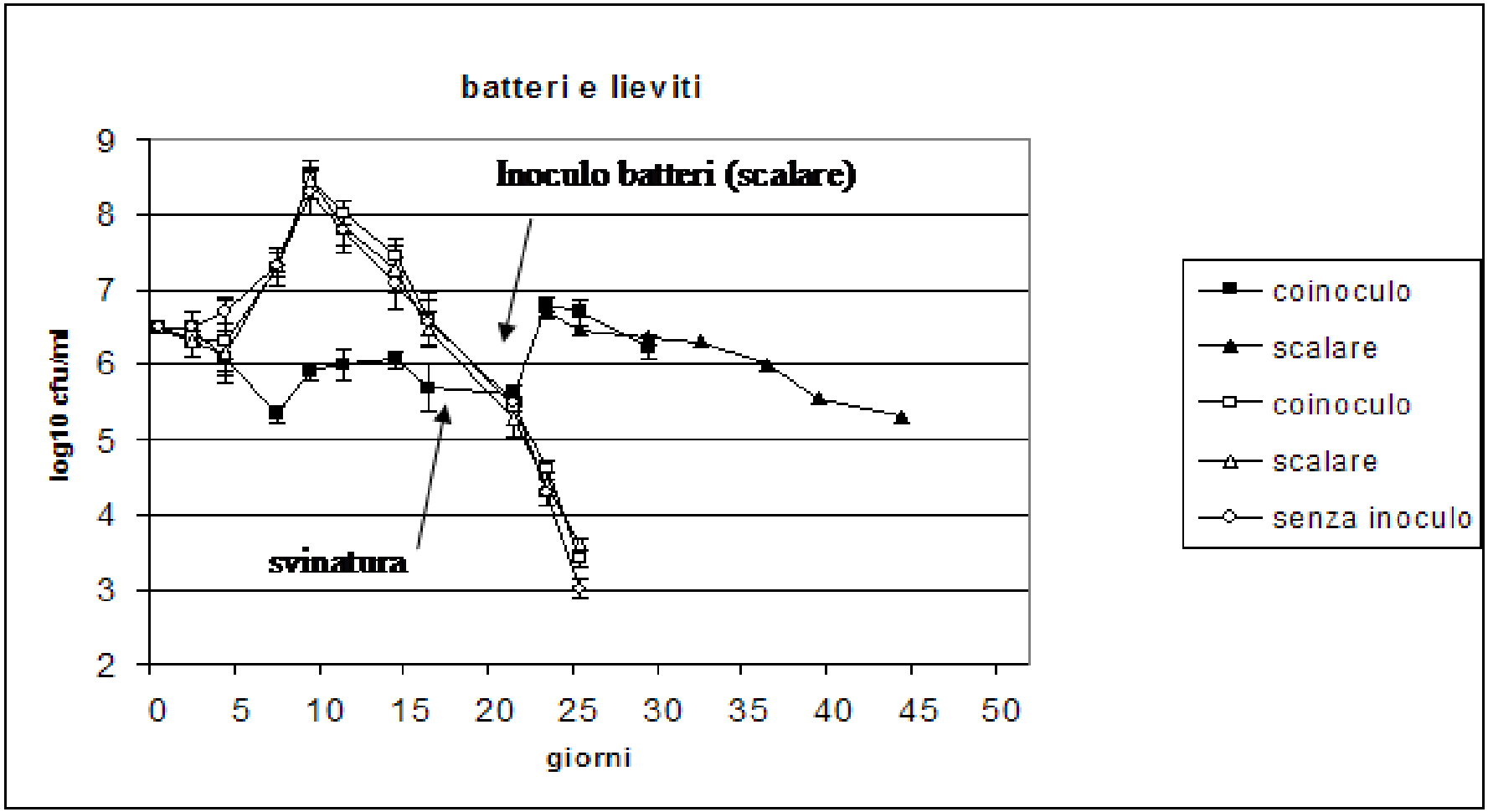
- Watch for SO2 (free&bound)
- Yeast Compatibility
- VA Production from Metabolism of Sugars
  - (not with V22, homofermentative strain)

What does the RESEARCH show?



(King and Beelmann 1986)

NO INFLUENCE OF O.Oeni on AF



Tosi, E[1]. & G. Zapparoli [2]
Dipartimento di Biotecnologie, Università degli Studi di Verona, Verona, Italy
The Australian & New Zealand Grapegrower & Winemaker
(February 2007, pp 71-77)

- As we know, aldehyde is especially responsible for green apple and vegetative sensations.
- In co-inoculation we have a very early consumption of acetaldehyde,
  - peak levels of acetaldehyde are significantly lower compared to a sequential inoculation
  - Same concept is valuable for other aldehydes and ketones.

- This explanation is based on results of our research collaboration with Ramón Mira de Orduna.

What about

HIGH PH REDS

(high risk)

?

Experimental set-up - 2005

Cabernet Sauvignon (RSA) (initial pH 3.77)

AF	Lalvin ICV D254	Lalvin Rhone 2056
MLF	MBR Alpha Lalvin VP41	MBR Alpha Lalvin VP41

Moment of inoculation:

A: co-inoculation yeast and bacteria

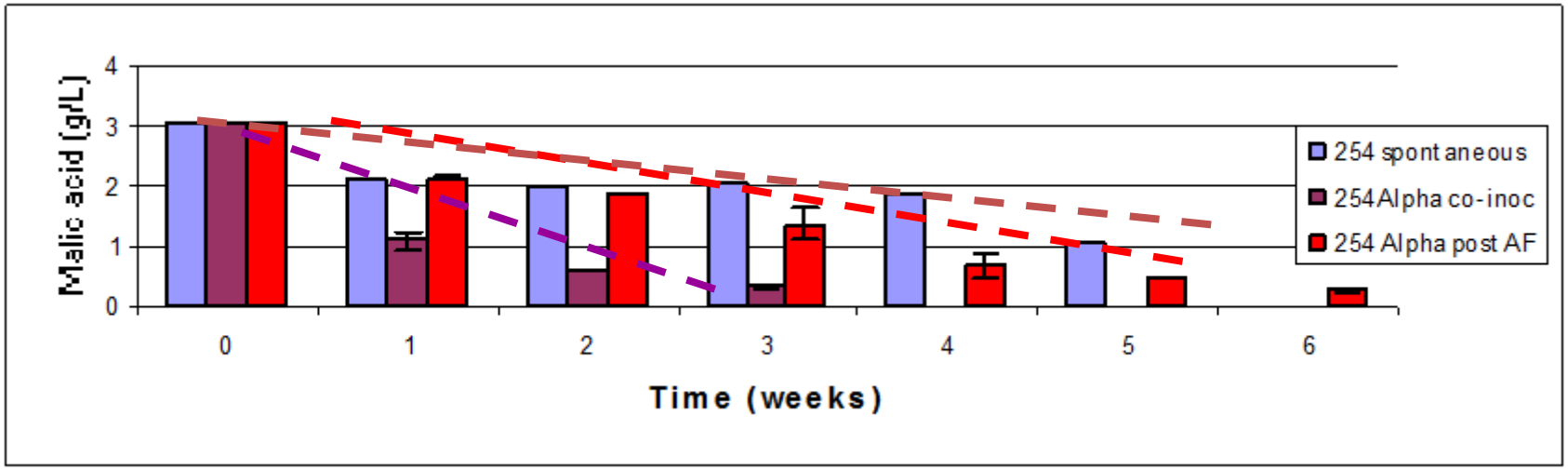
B: Inoculation of bacteria before the end of AF residual sugars < 30 g/L

C: Inoculation after AF

Control: Spontaneous MLF
All trials in duplicate

Analysis: FT-IR – malic acid degradation
HPLC – determination of biogenic amines

Speed of MLF

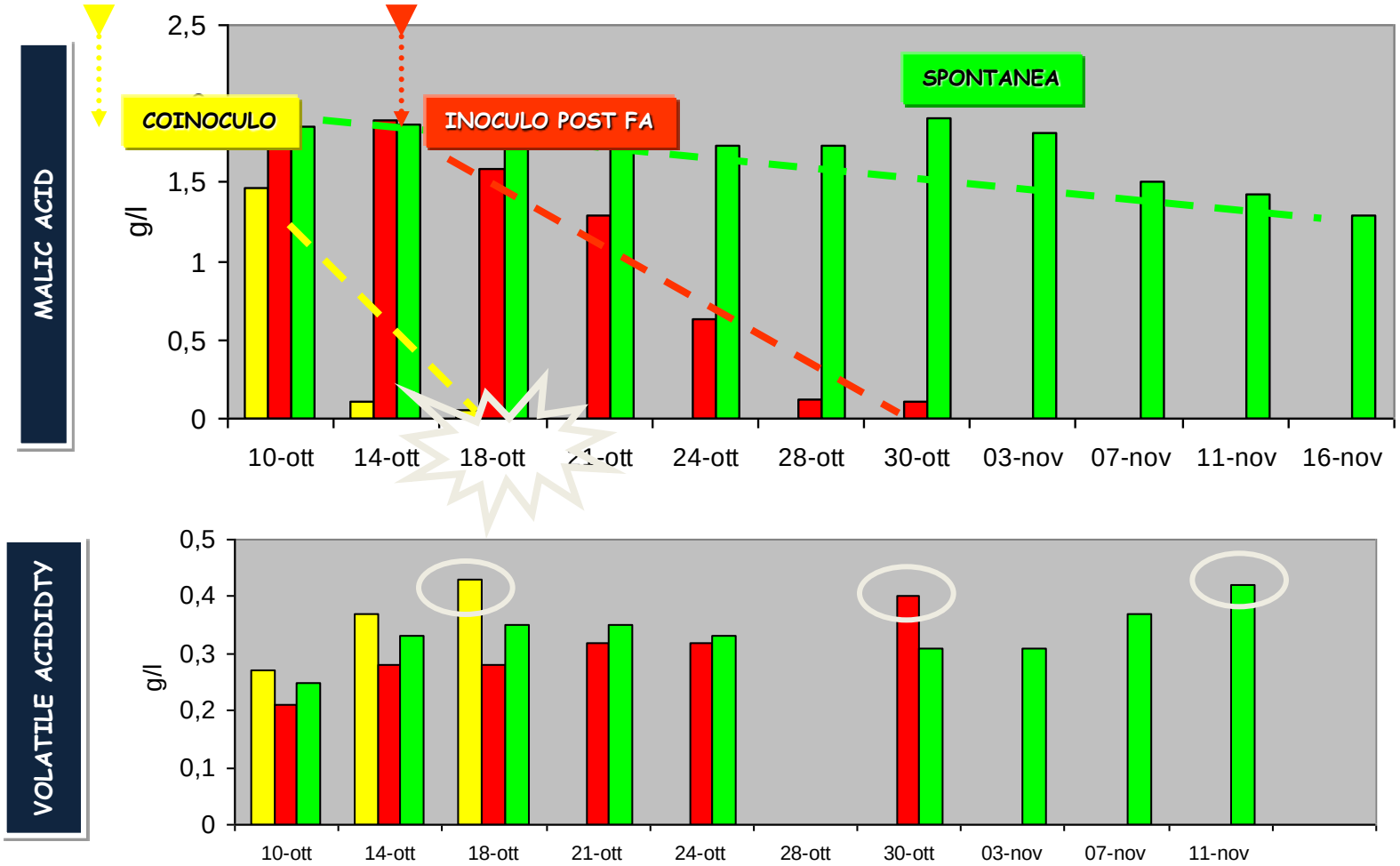


- With coinoculation,
  MLF much faster

MALOLACTIC FERMENTATION

DIFFERENT TIMING OF INOCULATION vs SPONTANE0US MLF

MONTEPULCIANO D'ABRUZZO-VINTAGE 2006 *C.Di Meo*



- **MLF MUCH FASTER**
- **NO SIGNIFICANT INCREASE IN VOLATILE ACIDITY !**

Improved nutrient formulations for *Oenococcus oeni*

Bacteria Nutrition

- How do you know what you need?
- Difficult as no “NOPA” for bacteria…..
- Focus on positives and make life as comfortable as possible!

Nutrient requirements for *O.oeni*

Nitrogen

Only in organic form (a-amino acids, peptides)
NO AMMONIUM SALTS

Vitamins

(nictotinic acid, biotine, thiamine,
pantotenic acid, etc.)

Trace elements

(Mg, Mn & K)

IMPORTANT

Important for the function of the bacteria
Absence of inhibitory compounds:
SO_2, pesticides, medium chain fatty acids etc.

WINE
SITUATIONS OF NUTRIENT DEFICIENCY

- Nitrogen limitation in the must + a short yeast autolysis = limited release of amino acids by yeast... for the nutrition of the bacteria
- Post-fermentation procedures (clarification, filtration, etc.)
- The yeast strain used for alcoholic fermentation

All these factors influence how "malolactic friendly" the wine is after alcoholic fermentation

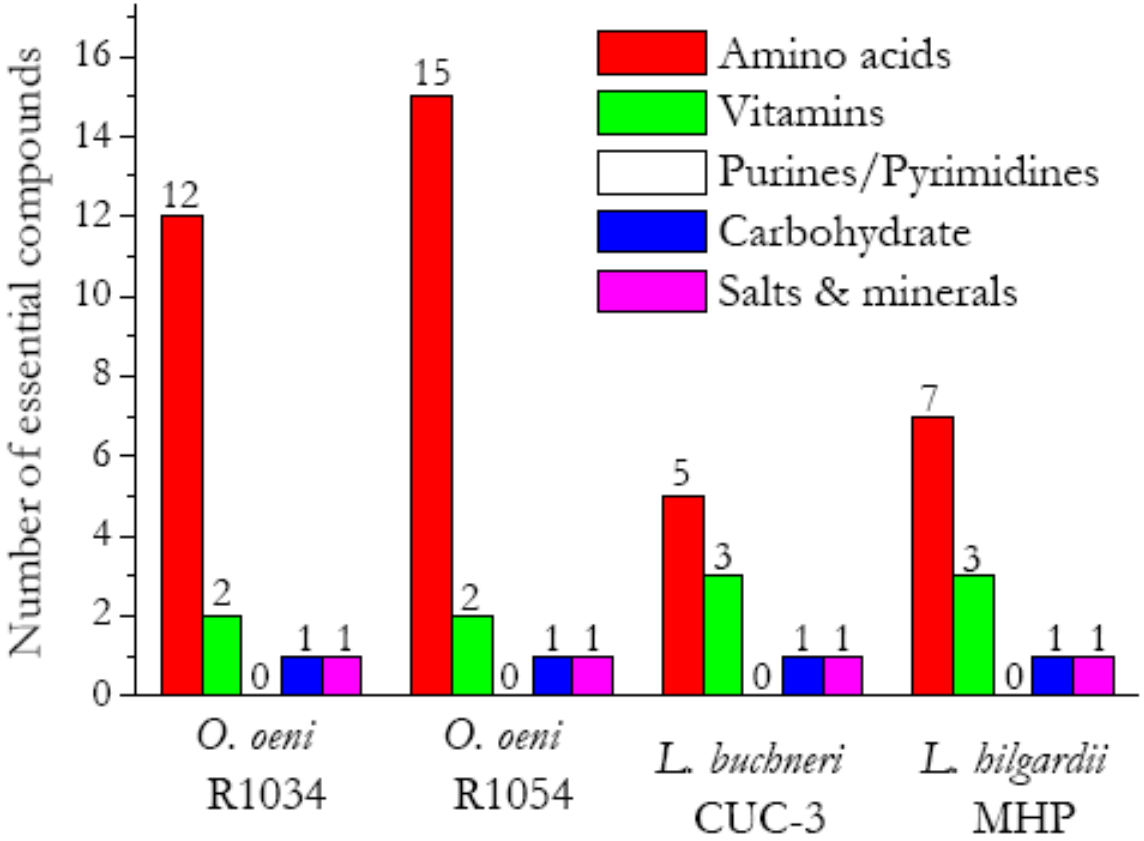
Table of compatibility with MLF

Level of compatibility	5	4	3	2	1	
	++	+	+ -	-	--	No information
Yeast strains	QA23	2056	EC1118		**ALB**	C1108
	ICV D254	**DV10**	**PM**	SIMI White	V1116 (K1)	C or R7
	71B	R2	ICV D21	2323	**WAM**	FC9
	AMH	M2	**BDX**	2226	Opale	
	W15	W	BRL97	RHST	Enoferm M1	T73
	VRB	QD145	SLO	T306		
	CSM	6U	Cross evolution	2056		Syrah
		CY3079	BGY	RA17		M69
		GHM	**MCS**	BM45		CGC62
		RC212	RQ15	BM4x4		CK
		ICV D80	**228**	BA11		
		VN	ICVD47			MCS
		43				**SVG**
		ICV GRE				
		RHST				
		Rhone 4600				
		CM				
		CS2				
		299				
		NEM				
		PMA				
		BC				
		CEG				

NUTRITIONAL NEEDS

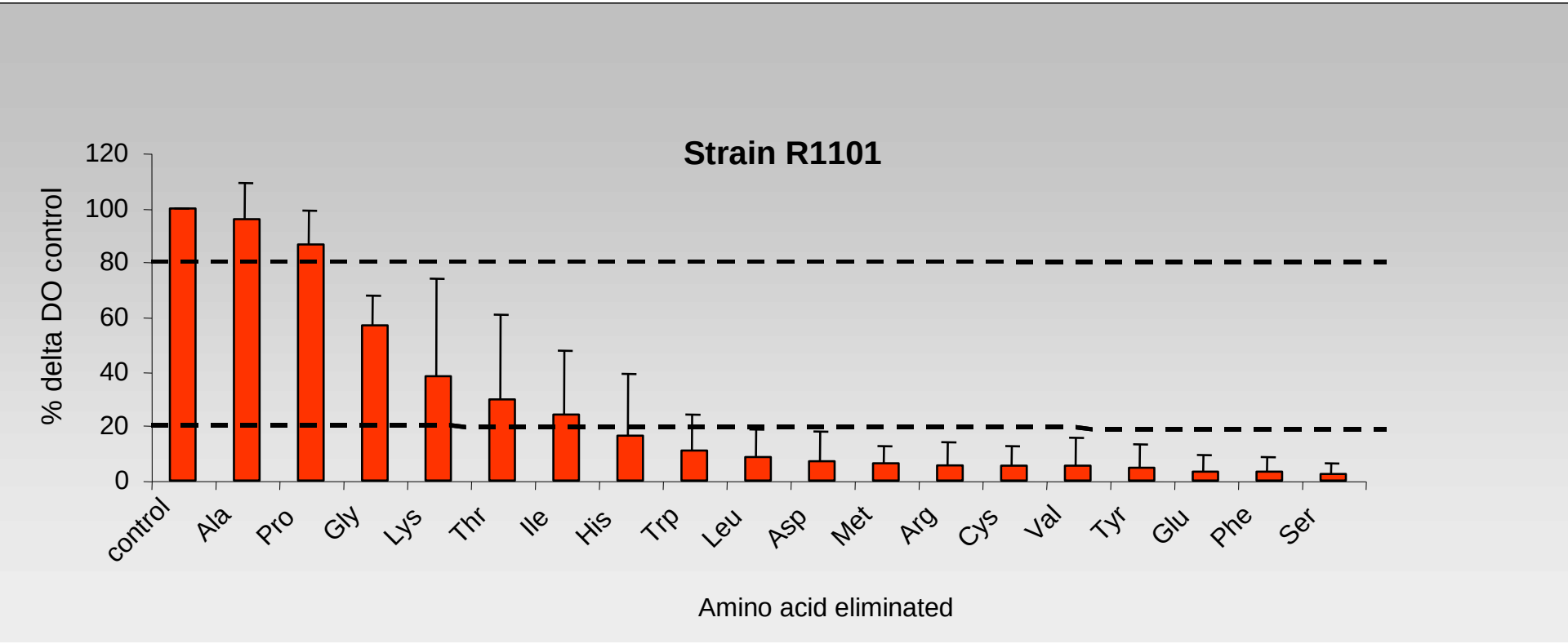
collaboration Cornell University – USA 2008 (R. Mira de Orduña)

Number of essential nutrients for four wine LAB determined with the single omission technique. Each strain was grown in a simplified version of the chemically defined medium



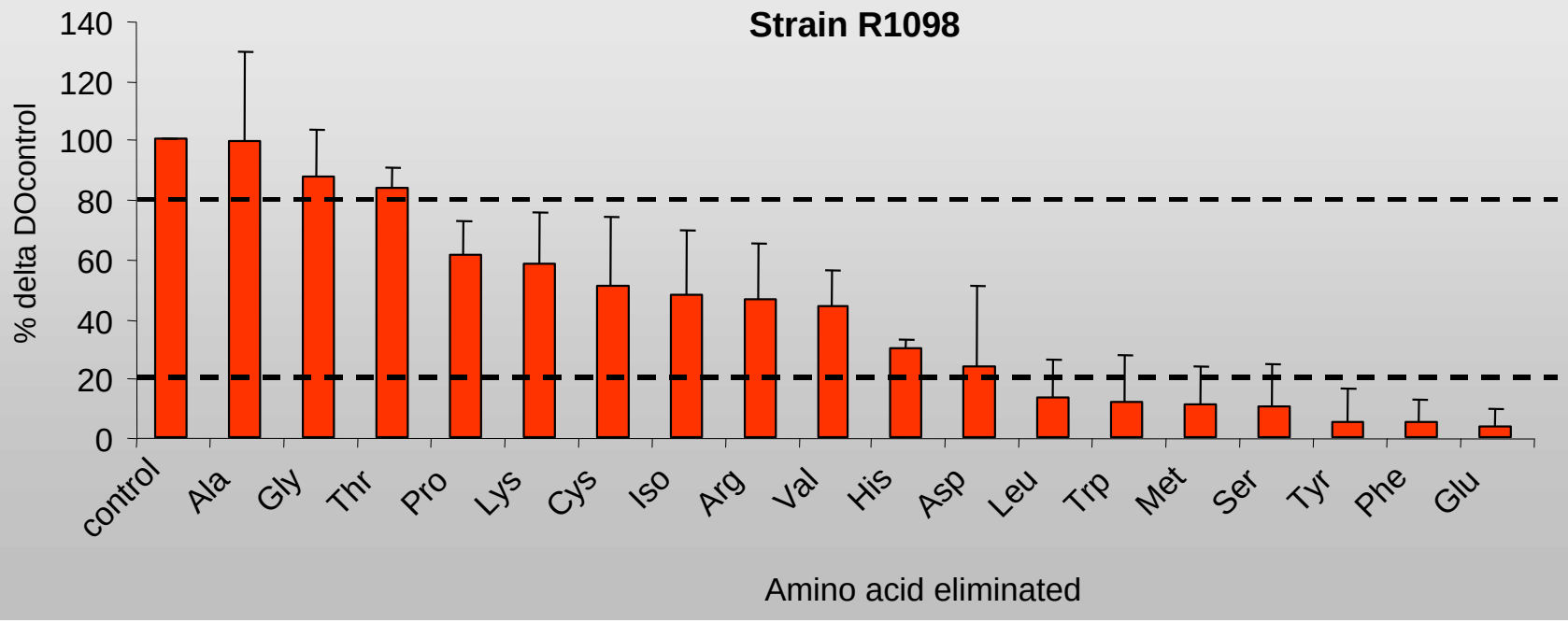
NUTRITIONAL NEEDS

Growth yield Oenococcus oeni strain Lalvin 31 on a synthetic medium lacking the indicated amino acid (Data are expressed as the percentage of OD 600 nm)



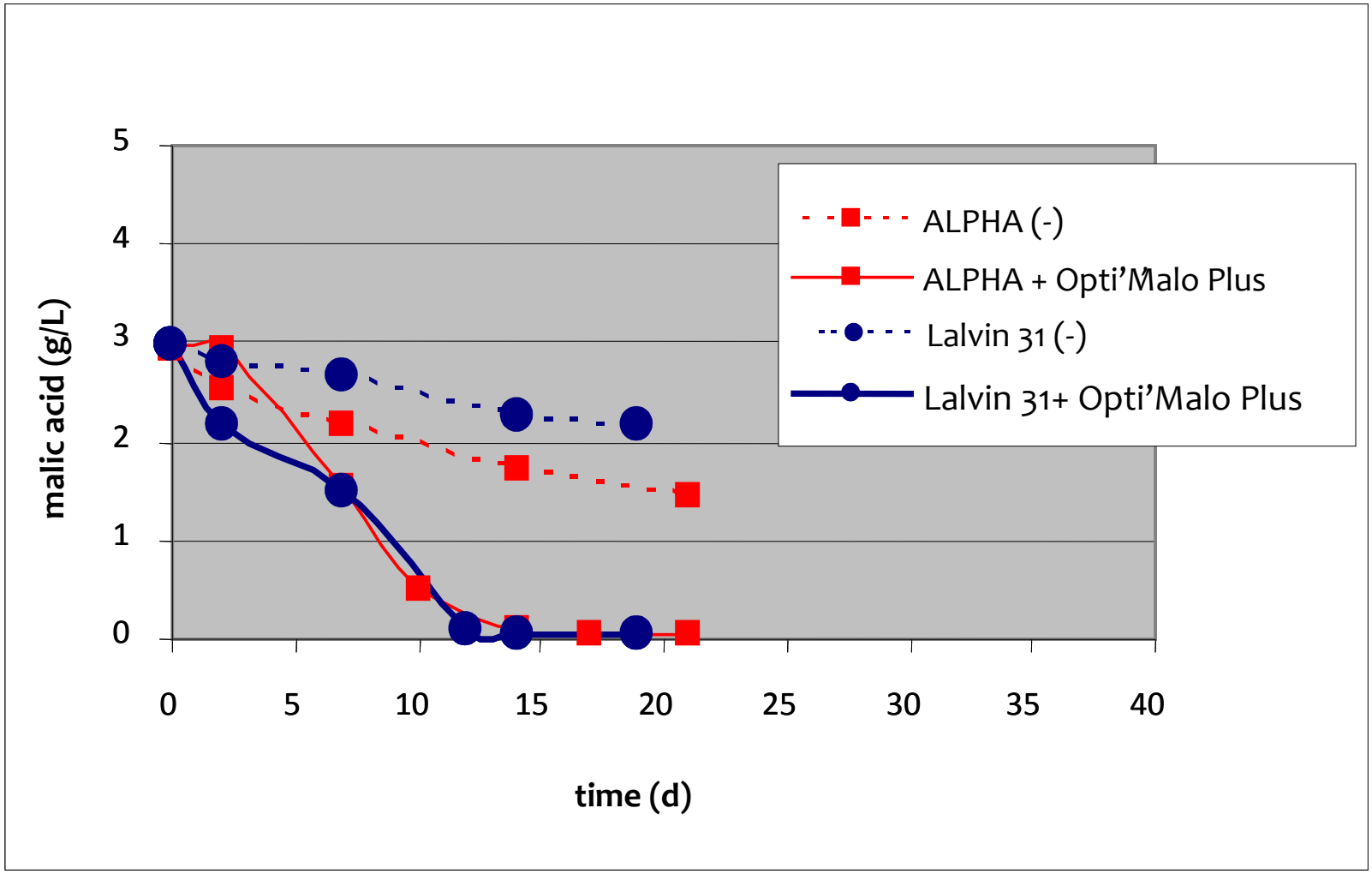
NUTRITIONAL NEEDS

Growth yield Oenococcus oeni strain ALPHA on a synthetic medium lacking the indicated amino acid (Data are expressed as the percentage of OD 600 nm)



NUTRITIONAL NEEDS:

Kinetics of malic acid degradation in a 2003 Cabernet Sauvignon (alcohol 13%vol, T-SO_2 35 ppm, pH 3,68) after direct inoculation with ML starter cultures with (+) and without (-) addition of ML nutrient Opti'Malo Plus



Comparisons

Rehydration Nutrients

- Acti-ML
  - Special inactivated yeast
    - Amino Acids
  - Peptides
  - Vitamins
  - Growth factors
  - Cellulose

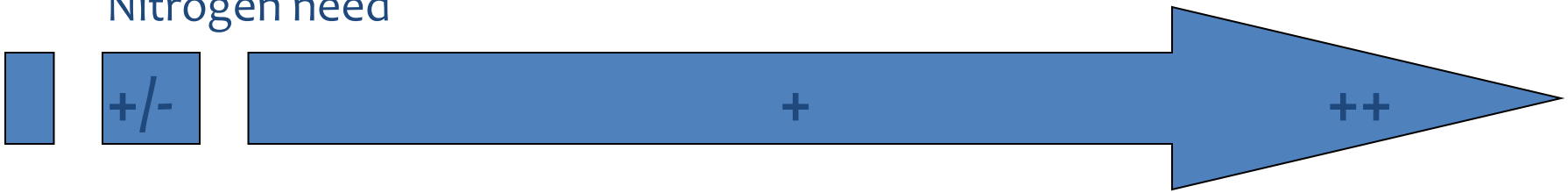
Fermentation Nutrients

- Opti-Malo Plus
  - Special inactivated yeasts
    - Amino Acids
  - Mineral Co-factors
  - Vitamins
  - Polysaccharides
  - Cellulose

Nutrient Need

O. oeni strain

Nitrogen need



VP41	ALPHA	PN4	Elios 1	Inobacter	Lalvin31	Beta
Will benefit. Especially in difficult conditions, high alcohol, low pH or high SO_2	Will benefit. Especially in difficult conditions, high alcohol, low pH	Will benefit. Especially in difficult conditions, high alcohol, low pH	Will benefit. Especially in difficult conditions	Obligatory in the pied-de-cuve step, but also benefitial in the base wine	ALWAYS Especially in low pH conditions	ALWAYS Especially in sequential inoculation

Bacteria sensory contribution to the MLF

pH & aroma in wine

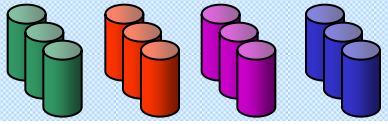
Limestone Coast

Clare Valley

AF ~ 550 kg vinimatic
Lalvin Rhone 2056
18-20°C

pH 3.3

MLB 1 **MLB 2** **MLB 3** **Non-MLF Control**



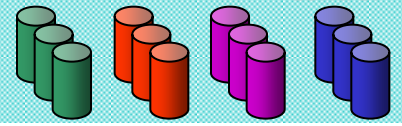
MLF duration: 20 days

Post-MLF pH adjustment → pH 3.5

15L, triplicate, 20°C

pH 3.7

MLB 1 **MLB 2** **MLB 3** **Non-MLF Control**



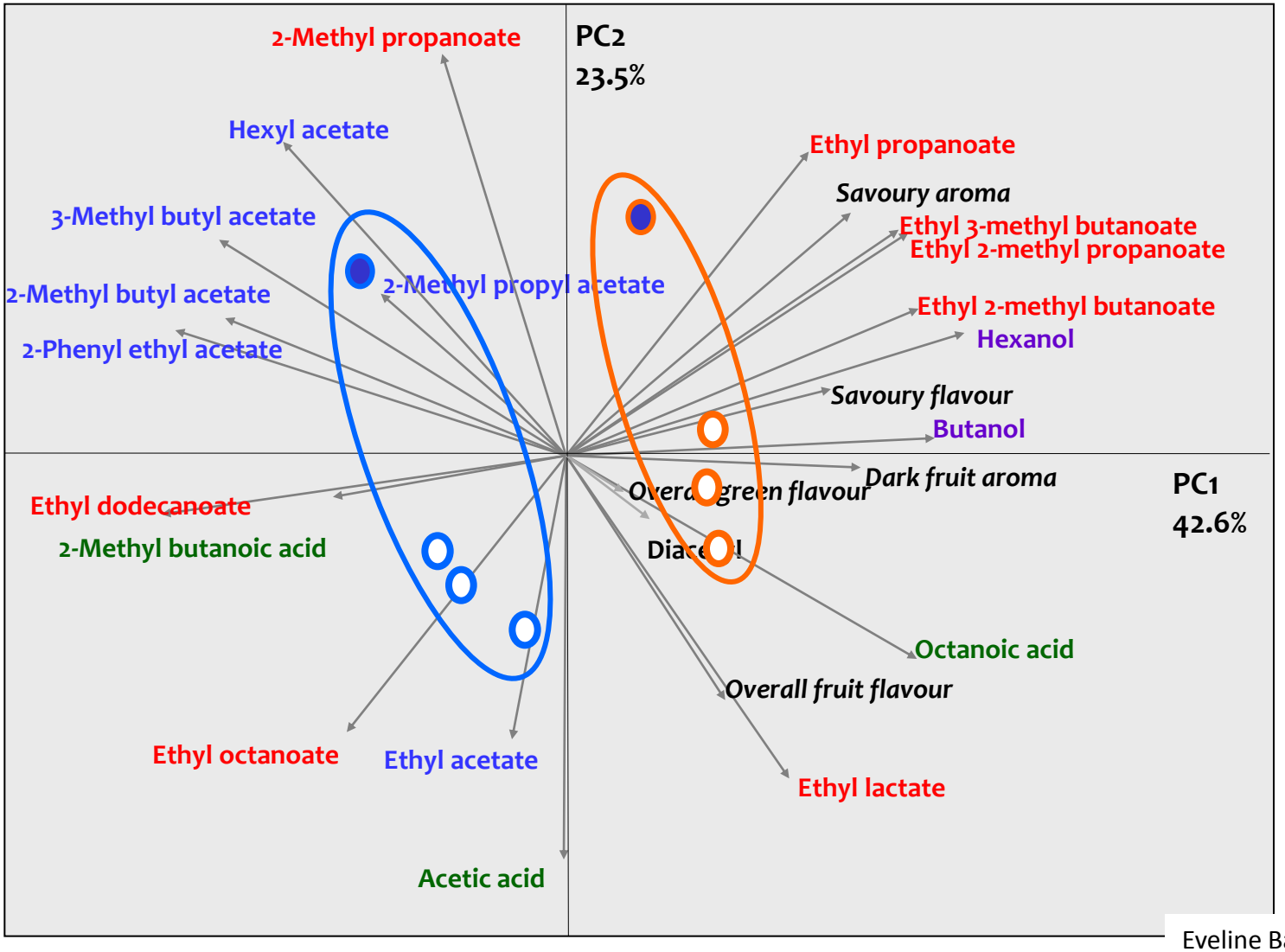
MLF duration: 12 days

Post-MLF pH adjustment → pH 3.5

Peter Costello

Eveline Bartowski, AWRI, 2009

Wine pH affects *O. oeni* metabolism



Limestone Coast

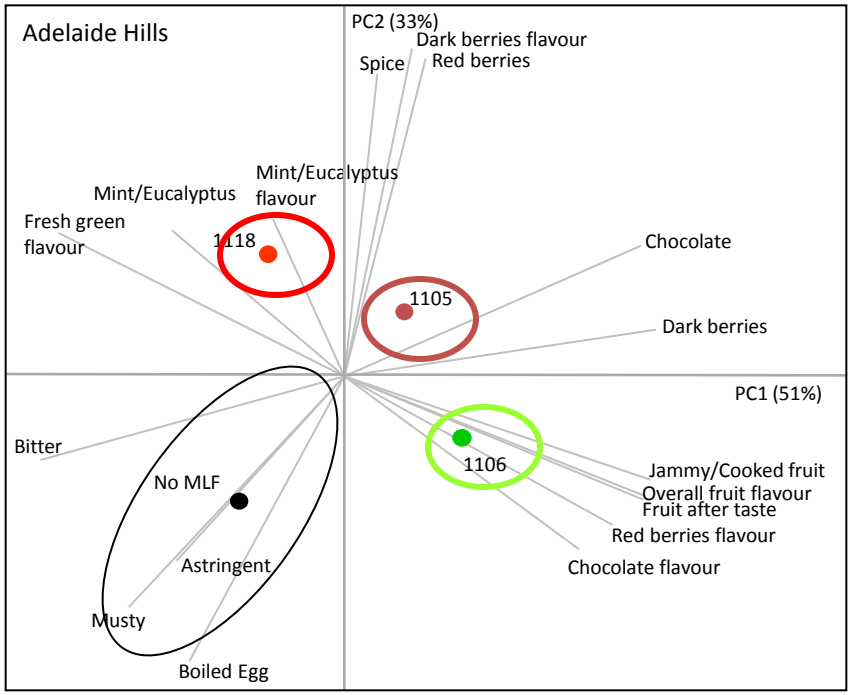
pH 3.3

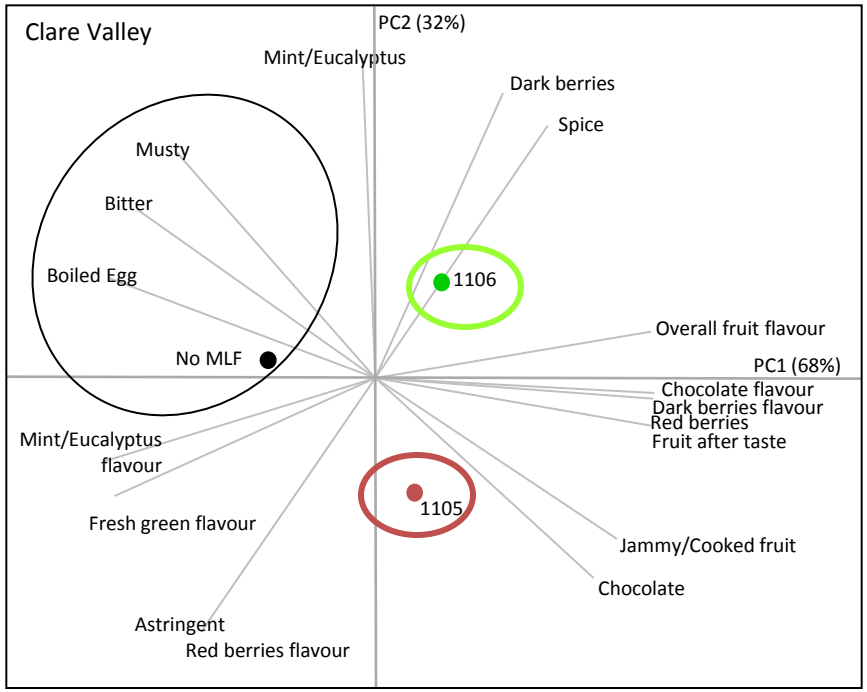
- No MLF
- MLF (3)

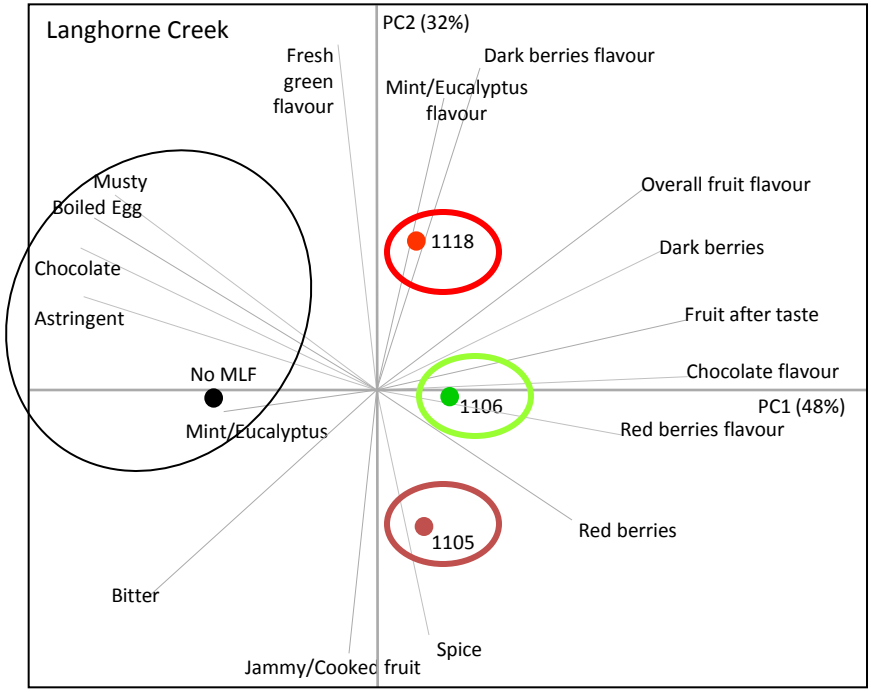
pH 3.7

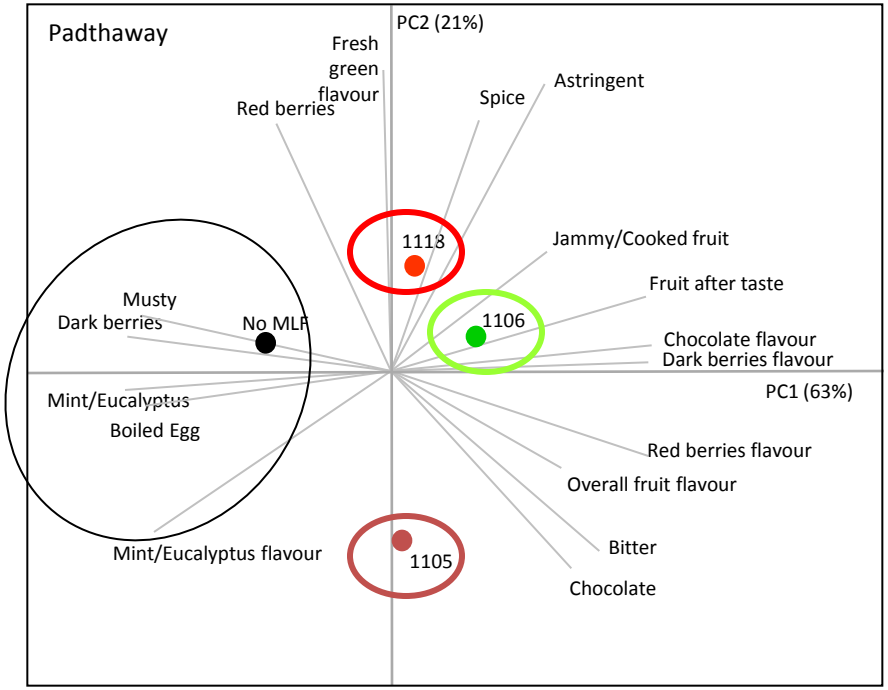
- No MLF
- MLF (3)

Eveline Bartowski, AWRI, 2009


Adelaide Hills
PC2 (33%)
Dark berries flavour
Spice
Red berries
Mint/Eucalyptus flavour
Mint/Eucalyptus
Fresh green flavour
1118
Chocolate
1105
Dark berries
PC1 (51%)
Bitter
1106
Jammy/Cooked fruit
Overall fruit flavour
Fruit after taste
Red berries flavour
Chocolate flavour
No MLF
Astringent
Musty
Boiled Egg

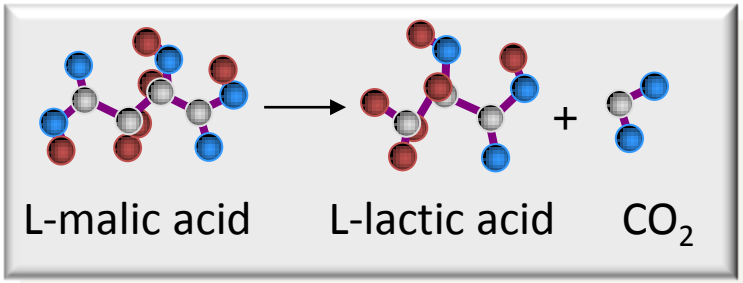

Clare Valley
PC2 (32%)
Mint/Eucalyptus
Dark berries
Spice
Musty
Bitter
Boiled Egg
1106
Overall fruit flavour
No MLF
PC1 (68%)
Chocolate flavour
Dark berries flavour
Red berries
Fruit after taste
Mint/Eucalyptus flavour
1105
Fresh green flavour
Jammy/Cooked fruit
Chocolate
Astringent
Red berries flavour


Langhorne Creek
PC2 (32%)
Fresh green flavour
Dark berries flavour
Mint/Eucalyptus flavour
Musty
Boiled Egg
Overall fruit flavour
1118
Dark berries
Chocolate
Astringent
Fruit after taste
No MLF
Chocolate flavour
1106
PC1 (48%)
Mint/Eucalyptus
Red berries flavour
1105
Red berries
Bitter
Spice
Jammy/Cooked fruit

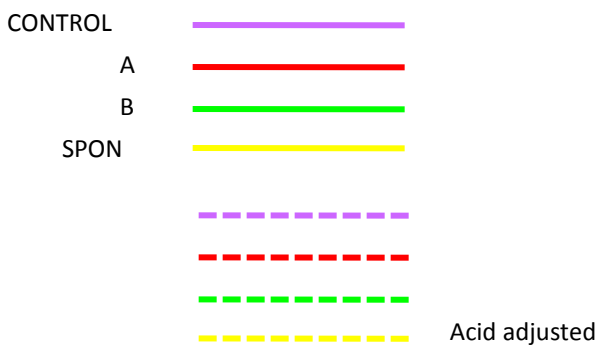

Padthaway
PC2 (21%)
Fresh green flavour
Red berries
Spice
Astringent
1118
Jammy/Cooked fruit
Fruit after taste
Musty
Dark berries
No MLF
1106
Chocolate flavour
Dark berries flavour
Mint/Eucalyptus
PC1 (63%)
Boiled Egg
Red berries flavour
Overall fruit flavour
Mint/Eucalyptus flavour
1105
Bitter
Chocolate

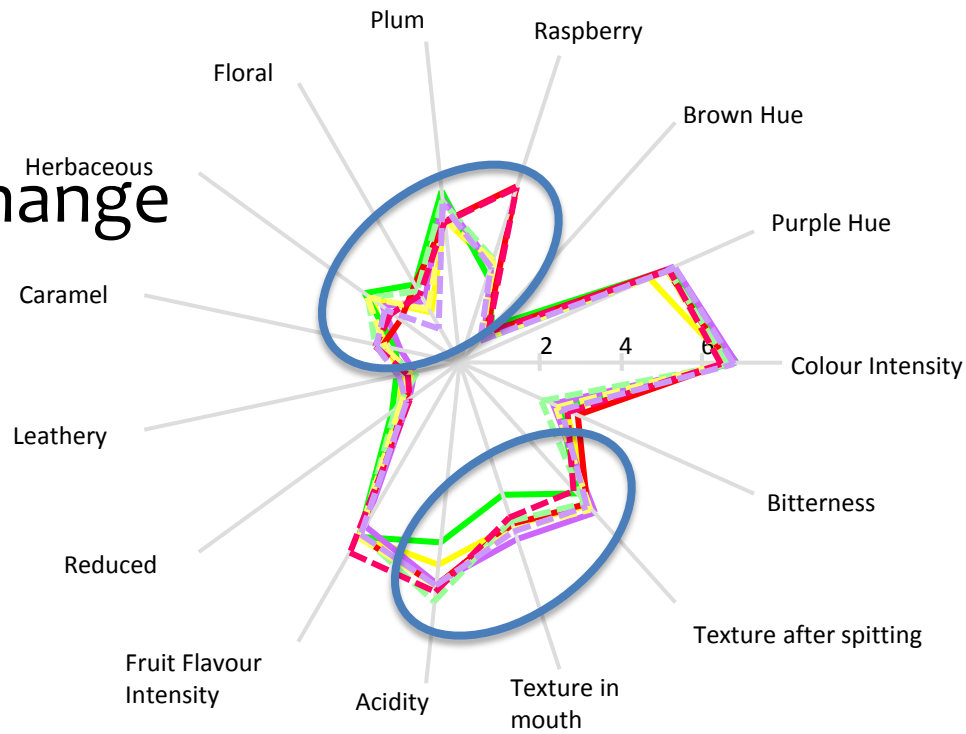
Malolactic fermentation

- Reduction in wine acidity



➢ More than just pH change





Eveline Bartowsky, AWRI, Neustadt 2010

Diacetyl

QUALITY

SENSORY IMPACT

In function of the wine type and the wine style

1 - 4 mg/L = impacts on complexity

> 5 - 7 mg/L = undesired buttery sensations

Rankine et al.,1969

Perception threshold

- Chardonnay 0.2 mg/L
- Pinot Noir 0.9 mg/L
- Cabernet Sauvignon 2.8 mg/L

Martineau & Henick-Kling, 1995

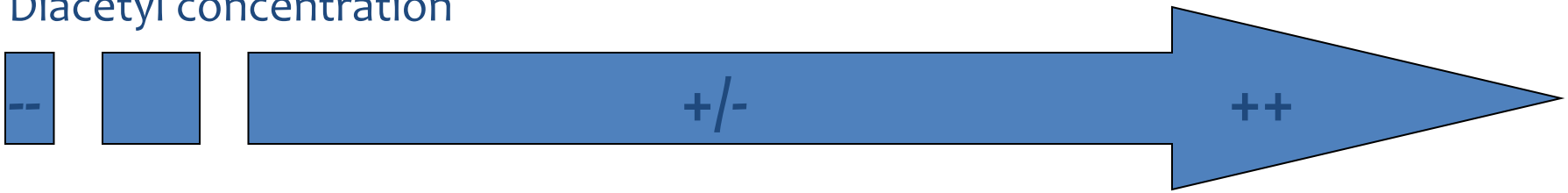
(threshold > red wines)

from Bartowsky & Henschke, 2004

Diacetyl - management during winemaking

O. oeni strain

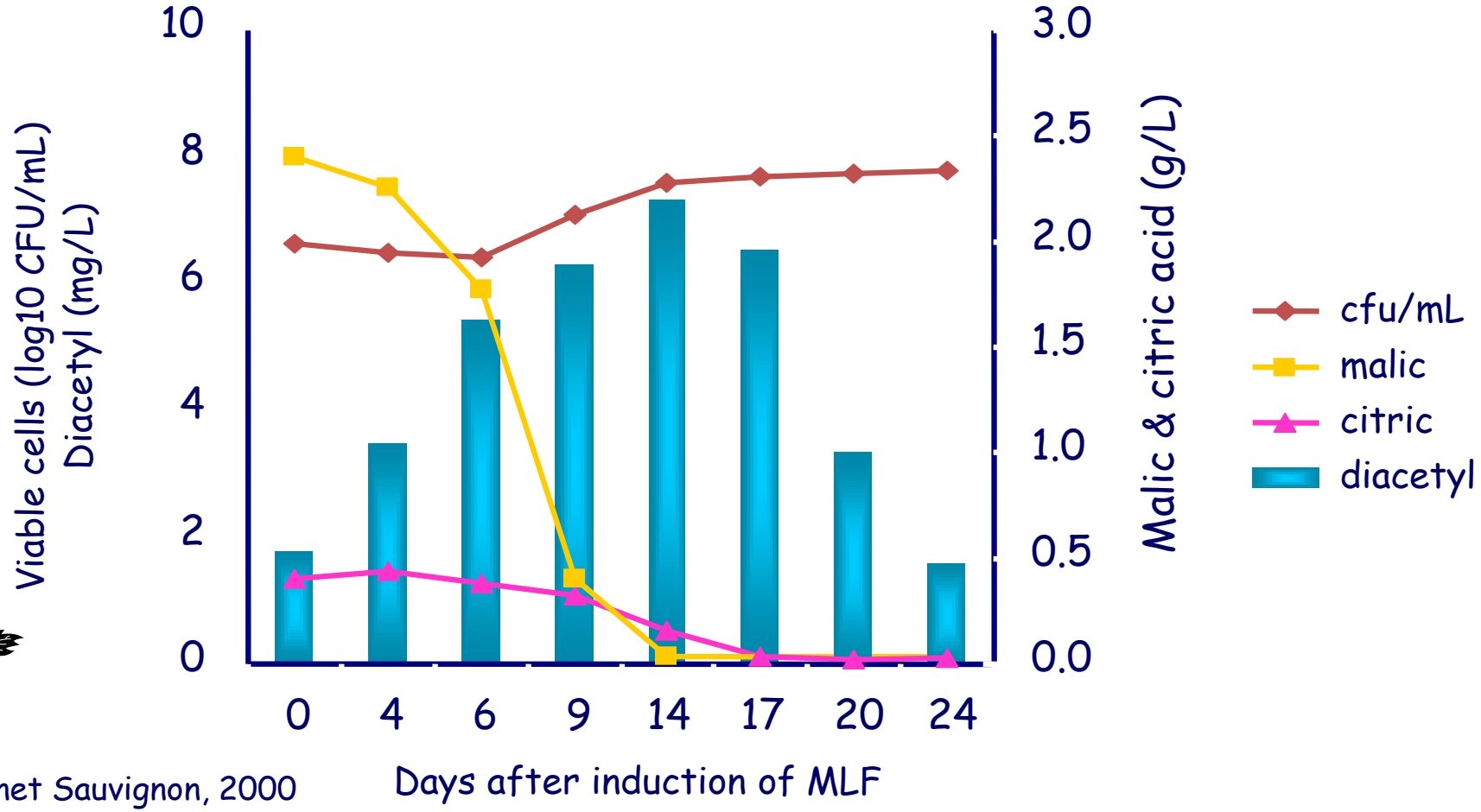
Diacetyl concentration



Exp. Strain C22L9	VP41	Elios 1	Alpha	Lalvin 31	Beta	PN4
Delayed attacks citric acid days after completion of malic acid	Only attacks citric acid after completion of malic acid.	Medium producer	Medium producer	Medium to low producer	In sequential inoculation: high producer	Early attack of citric acid (mid MLF)

Diacetyl

FORMATION DURING MLF



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

from Bartowsky & Henschke, 1999

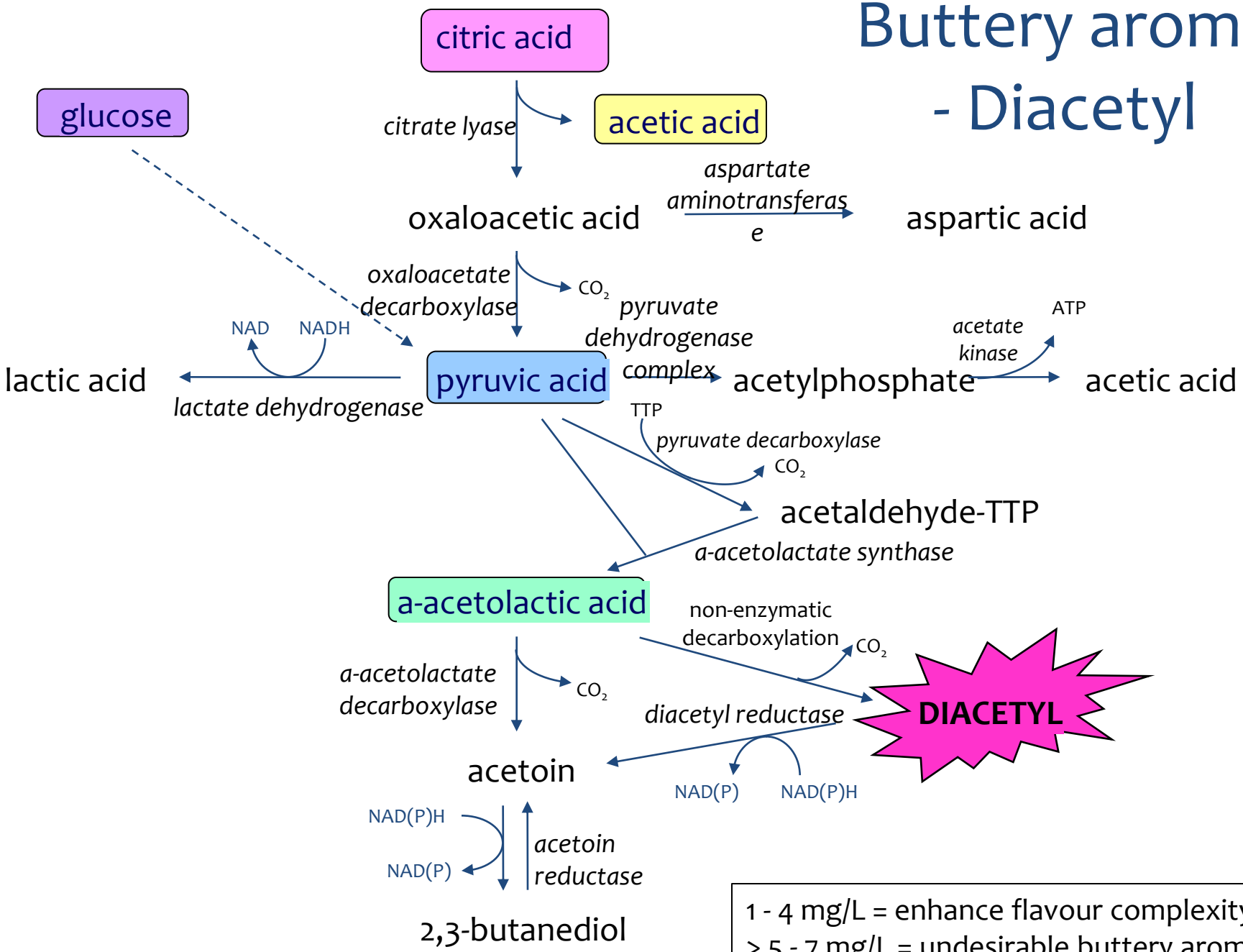
Diacetyl - management during winemaking

	Diacetyl conc^n
O. oeni strain	variable
wine type	white - lower red - higher
inoculation rate	10^4 - higher 10^6 - lower
fermentation time	longer MLF - higher

	Diacetyl conc^n
temperature	18°C - higher 25°C - lower
SO_2	binds to diacetyl - sensorially inactive
aeration	air - higher anaerobic - lower
contact with yeast lees	long contact- lower
pH	lower pH may favour

Eveline Bartowsky, AWRI, Trier 2008

Buttery aroma - Diacetyl

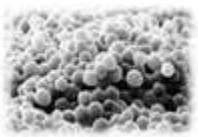


1 - 4 mg/L = enhance flavour complexity
> 5 - 7 mg/L = undesirable buttery aroma

Eveline Bartowsky, AWRI, 2004

In conclusion, diacetyl & MLF regime …

- Buttery character 
  - Diacetyl
  - ➢ Easily manipulated by the winemaker
    - *O. oeni* strain
    - Length of MLF
    - Temperature
    - Yeast (& bacterial) lees contact



- Co-inoculation of MLF with AF
  - Can reduce the time of malic acid metabolism
  - Can be used to modulate the aroma and flavour of wine
  - Length of MLF can influence the chemical composition of wine

➢ ***MLF inoculation regime can be used as a means to influence the wine style.***

Eveline Bartowski, AWRI, Neustadt 2010

In conclusion, fruity character …

- Changes in ester concentration is dependent upon several factors
  - ***O. oeni* strain**
  - Wine composition
  - MLF conditions
  - Viticultural region
  - Vintage
- Increase in total fruit berry concentration translates to an increase in berry related sensory descriptor terms
- MLF can be used to enhance the fruity berry characteristics of Cabernet Sauvignon

- ML strains behave similarly in other red varieties regarding fruity berry characters

THANK YOU

For your attention!

www.lallemandwine.com
www.scottlab.com

and there is a (free) APP for that!

Effective SO_2 and pH

Molecular SO_2 (%) = 100 / [10 $^{pH-1,81}$ + 1]

pH	Free SO_2 mg/l	% molecular SO_2 mg/l	Total molecular SO_2 mg/l
3	25	6,06 %	1,54
3,6	25	1,6 %	0,4
4	25	0,64 %	0,16

Effective SO_2 and pH

Molecular SO_2 (%) = 100 / [$10^{pH-1,81}$ + 1]

pH	Free SO_2 mg/l	% molecular SO_2 mg/l	Total molecular SO_2 mg/l
3	8,2	6,06 %	0,5
3,6	31	1,6 %	0,5
4	78	0,64 %	0,5

bactericide concentration

At pH = 3, mol. SO_2 = 6,06 % ⇒ 8,2 mg/l of free SO_2 will inhibit MLF

At pH = 4; mol. SO_2 = 0,64 %, ⇒ 78 mg/l of free SO_2 will inhibit MLF

Environmental Factors that Impact Malolactic Fermentation

	Good	Moderate	Difficult	Extreme
Alcohol (% vol)	<13	13 - 15	15 - 17	>17
pH	>3.4	3.1 - 3.4	2.9 - 3.1	<2.9
Free SO_2 (mg/L)	<8	8 - 12	12 - 15	>15
Total SO_2 (mg/L)	<30	30 - 40	40 - 60	>60

Molecular SO_2 (mg) based on 1 mg free SO_2 dependant of pH and different alcohol levels

Alcohol	pH										
%vol	2.80	2.90	3.00	3.10	3.20	3.30	3.40	3.50	3.60	3.70	3.80
0	0.078	0.063	0.051	0.041	0.033	0.026	0.021	0.017	0.013	0.011	0.008
1	0.081	0.066	0.053	0.043	0.034	0.027	0.022	0.017	0.014	0.011	0.009
2	0.085	0.069	0.055	0.044	0.036	0.029	0.023	0.018	0.015	0.012	0.009
3	0.089	0.072	0.058	0.047	0.037	0.030	0.024	0.019	0.015	0.012	0.010
4	0.093	0.075	0.061	0.049	0.039	0.031	0.025	0.020	0.016	0.013	0.010
5	0.097	0.078	0.063	0.051	0.041	0.033	0.026	0.021	0.017	0.013	0.011
6	0.100	0.081	0.066	0.053	0.043	0.034	0.027	0.022	0.017	0.014	0.011
7	0.104	0.085	0.069	0.055	0.044	0.036	0.028	0.023	0.018	0.014	0.012
8	0.109	0.088	0.072	0.058	0.046	0.037	0.030	0.024	0.019	0.015	0.012
9	0.113	0.092	0.075	0.060	0.048	0.039	0.031	0.025	0.020	0.016	0.013
10	0.118	0.096	0.078	0.063	0.050	0.040	0.032	0.026	0.021	0.017	0.013
11	0.122	0.100	0.081	0.065	0.053	0.042	0.034	0.027	0.022	0.017	0.014
12	0.127	0.104	0.084	0.068	0.055	0.044	0.035	0.028	0.023	0.018	0.014
13	0.132	0.108	0.088	0.071	0.057	0.046	0.037	0.030	0.024	0.019	0.015
14	0.138	0.113	0.091	0.074	0.060	0.048	0.039	0.031	0.025	0.020	0.016
15	0.143	0.117	0.095	0.077	0.062	0.050	0.040	0.032	0.026	0.021	0.016

The quantity of molecular SO2 can be calculated for each mg of free SO_2 with the help of a factor to be taken from the table above in relation to the wine pH and wine alcohol content. E.g. for a wine with 11 %vol and pH 3.1, which has 8 mg/L free SO_2 the mol. SO_2 will be:

8 mg/L free SO_2 x 0.65 = 0.52 mg/l molecular SO_2

The quantity of molecular SO_2 to protect the white wines from oxidations is > 0.50 mg/l the quantity to inhibit malolactic bacteria between 0.30 and 0.50. (O.oeni most sensitive)

INFLUENCE OF THE TEMPERATURE

Temperature	Total-SO_2 (mg/L)	Free SO_2 (mg/L)
0°C	412	68
15°C	412	85
30°C	412	100

At higher temperatures – liberation of more free SO_2 from bound SO_2 = higher antiseptical effect.

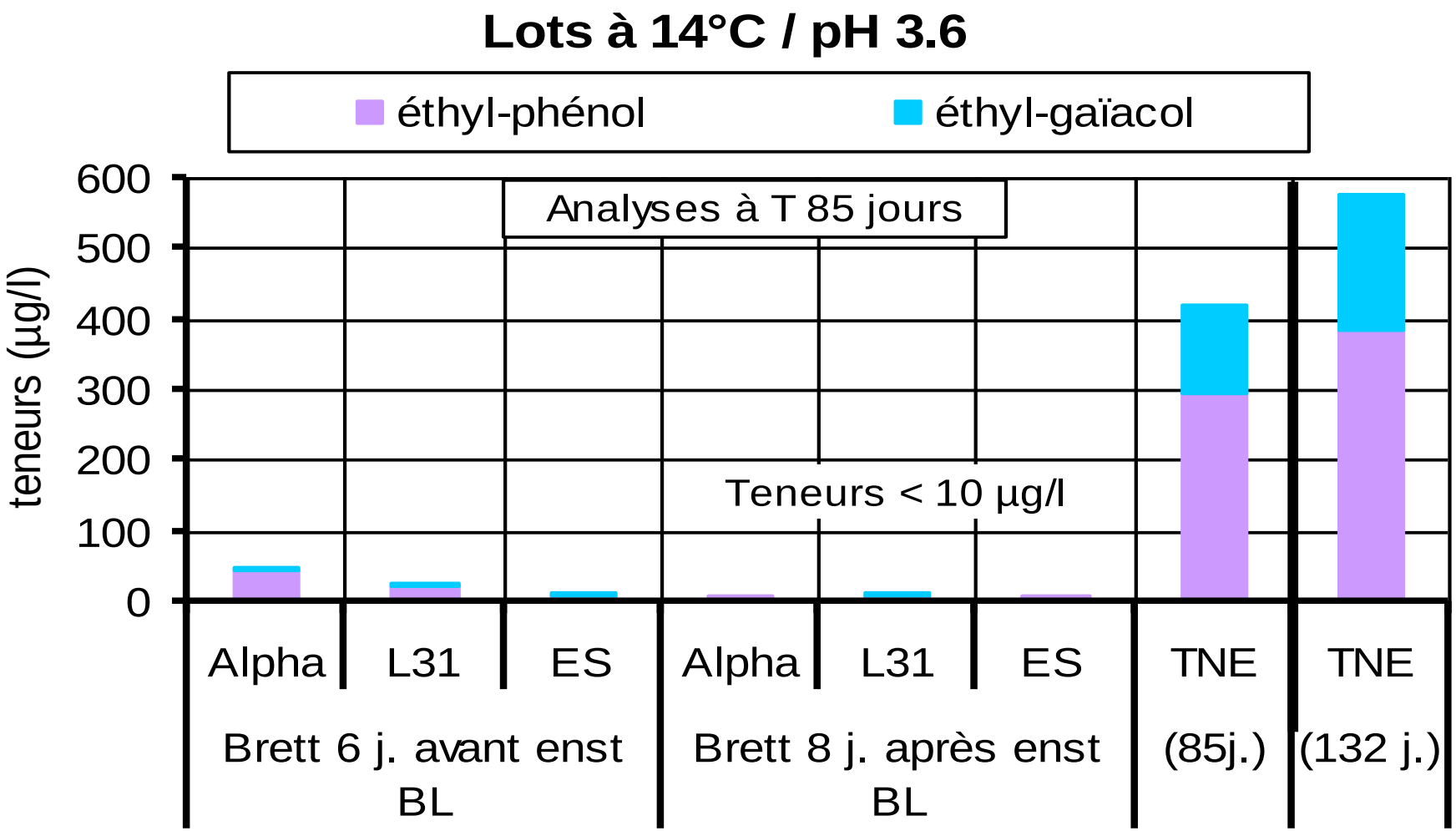
The antiseptically power of free SO_2 = molecular SO_2 (active form) is only a small fraction of it.

Similar effect for alcohol = at higher temperatures increasing toxic effect of ethanol

Additive effects: Alcohol & Temperature

Alcohol content of wine (% v/v)	✓ Temperature (°C) Which should not be exceeded
Lower than 14.5%	28
Higher than 14.5%	23

Volatile phenol content after MLF (wines inoculated pre-ML for the 'control')



VOLATILE PHENOLS *(Vincent Gerbaux et al. 2009, AJEV)*

Table 2. Volatile phenol (µg/L) 4-ethylphenol (EP) and 4-ethylguaiacol (EG) produced (Experiment 2, PN1 Wine)

	LACTIC ACID BACTERIA		
	No ML inoculation	MBR® 1	MBR® 2
	EP / EG	EP / EG	EP / EG
***Brettanomyces* inoculation**	**14°C – pH 3.3**		
When inoculated before MLF	148 / 353	41 / 119	68 / 178
When inoculated after MLF		28 / 68	30 / 82
	14°C – pH 3.6		
Before MLF	191 / 387	4 / 22	<1 / 11
After MLF		3 / 10	<1 / 10
	18°C – pH 3.3		
Before MLF	152 / 357	79 / 148	68 / 143
After MLF		<1 / < 1	<1 / <1
	18°C – pH 3.6		
Before MLF	185 / 398	<1 / < 2	<4 / < 6
After MLF		<1 / <1	<1 / <1

Influence of phenolic compounds deriving from wine grapes or enological tannins on vitality and activity of *O. oeni*

Collaboration with University of Bordeaux (Prof. Aline Lonvaud)

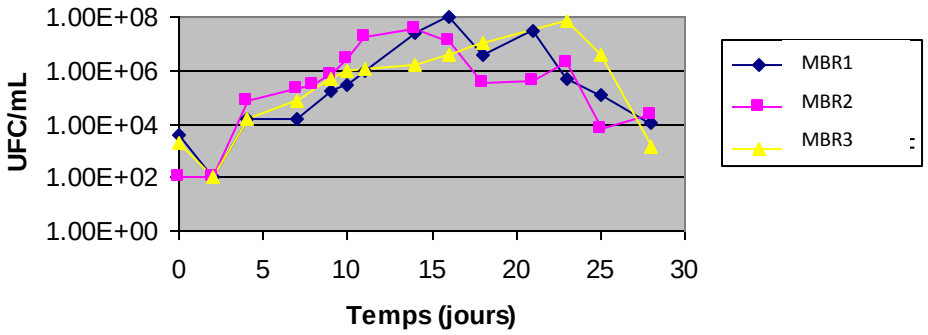
a) Investigation of different polyphenolic fraction from different grape varieties

b) Investigation of different enological tannins (grape seed tannins, grape skin tannins, oak tannins….)

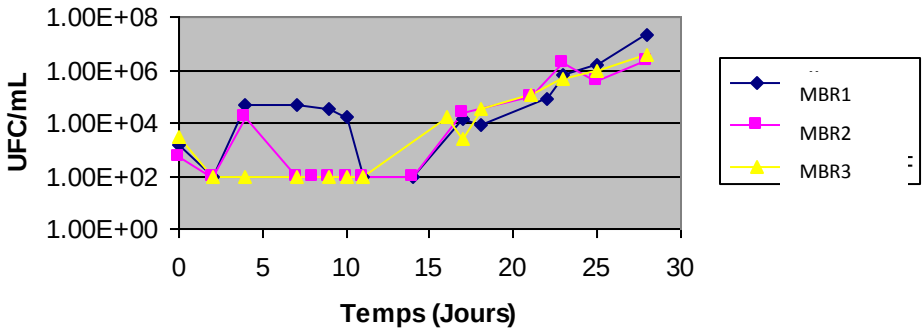
c) Investigation of nutrient formulations to overcome the inhibition

d) Investigation how to use the stimulating effect of some fractions

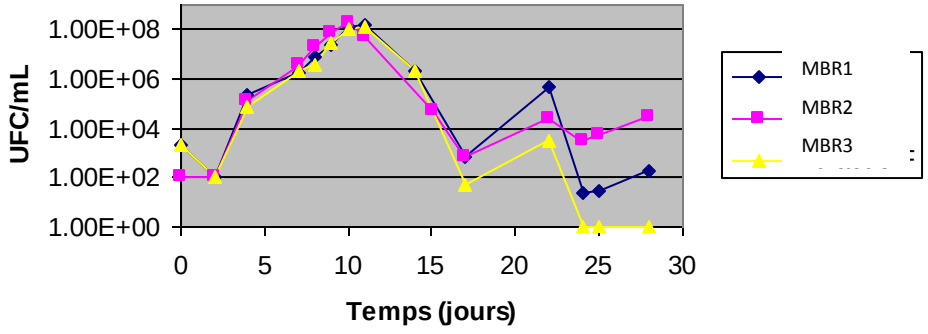
CONTROL



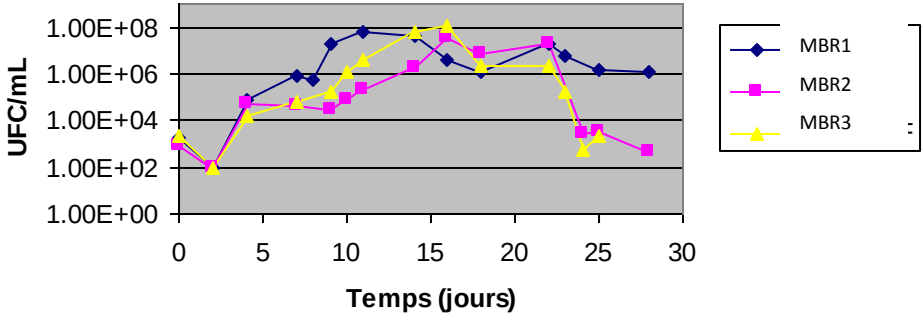
TANNIN EXTRACT



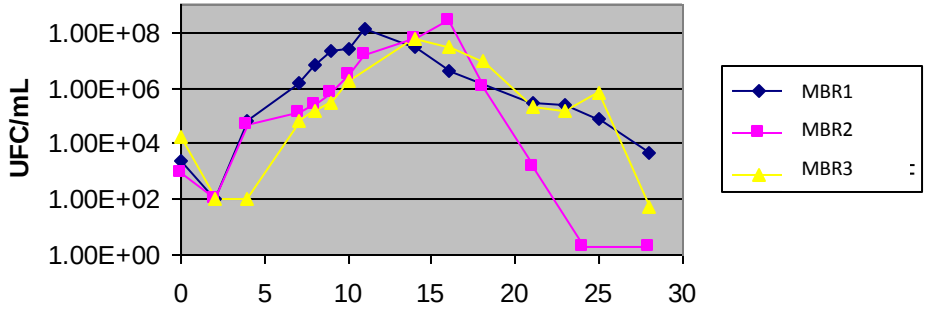
ANTHOCYANINS EXTRACT



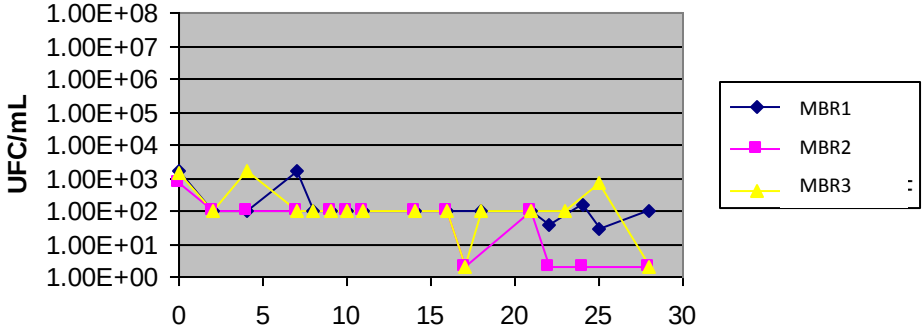
WINE EXTRACT



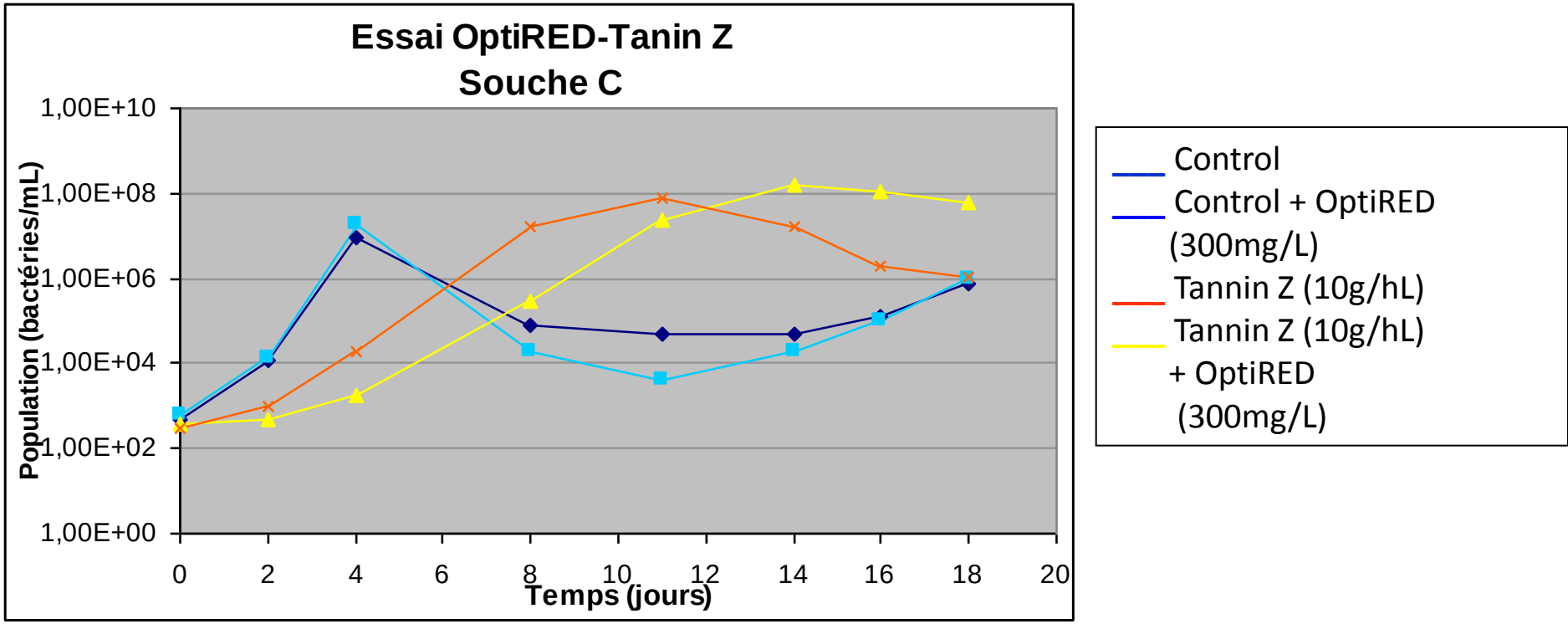
GRAPE SKIN TANNINS



GRAPE SEED TANNINS



- **Positive impact of nutrient addition containing mannoprotein fraction**



Rapid growth in presence of Opti'Red in comparaison to tanin Z only