



13 juillet 2020

REGULATION OF ORGANIC WINEMAKING AND DESIRE TO REDUCE INPUTS IN OENOLOGY



VIGNERONS BIO
NOUVELLE AQUITAINE

Vignerons Bio Nouvelle Aquitaine

Vignerons Bio Nouvelle Aquitaine VBNA

200 organic winegrowers

Follow-up of more than 100 growers in winemaking

*Animation of the network of organic wine advisors from the
New Aquitaine region: 65 public and private advisors*



VIGNERONS BIO
NOUVELLE AQUITAINE

Vignerons Bio Nouvelle Aquitaine : Our missions

*Regional union representative of the Organic Wines producer created in 1995
Today, more than 200 organic winegrowers are members
Accompanying the harvest until it is put on the market*



REPRÉSENTATION & DÉFENSE

bio winemakers' interests in regional, national and European viticulture and organic



PROMOTION

Promote and develop the collective image and awareness of organic wines and spirits among professionals and individuals



RECHERCHE & DÉVELOPPEMENT

Initiate and collaborate on research and experimentation programs aimed at improving the quality of Organic wines



ŒNOLOGIE

Contribute to the economic and technical development of organic wine production, by accompanying organic winegrowers individually and collectively

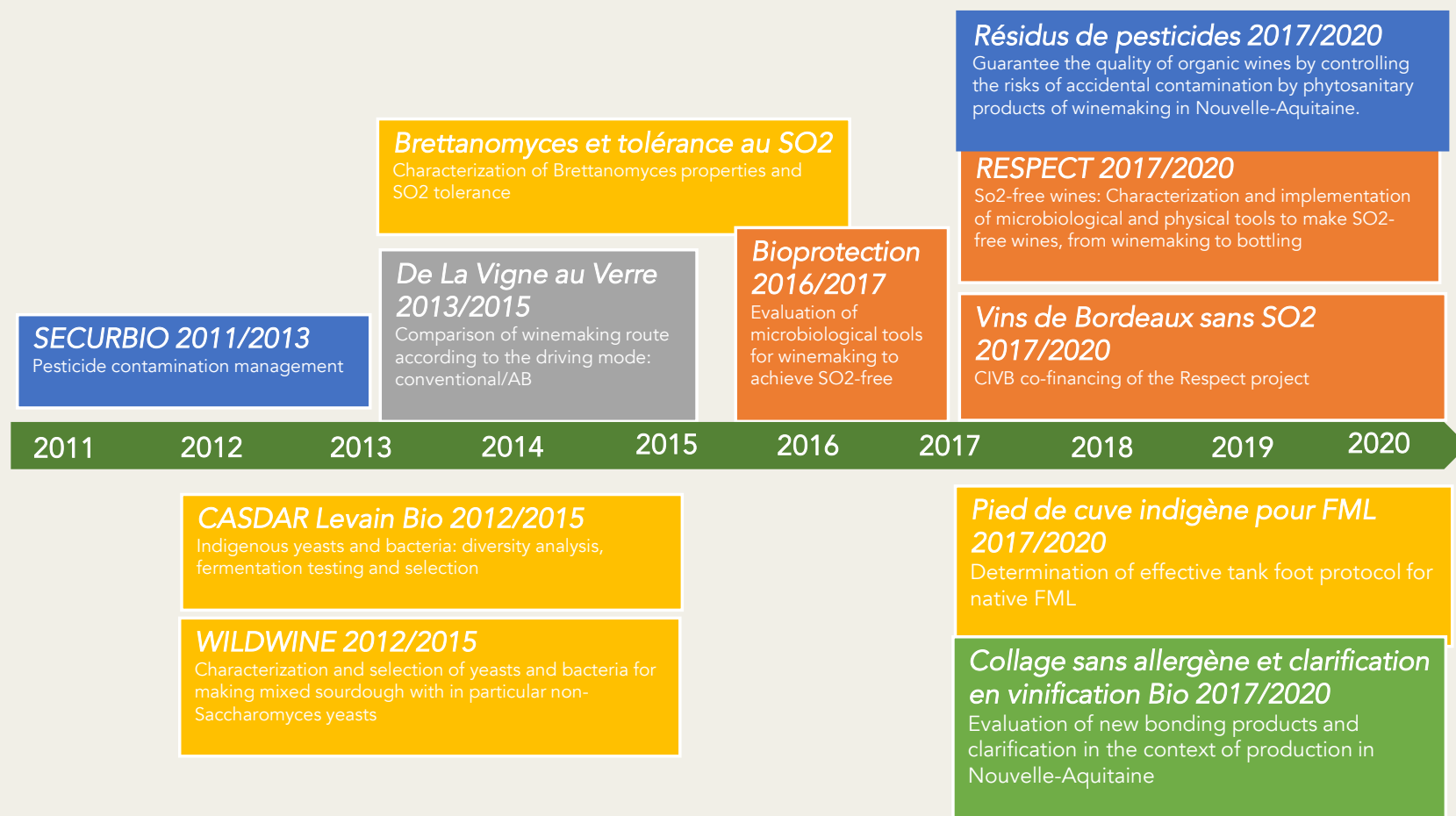


ECONOMIE



VIGNERONS BIO
NOUVELLE AQUITAINE

Research and experimentation projects



Les domaines de recherche :

Résidus pesticide

Microbiologie

Modes de production

Vinification sans SO₂

Nouveaux intrants



Programme pour accompagner à la réalisation de vins sans SO2

RESPECT 2017/2020

So2-free wines: Characterization and implementation of microbiological and physical tools to make SO2-free wines, from winemaking to bottling

RESAQ Viti Bio 2021

Participatory experimentation on copper reduction and evaluation of new phytosanitary inputs

MERGO 2020/2023

Erasmus +
Learning remote tasting and creating MOOCs

Vins de Bordeaux sans SO2 2017/2020

CIVB co-financing of the Respect project

Diffusion 2020/2021
Vins sans SO2/Colles/PDC
Bacteria/Pesticides/Copper/Bio control
Regional Funding FEADER 1.2

Vins Sans 2021/2023

Suite de RESPECT

So2-free wines: Livestock and gas management
Impact on the structure and aromatics of wines
Sweet Wines

Projet Chitosan 2021/2023

Suite de RESPECT

Management of brettanomyces
Management of malolactic fermentation in so2-free white wine

2020

2021

2022

2023

OenoBio
Erasmus +
Master européen en
viticulture œnologie Bio

Vinification des cépages résistants 2021/2023

Tools for mastering and evaluating the vinification of resistant grape varieties

Pesticides 2021/2023

Continuation of the Qualivin Bio program on pesticide contamination of organic wines and study of the specific case of phosphonic acid

COPPEREPLACE 2020/2023

Development and implementation of new ones technologies, products and strategies to reduce the application of copper in vineyards and to sanitize the contaminated soils in the SUDOE region

Les domaines de recherche :

Autres

Vinification

Diffusions

6

What is organic wine farming?

Brief history of Organic Farming

The organic farming movement developed in response to the advent of agrochemistry in the middle of the twentieth century, in the face of the development of pesticides and mineral fertilizers from synthetic chemistry.

Années 1920 : Birth of new schools of thought in Europe on the part of agronomists, doctors, farmers and consumers based on ethical and ecological principles with initiation of an alternative mode of agricultural production in criticism of the disappearance of the traditional peasantry

Années 1970 : Rise of the development of AB with the emergence of new sociological currents: resistance to the consumer society, awareness of the limits of the planet's resources with oil crisis...

1985 : Official recognition in France of Organic Farming allowing the approval with logo of AB products according to a national specification

1991 : Birth of a harmonized European regulation on AB, first crop production and then in 2000 for animal production

2012 : Birth of the European organic winemaking regulations, allowing to display "organic wine" and more: "wine from organically farmed grapes"

2022 : New European Regulation on Organic Farming



Les grands principes de l'AB

- ✓ allow soils to maintain their natural fertility
- ✓ respect the environment
- ✓ provide quality products
- ✓ favouring the autonomy of agricultural holdings
- ✓ establish direct relationships with consumers
- ✓ promoting employment in the agricultural sector



History of Organic Wine Regulation

2009 : ORWINE program launched by the European Commission to define specifications on organic winemaking regulations. Studies include scientific research programs and manufacturers/distributors/users/consumers surveys.

2012: Birth of the **European Regulation on organic winemaking**, to display the "organic wine" and not: "wine of grapes from organic farming" Regulation (EU) 203/2012

2014/2015: Opening of a request for evaluation of the organic winemaking regulations at the request of the Member States and the commission (evaluation of new oenological practices that were not yet authorized in 2010 and assessment of the authorized practices including techniques with a re-evaluation deadline, which led to the EGTOP report mentioned below)

2015 : EGTOP report "Final report on wine" on 17 November 2015 with a number of recommendations

2018 : New Organic Winemaking Regulation Implementing Regulation (EU) 2018/1584 amends Regulation (EU) 203/2012.

2018 : REGULATION EU 2018/848, which will repeal EC 834/2007 on 31/12/2020

The prohibited techniques are incorporated into the basic regulation, which will prevent any future changes.

Authorization of heating at 75°C

2018 à 2021 : Work on the construction of the implementing regulation

Oenological inputs will be authorized by implementing acts. New oenological processes may be introduced by delegated act.

Work on the alignment of the organic regulation with the new wine regulation (EU) 934/2019 carried out by the European Commission

Jan 2022 : Application of the new organic farming regulation EU 2018/848



VIGNERONS BIO
NOUVELLE AQUITAINE

A major evolution in 2018

Names of products or substances	Type of treatment targeted	Specific conditions and restrictions within the limits and conditions laid down in Regulation (EC) No 1234/2007 and Regulation (EC) No 606/2009
Yeast autolysates	Yeast nutrition	Only for fresh grapes, grape must, grape must in fermentation, partially fermented grape must from passerillé grapes, concentrated grape must, new wine still in fermentation
Vegetable proteins from potatoes (2) Chitosan derived from <i>Aspergillus niger</i> Levurian protein extracts (2) (EPL)	Clarification	For the treatment of wines, the limit of use of chitosan is a maximum of 100 g/hl. For the treatment of musts and white wines and rosé wines, the limit for the use of EPL is 30 g/hl and for the treatment of red wines, 60 g/hl.
Yeast mannoproteins	Tartaric stabilization	For partially fermented must used for direct human consumption in the unaltered state and the products defined in points 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008
Chitosan derived from <i>Aspergillus niger</i>	Wine stabilization	a) Reduction of the content of heavy metals, in particular iron, lead, cadmium and copper b) Prévention de la casse ferrique, la casse cuivrique c) Reduction of potential contaminants, in particular ochratoxin A d) Reduction of populations of undesirable microorganisms, including <i>Brettanomyces</i>, by chitosan-only treatment <u>Prescriptions:</u> ♦ The doses to be used shall be determined after prior testing. The maximum dose of use must be less than or equal to: - 100 g/hl for applications (a) and (b) - 500 g/hl for application (c) - 10 g/hl for application (d) - ♦ Sediments are removed by physical processes.
Inactivated yeasts (LSI)	Yeast nutrition/breeding	



VIGNERONS BIO
NOUVELLE-AQUITAINE

New wine regulation

RÈGLEMENTS

RÈGLEMENT DÉLÉGUÉ (UE) 2019/934 DE LA COMMISSION

du 12 mars 2019

complétant le règlement (UE) n° 1308/2013 du Parlement européen et du Conseil en ce qui concerne les zones viticoles où le titre alcoométrique peut être augmenté, les pratiques œnologiques autorisées et les restrictions applicables à la production et à la conservation de produits de la vigne, le pourcentage minimal d'alcool pour les sous-produits et leur élimination, et la publication des fiches de l'OIV

LA COMMISSION EUROPÉENNE,

vu le traité sur le fonctionnement de l'Union européenne,

vu le règlement (UE) n° 1308/2013 du Parlement européen et du Conseil du 17 décembre 2013 portant organisation commune des marchés des produits agricoles et abrogeant les règlements (CEE) n° 922/72, (CEE) n° 234/79, (CE) n° 1037/2001 et (CE) n° 1234/2007 du Conseil (¹), et notamment son article 75, paragraphe 2, et son article 80, paragraphe 4,

considérant ce qui suit:

New Regulation 934/2019 and 935/2019 replacing 606/2009



Key point of Organics wine regulation

A number of wine inputs must be organic in origin, if available:

Wine Regulation 934/2019					Organic wine regulation 2164/2019
	Substance	Detail	additif	auxiliary	
4	Activators of alcoholic and malolactic fermentation				
4.6	Yeast autolysates			x	Authorized Organic if available
4.7	Yeast bark			x	Authorized Organic if available
4.8	Inactivated yeasts			x	Authorized Organic if available
5	Clarifying agents				
5.1	Food gelatin			x	Authorized Organic if available
5.2	Wheat protein			x	Authorized Organic if available
5.3	Protein from peas			x	Authorized Organic if available
5.4	Protein from potatoes			x	Authorized Organic if available
5.5	glue			x	Authorized Organic if available
5.12	Tanins			x	Authorized Organic if available
5.15	Levurian protein extracts			x	Authorized Organic if available
6	Stabilizing agents				
6.4	Tanins				Authorized Organic if available
6.8	gum arabic		x		Authorized Organic if available
9	Fermentation agents				
9.1	Yeast	not gmo-derived		x	Authorized Organic if available
9.2	Lactic acid bacteria	not gmo-derived		x	Authorized Organic if available

A list of organic wine input is available in France. Produced each year by France Vin Bio in partnership with UFLIO and OENOPPIA validated and disseminated by INAO



Liste des intrants œnologiques issus de matières premières biologiques conformément à l'article 29 quater du R(CE) 889/2008 et distribués en France.

Mise à jour pour 2018

Substance	AB	UE	France Vin Bio	UFLIO	OENOPPIA
Activants de fermentation alcoolique et malolactique					
Clarifiants					
Stabilisants					
Agents de fermentation					
Autres					

Non-exhaustive list of prohibited organic inputs



Wine Regulation 934/2019					Organic Regulation 2164/2019
	Substance	Detail	additive	auxiliary	
1	regulator				
1.2	Malic acid (D, L-; L-)	Acide L- malique	X		Forbidden
1.7	Calcium tartrate			X	Forbidden
1.9	Potassium carbonate			X	Forbidden
2	Preservatives and antioxidants				
2.4	Potassium sorbate		X		Forbidden
2.5	Lysozyme		X	X	Forbidden
2.7	Dimethyl dicarbonate (DMDC)		X		Forbidden
3	Sequesters				
3.2	Selective vegetable fibres			X	Forbidden
4	of alcoholic and malolactic fermentation				
4.1	Microcrystalline cellulose			x	Forbidden
4.3	ammonium sulphate			x	Forbidden
4.4	Ammonium bisulphite			x	Forbidden
4.9	Inactivated yeasts with guaranteed glutathione levels			x	Forbidden
5	agents				
5.11	Kaolin			x	Forbidden
5.14	Chitin-glucan derived from Aspergillus niger			x	Forbidden
5.16	Polyvinylpyrrolidone			x	Forbidden
5.17	alginate			x	Forbidden
6	agents				
6.2	tartrate			x	Forbidden
6.5	ferrocyanide			x	Forbidden
6.6	phytate			x	Forbidden
6.9	D, L- tartaric acid or its neutral potassium salt			x	Forbidden
6.11	Carboxymethylcellulose		x		Forbidden
6.12	Polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)			x	Forbidden
6.13	Potassium polyaspartate		x		Forbidden
7	Enzymes				
7.1	Urease			x	Forbidden
7.7	Betaglucanase			x	Forbidden
7.8	Glycosidase			x	Forbidden
10	Correction of defects				
10.1	Copper sulphate, pentahydrate			x	Forbidden
10.4	Chitin-glucan derived from Aspergillus niger			x	Forbidden
11	practices				
11.3	Caramel		x		Forbidden
11.4	Allyl isothiocyanate			x	Forbidden
Technic					
5	Elimination of sulphur dioxide by physical processes				Forbidden
9	Pure paraffin discs impregnated with allyl isothiocyanate				Forbidden
10	Electrodialysis treatment				Forbidden
12	Correction of the alcohol content of wines	dealcoholization			Forbidden
13	Cation exchangers for tartaric stabilization				Forbidden
14	Electromembran treatment				Forbidden
15	Cation exchangers for acidification				Forbidden
16	Membrane coupling				Forbidden
17	Membrane contactors				Forbidden
18	Membrane technology associated with activated carbon				Forbidden
19	Filter plates containing Y-faujasite zeolites				Forbidden

List of inputs and techniques



VIGNERONS BIO
NOUVELLE AQUITAINE



Le SO2

The rule laid down is a reduction of 50 mg/L in the total SO2 content for dry wines (<2 g/L of residual sugars) and 30 mg/L for other wines compared to the limits of the COM

SO2 mg/l	Rgt Européen OCM Viti-Vini N° 479/2008	(1) Rgt Européen BIO (CE) 889/2008 (2019)
	Total	Total
Dry red wines (< 2g/l sugar)	150	100
Dry red wines (≥ 2g/l and < 5g/l sugar)		120
Red wines (≥ 5g/l sugar) and semi-dry	200	170
Dry white wines/rosés (< 2g/l sugar)	200	150
White wines/dry rosés (≥ 2g/l and < 5g/l sugar)		170
wines (≥ 5g/l sugar) and semi-dry	250	220
wines for certain PDOs (so-called "sweet")	400	370
wines (>12 and < 45g/l sugar)	300	270
wines (≥ 45g/l sugar)	200	170
wine (TAV > 15%)	200 (sucre ≥ 5g/l)	170 (sucre ≥ 5g/l)
Sparkling	235	205
Quality effervescent	185	155



VIGNERONS BIO
NOUVELLE AQUITAINE

Regulatory news

The organic winemaking regulations are built on the basis of the general wine regulations. However, the general wine regulation has evolved considerably during its last revision. The work set in motion by the European Commission is to achieve the correspondence between this new wine regulation and the wine regulation

Usable filtration soils (perlites, cellulose, diatom earths) no longer appear in the general wine regulations (934/2019), are still present in Regulation No. 2164/2019, but no longer appear in the draft annex to the future organic regulation. Legal analysis: all oenological practices authorized under the general regulations are authorized under the organic regulation unless otherwise specified. (art 1.2 of Part VI of Annex II of 848/2018): therefore there is no questioning of these substances

Part D: Authorized products and substances for the production and conservation of organic grapevine products of the wine sector referred to in point 2.2. of Part VI of Annex II to Regulation (EU) 2018/848

Products and substances	References in Annex I to Regulation (EU) 2018/848	Specific conditions and limits
Air	Part A, Table 1, points 1 and 8	
Gaseous oxygen	Part A, Table 1, point 1 Part A, Table 2, point 8.4	
Argon	Part A, Table 1, point 4 Part A, Table 2, point 8.1	May not be used for bubbling
Nitrogen	Part A, Table 1, points 4, 7 and 8 Part A, Table 2, point 8.2	
Carbon dioxide	Part A, Table 1, points 4 and 8 Part A, Table 2, point 8.3	
Pieces of oak wood	Part A, Table 1, point 11	
Tartaric acid (L(+)-)	Part A, Table 2, point 1.1	
Lactic acid	Part A, Table 2, point 1.3	
Potassium L(+)-tartrate	Part A, Table 2, point 1.4	
Potassium bicarbonate	Part A, Table 2, point 1.5	
Calcium carbonate	Part A, Table 2, point 1.6	
Calcium sulphate	Part A, Table 2, point 1.8	
Sulphur dioxide	Part A, Table 2, point 2.1	(a) The maximum sulphur dioxide content shall not exceed 150 milligrams per litre for red wines as referred to in point A.1 (a) of Part B of Annex I to Regulation (EU) 2018/848 and 4 mg/L for white wines.
Potassium bisulphate	Part A, Table 2, point 2.2	
Potassium metabisulphate	Part A, Table 2, point 2.3	

Enzymes: the current organic regulation indicates that pectolytic enzymes are allowed in clarification only: the new wine regulation allows to specify the enzymes that it includes in this list: pectin lyases; pectin methylsterase; polygalacturonase; hemicellulase; cellulase. A request to extend these same enzymes in maceration for juice extraction is under construction

Actualité réglementaire

- Fresh lees: They had been forgotten in the European Commission's alignment proposal
This point has been amended by the European Commission and the fresh lees have been incorporated into a new alignment proposal
- *Acidification: A request to add the use of malic acid in its natural form (L-malique) in organic has been made by France Vin Bio. It is currently being analyzed by the Organic WINE COMMISSION INAO for a possible French request*

► Cellulose microcristalline :

It was considered an adjuvant in oenology and did not appear as an input in the old wine regulations. However, this is an input that has been added to the new wine regulations.

In fact it is for the moment banned in Organic because not appraised by the EGTOP expert group of the European Commission and not validated by the commission. Microcrystalline cellulose is also due to be replaced shortly by food cellulose in the wine regulations.

A request for the addition of food cellulose to the organic regulation is being discussed at the INAO for the construction of a possible application to Europe

In the meantime, we must be vigilant about the composition of the products used. Microcrystalline cellulose is present in some compositions of inactivated dry yeasts and some chitosan formulations (especially in effervescent tablet form). The wines in which those products would have been used fall within the scope of the infringement 284 which provides for the downgrading of the lot at first finding.

- Electrodialysis: Reintroduction of the technique into organic regulations following the manufacturer's request
This technique is enshrined in the basic regulation which has already been voted on by the committee, the Council and Parliament. The committee recently recalled that there would be no possible amendment to the basic text.
France vin Bio has taken a stand against this request.

Part D: Authorised products and substances for the production and conservation of organic grapevine products of the wine sector referred to in point 2.2. of Part VI of Annex II to Regulation (EU) 2018/948

Products and substances	References in Annex I to Regulation (EU) 2018/948	Specific conditions and limits
Air	Part A, Table 1, points 1 and 8	
Gaseous oxygen	Part A, Table 1, point 1 Part A, Table 2, point 8.4	
Argon	Part A, Table 1, point 4 Part A, Table 2, point 8.1	May not be used for bubbling
Nitrogen	Part A, Table 1, points 4, 7 and 8 Part A, Table 2, point 8.2	
Carbon dioxide	Part A, Table 1, points 4 and 8 Part A, Table 2, point 8.3	
Pieces of oak wood	Part A, Table 1, point 11	
Tartaric acid (L(+)-)	Part A, Table 2, point 1.1	
Lactic acid	Part A, Table 2, point 1.3	
Potassium L(+)-tartrate	Part A, Table 2, point 1.4	
Potassium bicarbonate	Part A, Table 2, point 1.5	
Calcium carbonate	Part A, Table 2, point 1.6	
Calcium sulphate	Part A, Table 2, point 1.8	
Sulphur dioxide	Part A, Table 2, point 2.1	(a) The maximum sulphur dioxide content shall not exceed 150 milligrams per litre for red wines as referred to in point A.1 (a) of Part B of Annex I to Regulation (EU) 2018/948 and 4 mg/L
Potassium bisulphate	Part A, Table 2, point 2.2	
Potassium metabisulphate	Part A, Table 2, point 2.3	

« Organic wine, Biodynamics, HVE, nature, vegan? »

Vin Bio, vin Biodynamique, vin méthode nature

MENTION	Vin Biologique	Vin Biodynamique	Vin méthode nature
REGLEMENTATION	European Regulations 834/2007, 889/2008, 203/2012	European Bio regulations + private specifications (Demeter, Biodyvin ...)	European Bio Regulations + private specifications (Wine nature method)
COORDINATEUR	European institutions In France: INAO	Private associations e.g. Demeter, Biodyvin (international union of winegrowers in biodynamic cultivation)	Private union Syndicat de défense du vin naturel (only one recognized to date)
ENGAGEMENTS	Organic Principles Compliance	Compliance with ab+ principles build a balanced plant/environment production system taking into account celestial and terrestrial forces	Compliance with ab principles + manual harvesting + no oenological input + no physical technique + no sulphites before and during fermentations
CONVERSION	3 years	3 years	Not
CERTIFICATION	Oui	Oui	Oui
ETIQUETAGE	Mention "Organic Wine" Mandatory European logo Optional French AB logo	Authorized information: "Vin Demeter" 'Biodynamic wine (certified by [...]) ' AB certified wine and biodynamic controlled »	Affixing one of the 2 logos "Wines method nature"



La Biodynamie



In the vineyard : same permissions as in AB with the addition of:
 copper restricted to 3 kg/ha/year
 all the crops of the domain must be in Biodynamics
 biodynamic preparations (500 and 501 on the estate, 502 to 507 in compost)
 Domain must act to maintain biodiversity



Au chai :

Vinification

Fermentation

			
Protection de la vendange	Dioxyde de soufre (SO2)	✓	✓
	Acide ascorbique	✓	✗
Enzymage, débourage, extraction du jus	Enzyme pectolitique	✓	✗
	Enzyme d'extraction	✗	✗
Aération	Air / oxygène	✓	✓
Fermentation alcoolique	Levures exogènes	✓	✗*
Nutrition des levures	Ecorce de levures	✓	✓
	Thiamine	✓	✗
	Phosphate diammonique	✓	✗
	Bisulfite d'ammonium	✗	✗
Fermentation malolactique	Bactéries lactiques exogènes	✓	✗*

* une dérogation peut être accordée dans des cas bien précis, motivés et justifiés et après étude de la demande par la commission cahier des charges de Demeter France



Vin vegan

- ✓ means without the addition of animal inputs

✓

In the vineyard, no limitation.

(In biodynamics, the question arises of preparations 500 to 507)

In the cellar, certain oenological inputs not usable, in particular bonding product based on: Albumin/ovalbumin => egg/Fish glue/gelatin/Casein => skimmed milk/Oenological coal => varied origin including some animal

- ✓ Term and logo VEGAN royalty-free, no need to be certified to use them. => be able to prove its practices via its traceability in case of control or at the request of the consumer.
- ✓ Several brands offer product certification and the use of the logo associated with the brand.
- ✓ Certification becomes necessary when a certificate is requested from the winegrower by a professional buyer, in particular for export.

✓



Natural Wine

NOTRE CHARTE D'ENGAGEMENT

1 • 100 % des raisins (de toutes origines : AOP, Vin de France, etc.) destinés à un vin qui se revendique « Vin méthode nature » se doivent d'être issus d'une agriculture biologique engagée et certifiée (Nature & Progrès, AB, ou 2^e année de conversion AB à minima).

2 • Les vendanges sont manuelles.

3 • Les vins sont vinifiés uniquement avec des levures indigènes.

4 • Aucun intrant n'est ajouté.

5 • Aucune action de modification volontaire de la constitution du raisin n'est autorisée.

6 • Aucun recours aux techniques physiques brutales et traumatisantes (osmose inverse, filtrations, filtration tangentielle, flash pasteurisation, thermovinification...) n'est permis.

7 • Aucun sulfite n'est ajouté avant et lors des fermentations.
(Possibilité d'ajustement - de l'ordre de : $\text{SO}_2 < 30 \text{ mg/l H}_2\text{SO}_4 \text{ total}$, quels que soient la couleur et le type de vin - avant la mise; obligation d'information d'adjonction de sulfites, mentionnée sur l'étiquette via un logo dédié.)

8 • Lors d'un « salon des Vins méthode nature » les vignerons comme les organisateurs s'engagent à présenter la charte à côté des bouteilles; les cavistes indépendants sont encouragés à faire de même, dans la mesure du possible, au sein de leur établissement.

9 • Utilisation d'un logo d'identification.



10 • L'engagement se fera lors de la mise en « commercialisation » (obligation de résultat) par une « déclaration sur l'honneur », faisant suite à l'avis du bureau du Syndicat; il sera demandé chaque année pour chaque cuvée (lot clairement identifié).

11 • Les cuvées non « Vins méthode nature » doivent être clairement identifiables (étiquetage différencié) chez les signataires.

12 • Les signataires s'engageront en leur nom propre et toutes les informations demandées seront mises en ligne.

Cet engagement est issu d'une association collégiale.

Notre syndicat

Le syndicat des Vins Nature'l existe depuis septembre 2019 pour fédérer une large communauté autour des valeurs (artisanat, transparence, indépendance, dimension sociale) et des principes d'élaboration et de diffusion du « Vin méthode Nature ».

Nos objectifs opérationnels

- Défendre le vin nature et ses membres auprès des institutions publiques et organisations
- Communiquer sur la charte d'engagement et le logo « Vin méthode Nature »
- Faire vivre une communauté (vigneron, professionnel du vin, consommateur) autour du vin nature

Vin
méthode
Nature

Procédure d'engagement du vigneron.e

ÉTAPE 1

ADHÉRER au Syndicat de défense des vins Nature'l

- Adhésion de 30 € par an
- Via un formulaire en ligne sur internet

ÉTAPE 2

ENGAGER les cuvées avant le 10 décembre

- (date de déclaration de récolte)
- Via un formulaire en ligne sur internet
- Nombre de cuvée et volume maximum (possibilité de réduire les cuvées et les volumes ensuite mais pas de les augmenter)

ÉTAPE 3

TRANSMETTRE au Syndicat une analyse cofrac « Frantz Paul » par lot

- 1 analyse par cuvée ou par lot après mise en bouteille (en gardant les documents de traçabilité du vin et cahier de cave)
- À réaliser à la charge du vigneron

ÉTAPE 4

VÉRIFICATION de l'analyse par le Syndicat

- Conformité de l'analyse
- Respect de la charte d'engagement

ÉTAPE 5

VALIDATION par le Syndicat

- Confirmation au vigneron de l'utilisation du logo en fonction de l'analyse (-10 ou -30)
- Convention d'utilisation du logo
- Transmission d'une facture de 20 € par cuvée engagée



VIGNERONS BIO
NOUVELLE AQUITAINE

Input and technics use on the estate

SURVEY ON THE OENOLOGICAL PRACTICES OF ORGANIC
WINEGROWERS
IN FRANCE
MILLESIME 2020



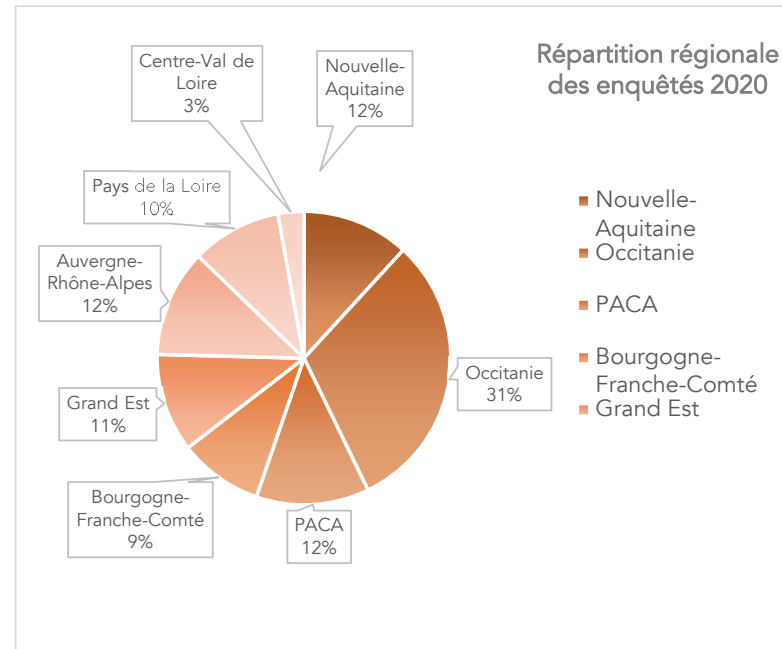
itab

l'Institut de l'agriculture
et de l'alimentation biologiques

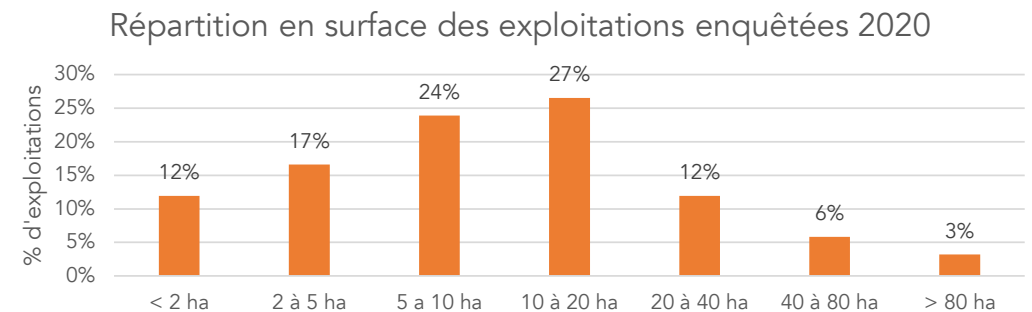
- The survey on oenological practices has been carried out since 2012.
- It makes it possible to take stock of the vintage that has just passed by highlighting the use of inputs and techniques by organic winegrowers in Oenology.
- In recent years we have also added a very short survey on the phytosanitary campaign.
- Its renewal every year makes it possible to follow the evolution of the practices of organic winegrowers in France
- It also constitutes a working basis to build the technical arguments that will be used to evolve the regulations for the benefit of organic winegrowers.

Description of the sample

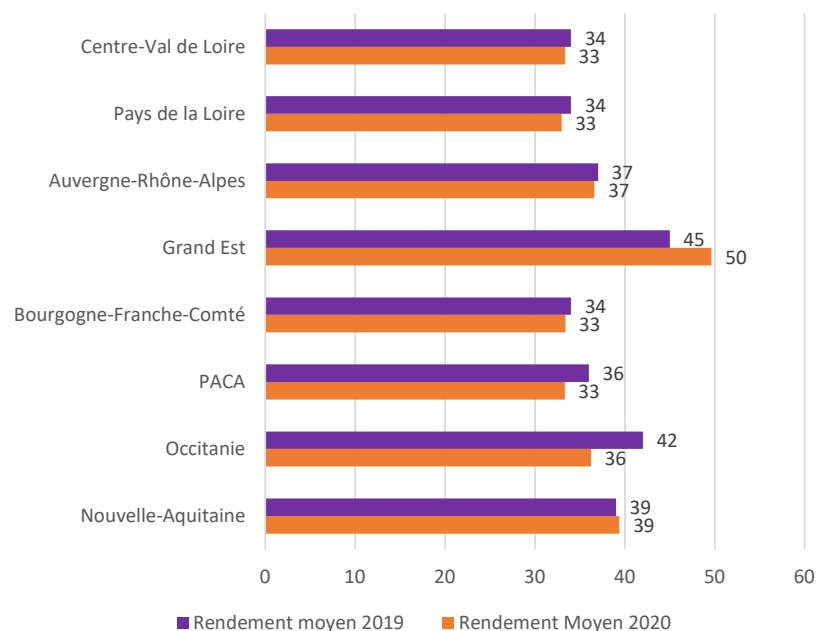
343 organic
winegrowers,
spread over
the entire
territory



The average
size of the
properties
surveyed is
28.83 ha



Rendement moyen en Hl/Ha

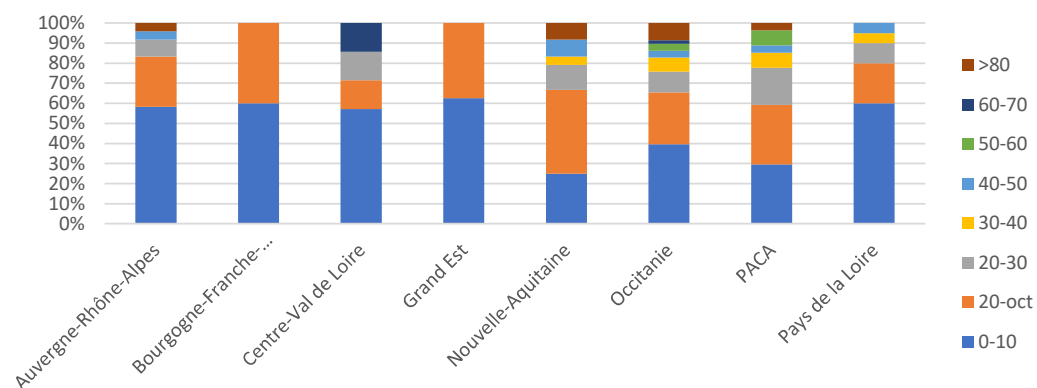


Slightly lower yields in almost all regions, in line with climatic conditions and field observations.

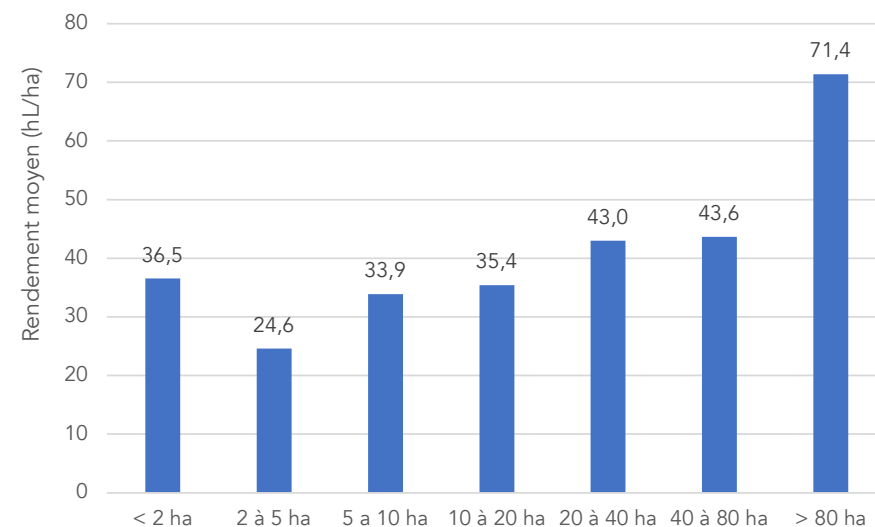
This situation had also been confirmed during the campaign review carried out in the Organic INAO wine commission by all the professionals around the table.

The size of the farms which is dependent on the region of production, the type of wine achieved impact on the yields produced. Indeed the yield is more important on the properties with large surfaces

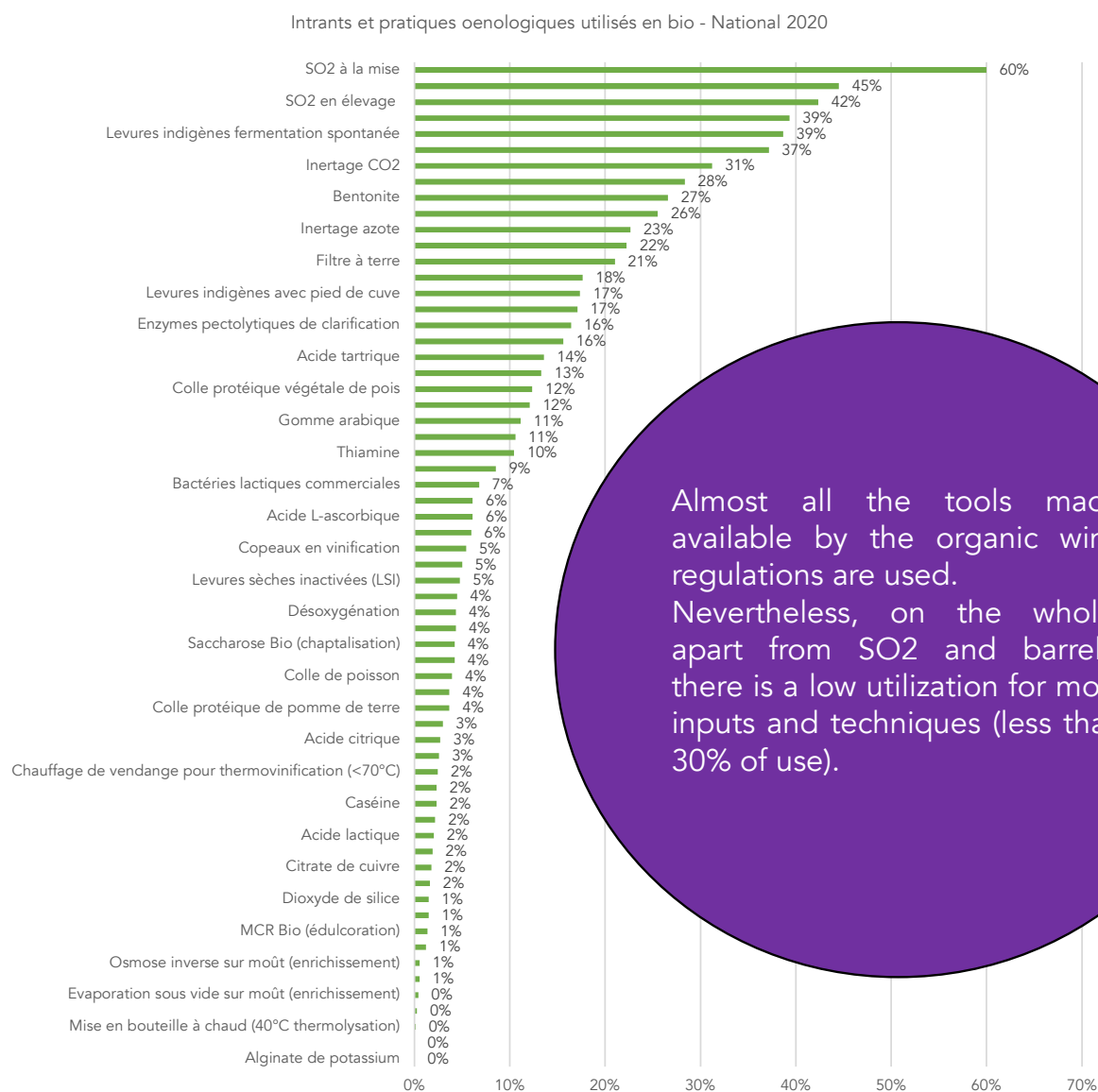
Repartition de la taille des exploitations par région



Rendements moyens 2020 en fonction de la taille d'exploitation



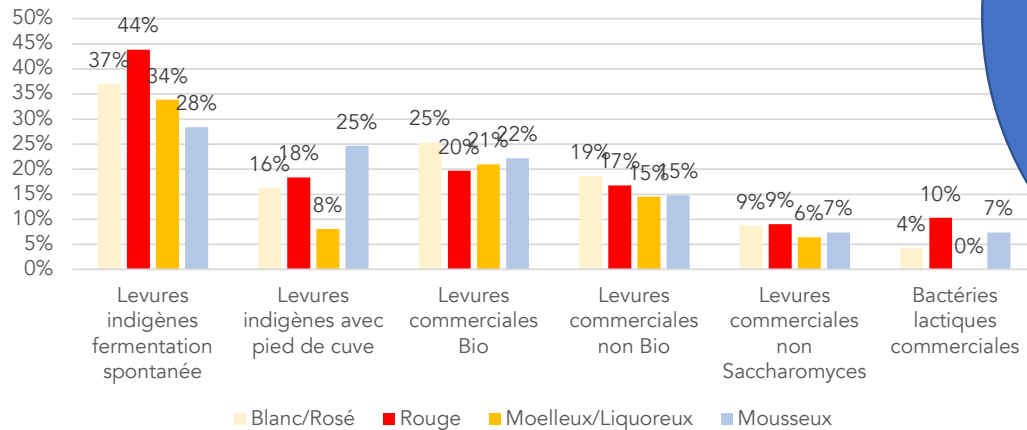
Input/Technical Uses in 2020



Focus par types d'intrants en 2020

Yeasts and bacteria

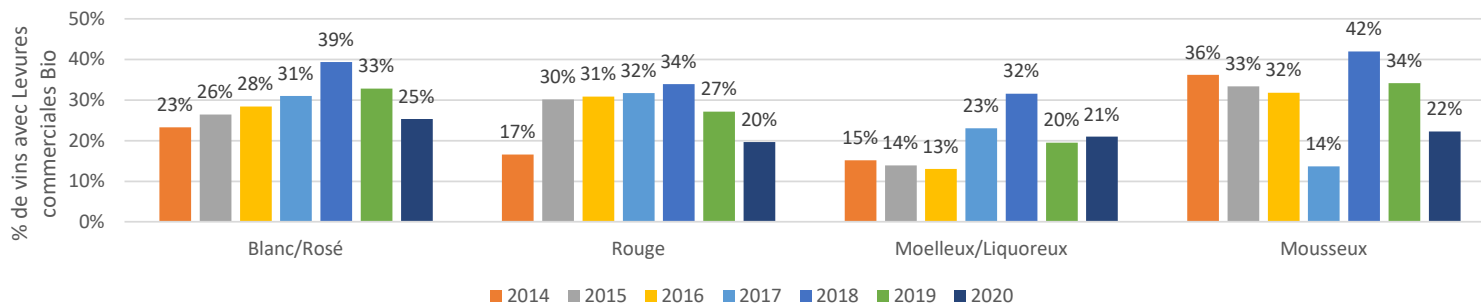
Color distribution of yeast and bacteria use - 2020



The use of native fermentation remains lower in white/rosé than in red.

The impact of yeast being more important on the aromatic profile and the very important use of a cold phase to settling the mouts in white encourage winegrowers to resort to commercial yeasts to ensure good fermentation and good quality.

Evolution use Organic commercial yeasts by color since 2014



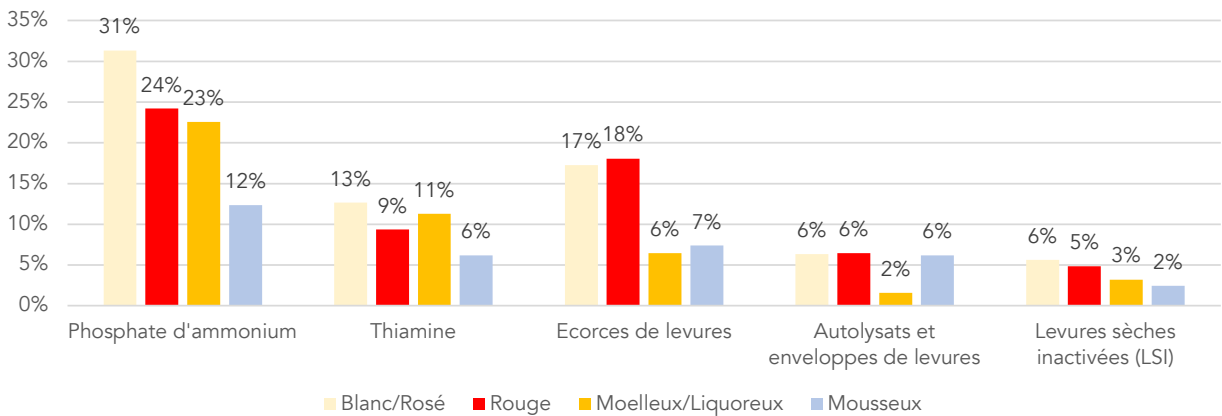
If we look a little more ready the specific use of organic yeasts we find that there is little evolution of use.

Focus by input types in 2020

Nutrition and clarification

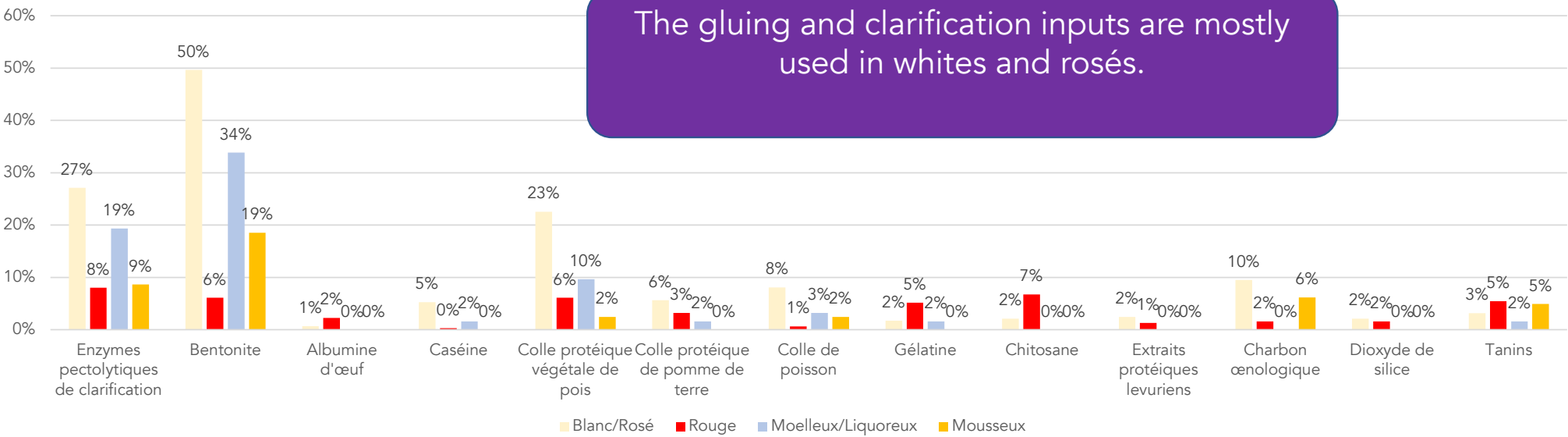
There is a stronger use of nutrition in white especially via ammonium phosphate.

Color distribution of the use of fermentation regulators - 2020



Color breakdown of the use of bonding products and clarification - 2020

The gluing and clarification inputs are mostly used in whites and rosés.

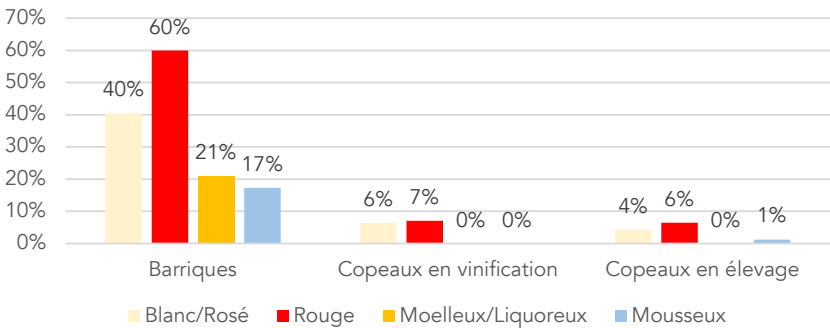


Focus by input types in 2020

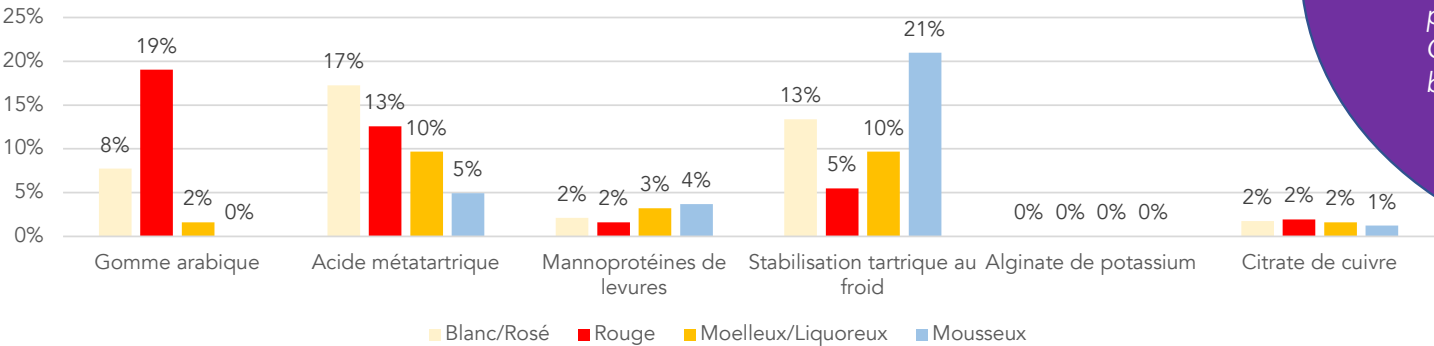
Winemaking auxiliaries

There is a limited use of chips in winemaking and ageing, even in red. The barrel remains mostly used in red unsurprisingly, followed by white and sweet.

Color distribution of wood use - 2020



Color distribution of the use of stabilizers and wine treatments - 2020



Only a small part of the winegrowers use techniques for tartaric stabilization. The most used methods being meta tartaric acid or cold treatment this method being the most effective to ensure that there are no export problems. Gum arabic is widely used in red because it is not very restrictive to use

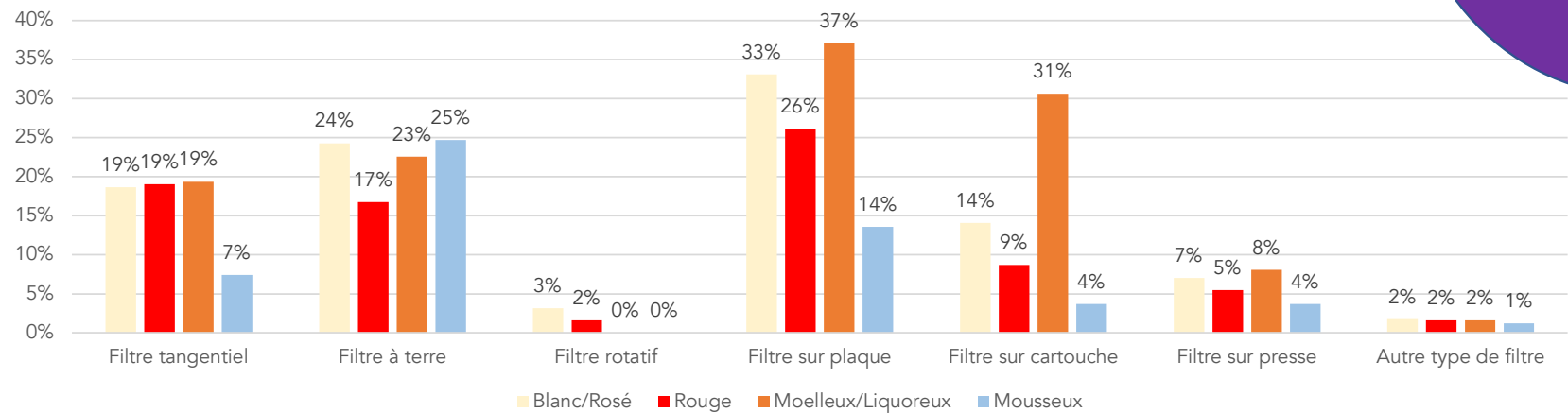
Focus by input types in 2020

Filtration

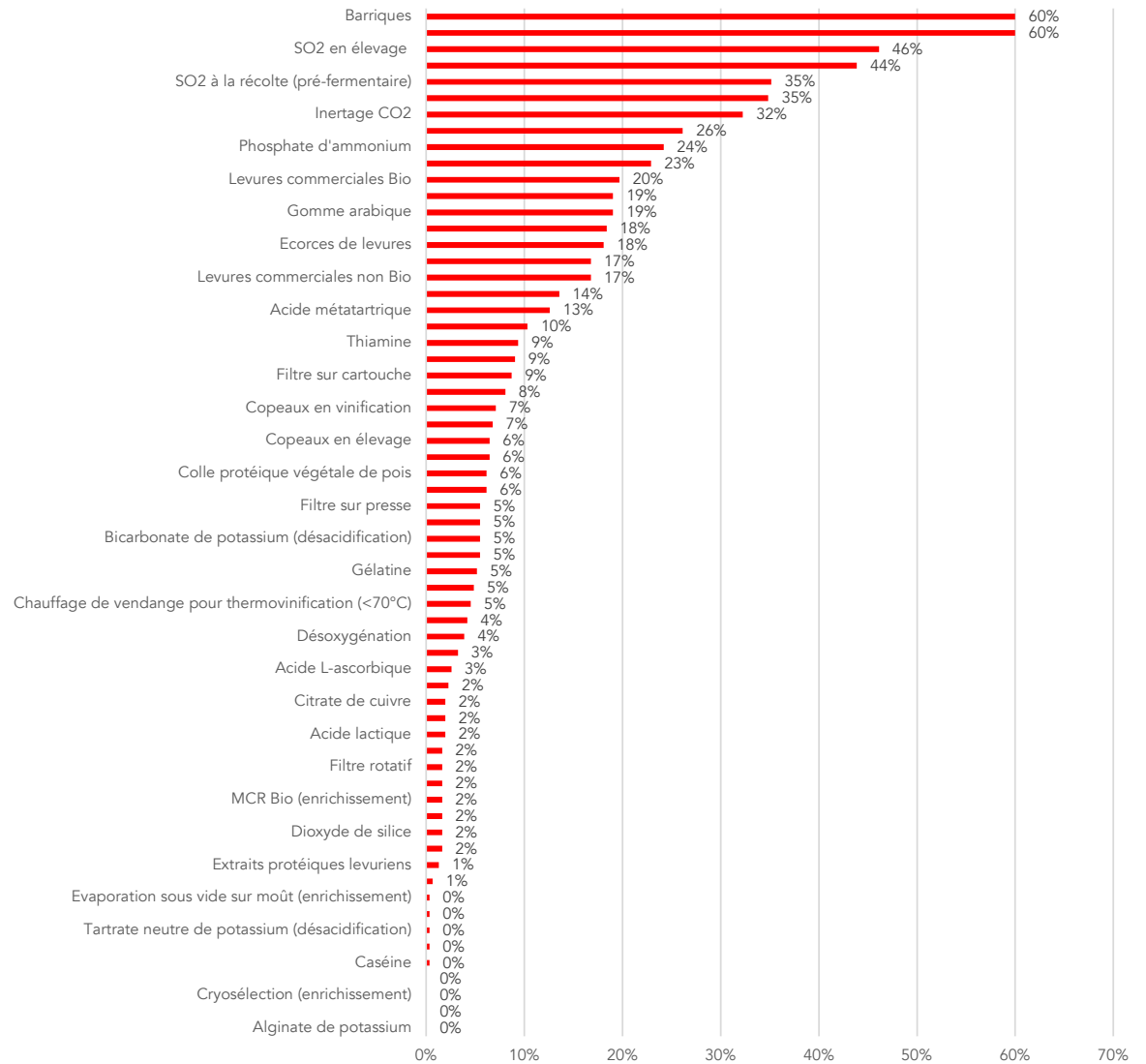
Tangential filtration is becoming more democratic

Plate filtration remains the majority because it is it that is used at the time of bottling.

Color distribution of filtration use - 2020



Oenological inputs and practices used in organic in Red - National 2020



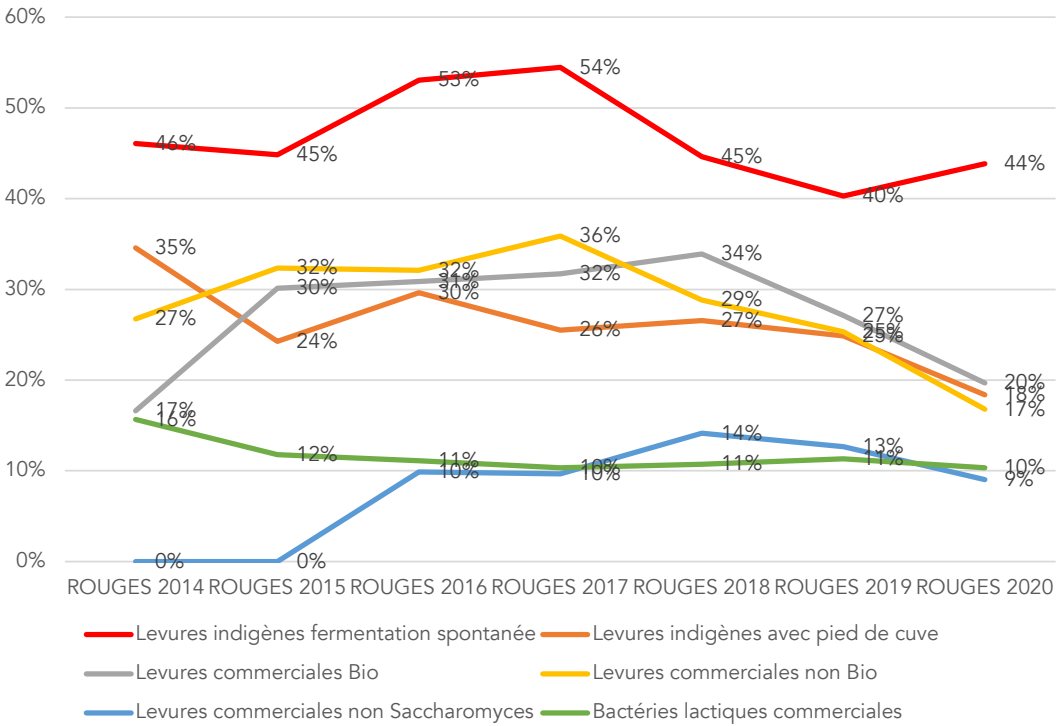
Focus on Red Wines

Focus red wines

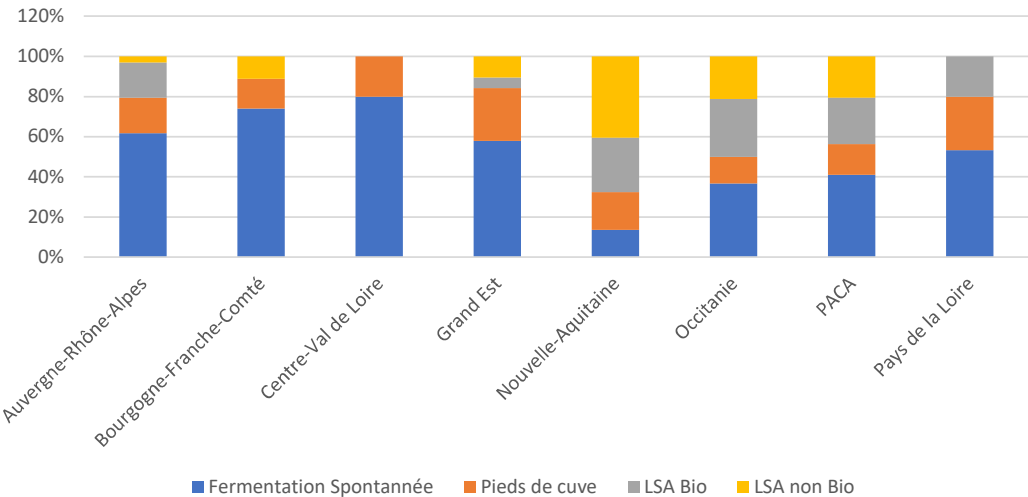
Yeasts and bacteria

Indigenous fermentation, although majority has seen its use decrease since 2018, probably due to the difficulty of fermentability of musts.

Evolution use Yeasts/Bacteria in Red



Distribution of fermentation types in red by region



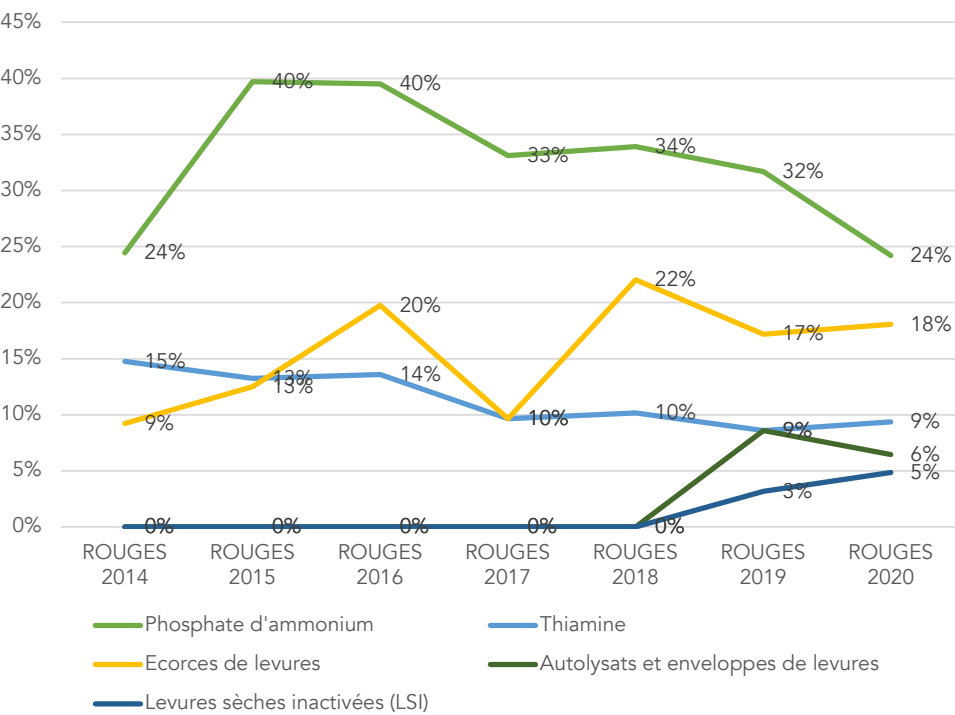
Warmer regions which are also areas of larger production in terms of farm size make greater use of fermentations with LSAs.

Focus red wines

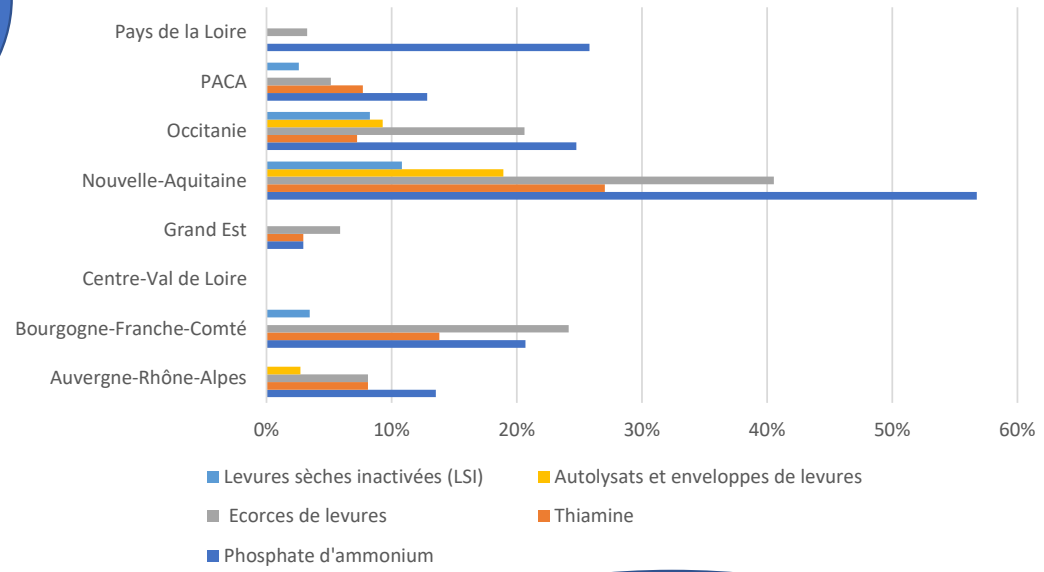
Nutrition

Mineral nutrition remains the majority because it is a priority in case of resorting to nutrition for the fermentation of wines. The fall in 2020 is probably due to the change of people surveyed.

Red fermentation regulators



Distribution of red wine nutrition according to regions



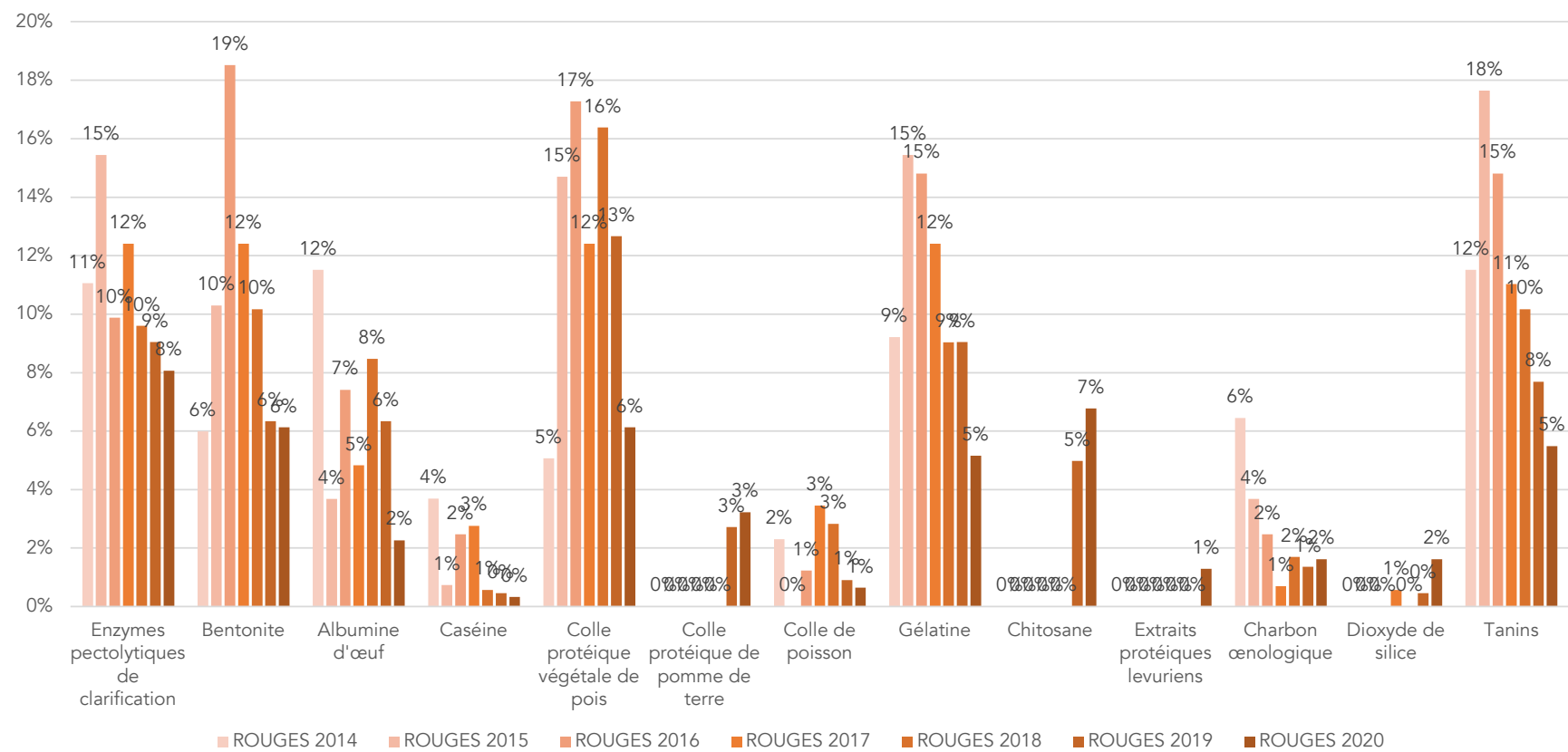
The level of use of nutrition may also need to be linked to the type of oenological monitoring of the different regions. The new Aquitaine region has a very important network and support in oenology. Oenologists tend to take less risk in the somewhat difficult years with high degrees as in 2020 in Nouvelle Aquitaine.

Focus red wines

All the possibilities of bonding inputs are used, varying according to the years but with percentages much lower than those of whites and rosés.

Collages/clarification

Red Collage/Clarification



Focus red wines

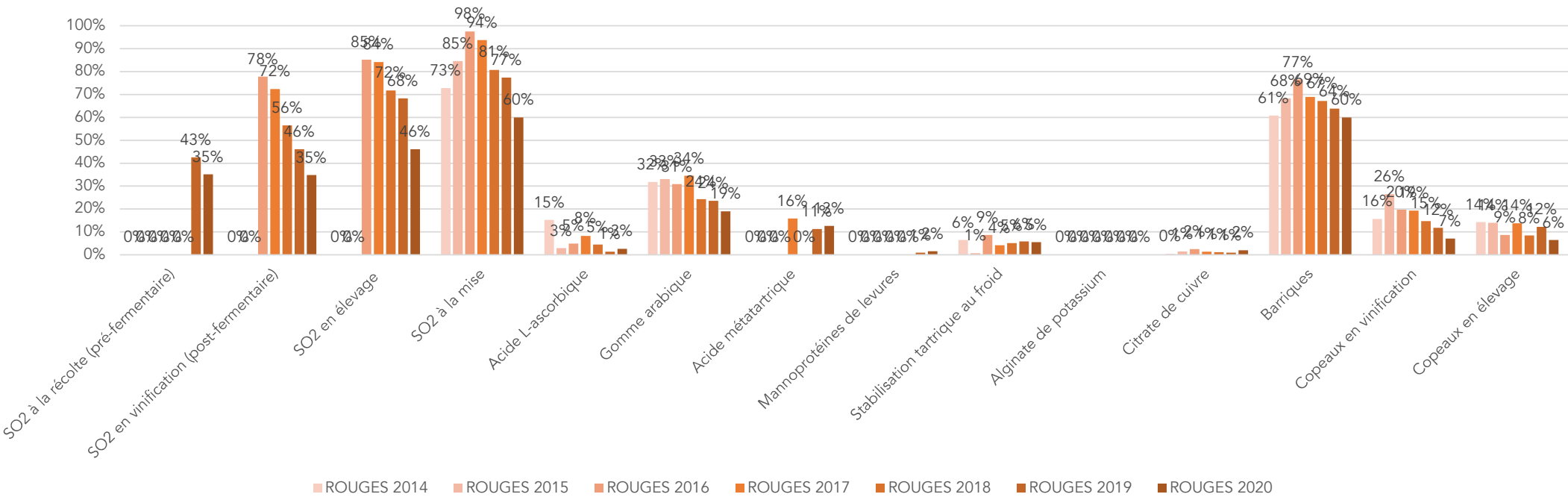
Collages/clarification

SO2 and barrels are very widely used in red.

There is also a fairly significant use of gum arabic.

Chips seem to be little used by organic winegrowers.

Red winemaking auxiliaries



Conclusions

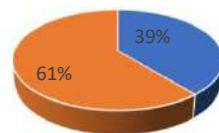
- The practices of organic winegrowers on the 2020 vintage change little compared to previous vintages.
- The uses of permitted inputs and techniques remain low (less than 30% if we exclude SO₂ and barrels and the whole range of tools made available by the Organic Wine regulation is used. The use of inputs or techniques is therefore generally reasoned in Organic.
- This survey confirms the existence of several schools of winemaking in Organic: on the one hand organic winemakers who try to do without inputs as much as possible. On the other hand, organic winegrowers who use a wider range of inputs to obtain a specific product profile, regular, constant over time, in order in particular to meet certain export requests.
- The same phenomenon is also observed on the management of SO₂, the development of organic cuvées "without added sulphites" responding to a growing demand, on the three colors. On these cuvées without SO₂ it seems to emerge 2 families. Wines produced in smaller volumes with winemakers approaching the specifications of natural wine methods. And wines produced in larger volumes that have to use certain inputs such as yeasts or nitrogen nutrition to ensure the quality of these wines.

Wines without sulfite

Wines Without Added Sulphite

More than a third of organic winegrowers in France make so2-free cuvées

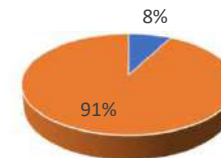
Make yourself a cuvée without SO2



■ Oui ■ Non

However, the phenomenon is anecdotal in bulk carriers

Make yourself bulk without SO2

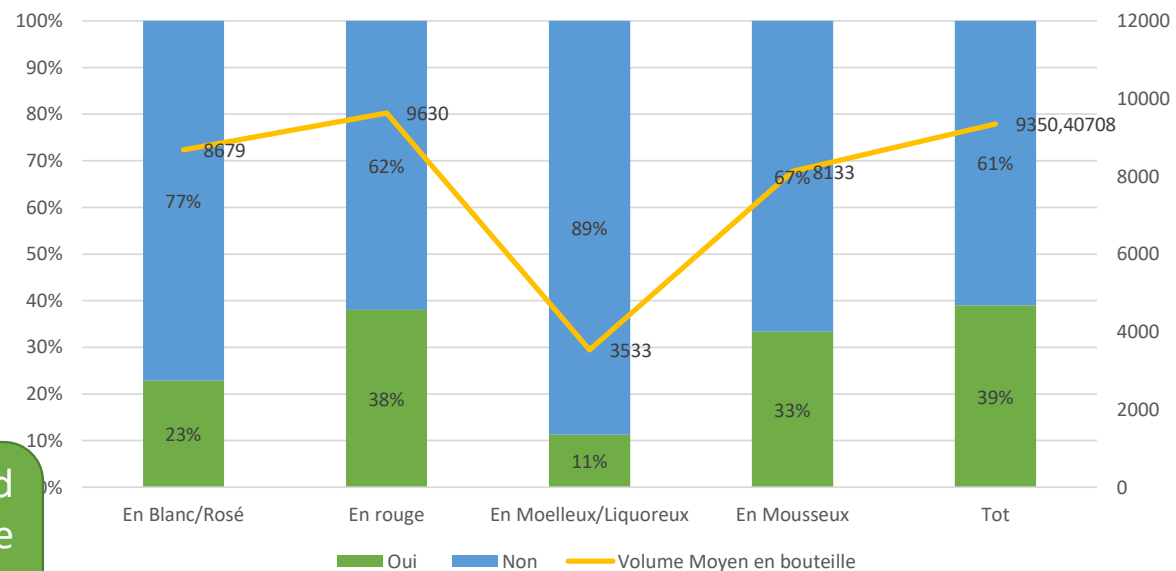


■ Oui ■ Non

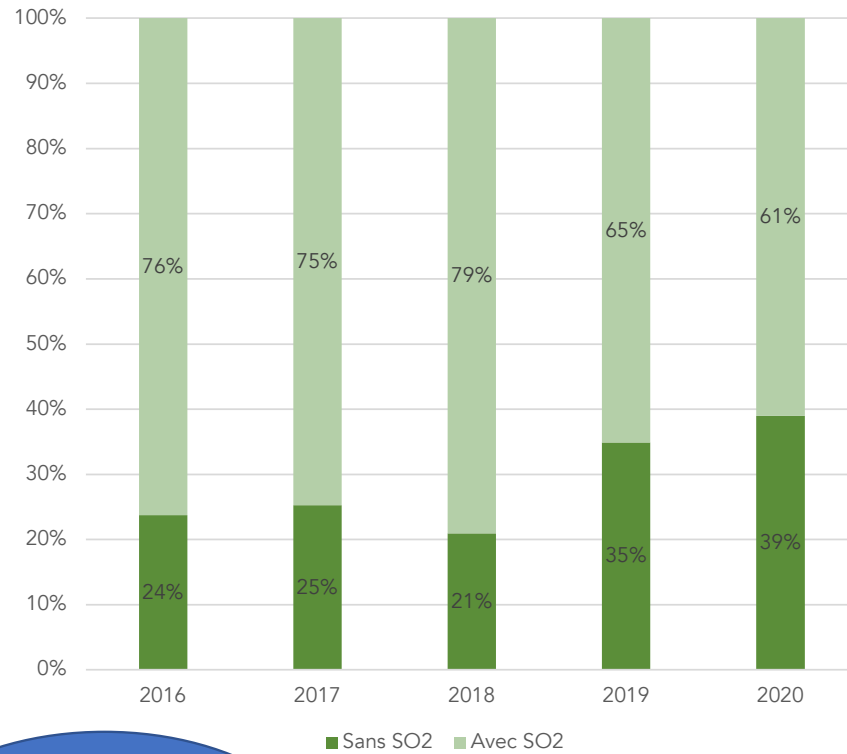
Cuvées without SO2 are made mostly on red wines, the technique being easier to master.

The average volumes produced in white and red are beginning to be relatively large. We have averages around 10,000 bottles for a few years in the survey.

Make yourself a bottle cuvée without SO2



% Cuvées with or without SO2 per vintage

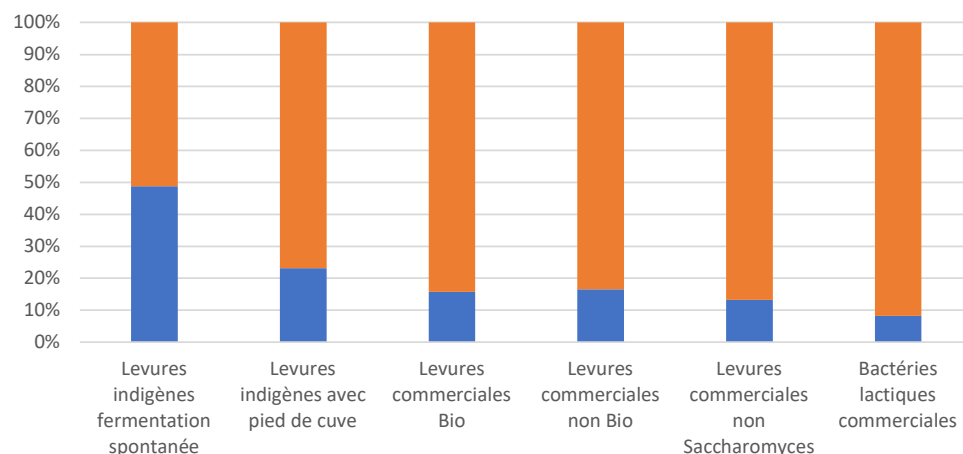


A steady increase
in the production
of SO2-free cuvée

Breakdown of the volumes produced by producers of red wine without added sulphite from the survey

Tranche de volume en Hl	NOMBRE	Pourcentage
0-500	84	69%
500-1000	12	10%
1000-2000	12	10%
2000-4000	6	5%
>6000	7	6%

Yeasts and bacteria

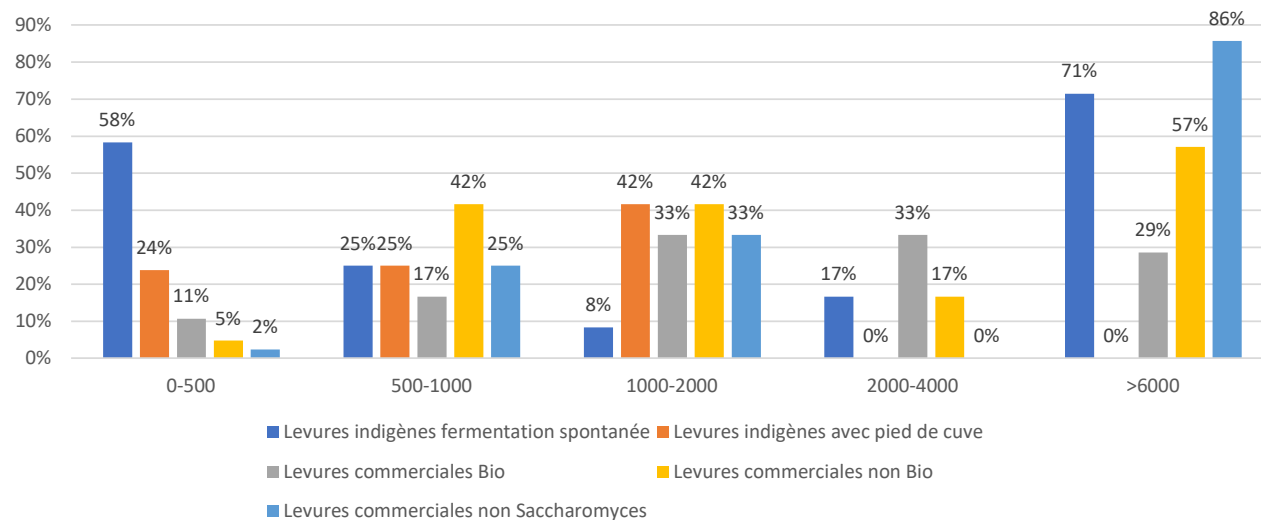


Concerning yeasts

We find a significant proportion of native yeasts with spontaneous fermentations and tank feet.

We find here the users of yeasts non-saccharomyces

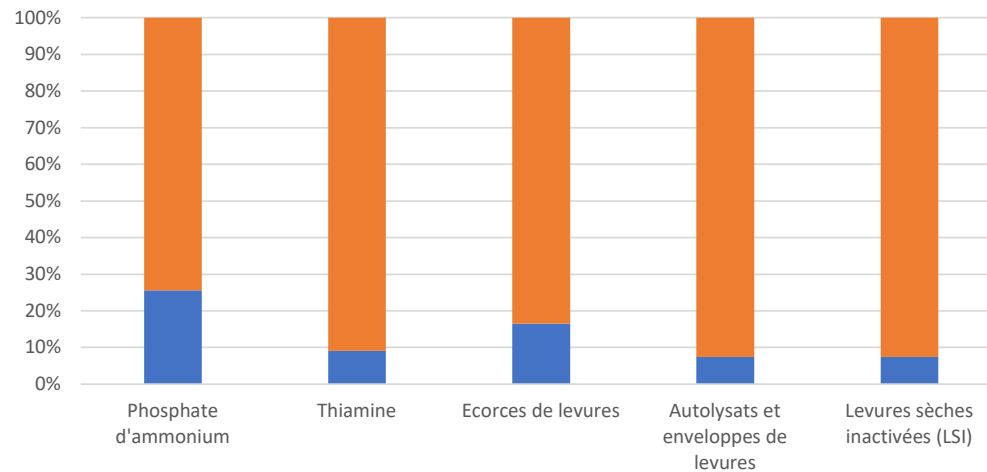
Use of yeasts in red wines without SO2 according to the volumes produced



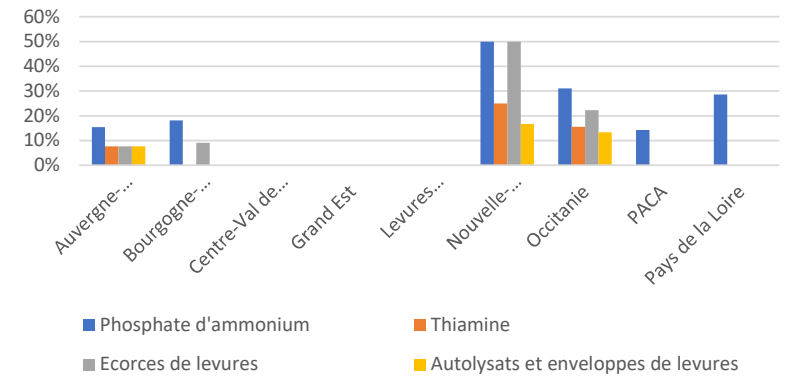
The use of commercial yeasts increases with the size of the production as well as the use of yeasts non-saccharomyces.

This is undoubtedly in order to secure the quality of the batches carried out

Nitrogen Nutrition



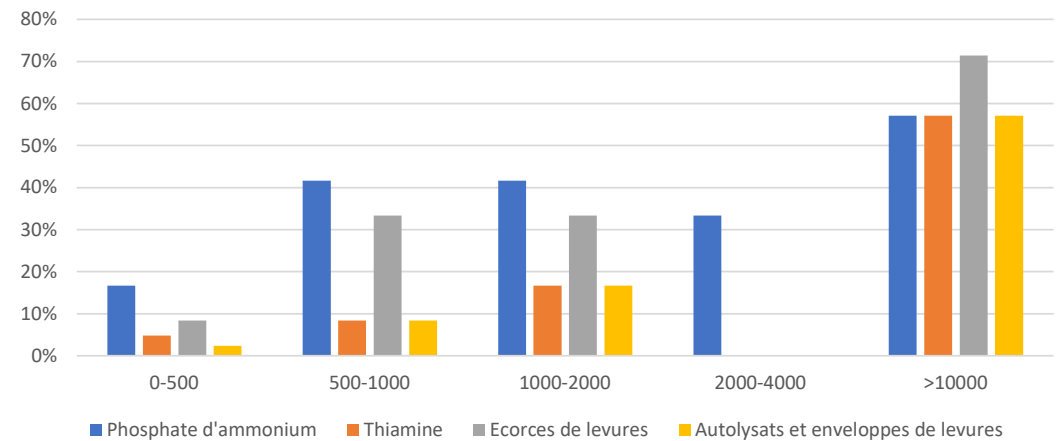
Nutrition of SO2-free red wines by region

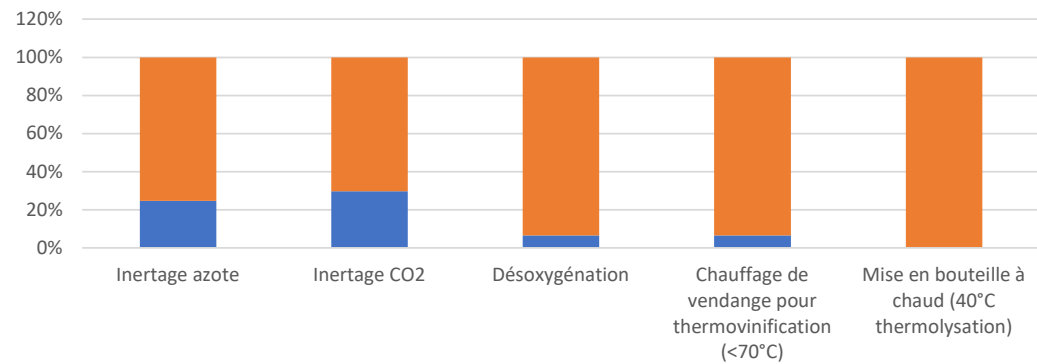


There is overall little use of nutrition approaching in this respect the specifications of wines natural method.

But as before we find a disparity between the regions and the types of wines made. There is a higher use of nutrition as well as yeast bark in regions with higher production and larger structure sizes

Use of inputs on so2-free red wine as a function of production



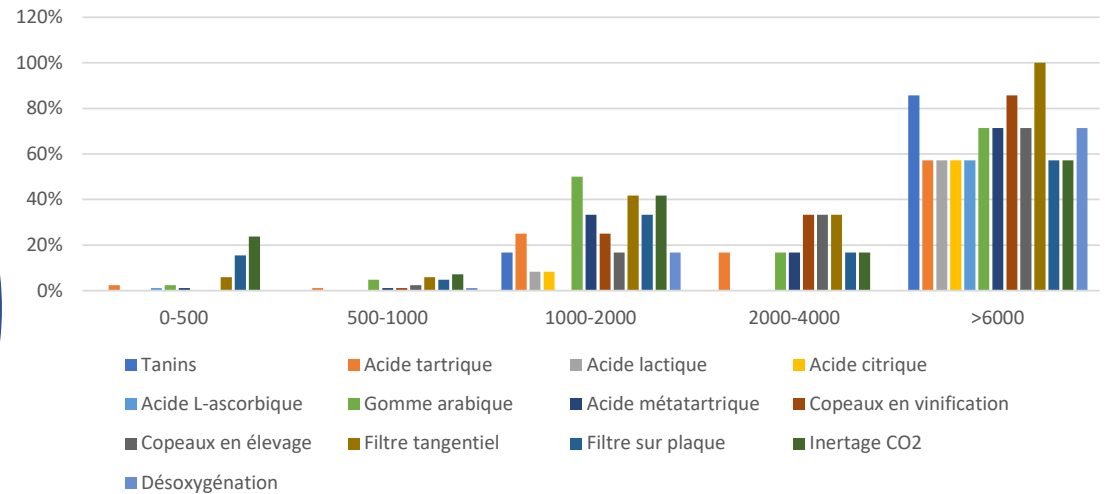


The use of gases is still quite low and this is in line with the field findings of a need for support on the management of dissolved gases for this type of wine.

There is greater use on other inputs for large volume producers. In particular, there is greater use of tangential filtration as well as chips and acidification.

Bar chart showing the percentage of various substances in different particle size ranges (0-500, 500-1000, 1000-2000, 2000-4000, >6000). The substances include Tanins, Acide L-ascorbique, Copeaux en élevage, Désoxygénation, Acide tartrique, Gomme arabique, Filtre tangential, Acide lactique, Acide citrique, Acide méatartrique, Copeaux en vinification, Filtre sur plaque, and Inertage CO2.

Substance	0-500	500-1000	1000-2000	2000-4000	>6000
Tanins	15%	5%	15%	85%	55%
Acide L-ascorbique	1%	1%	1%	55%	55%
Copeaux en élevage	0%	1%	1%	35%	55%
Désoxygénation	0%	0%	0%	15%	70%
Acide tartrique	2%	1%	25%	15%	55%
Gomme arabique	2%	5%	50%	15%	70%
Filtre tangential	5%	5%	40%	35%	100%
Acide lactique	0%	0%	8%	35%	55%
Acide citrique	0%	0%	8%	35%	55%
Acide méatartrique	0%	0%	15%	35%	55%
Copeaux en vinification	0%	0%	25%	35%	85%
Filtre sur plaque	0%	0%	15%	15%	55%
Inertage CO2	25%	5%	40%	15%	55%



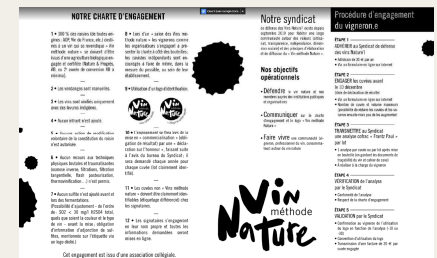
Contexte

A consumer and legislator's demand for naturalness on food products

A willingness not only for consumers but also for winemakers to reduce inputs

A need/desire to create new product profiles for their range and the story telling of winemakers and their products

A technical challenge



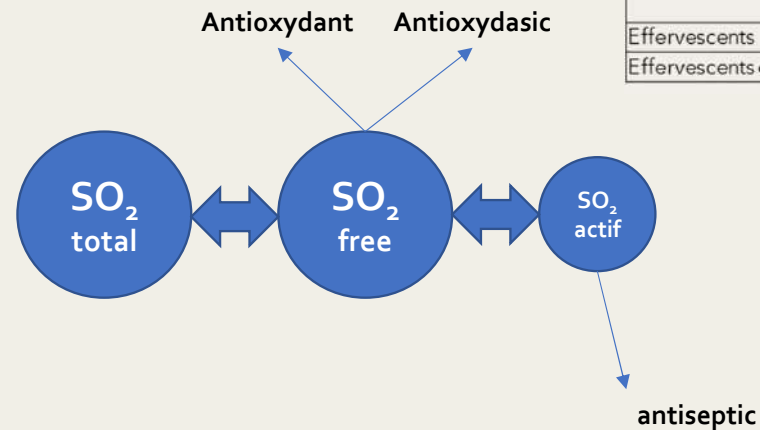
VIGNERONS BIO
NOUVELLE AQUITAINE

SO2

Broad spectrum of action:

- Antioxydant
- Antioxydasic
- Microbiological stabilization

- Only a small part of the total SO2 is active
-
- MCO Recommendations: 0.7 mg/kg body weight

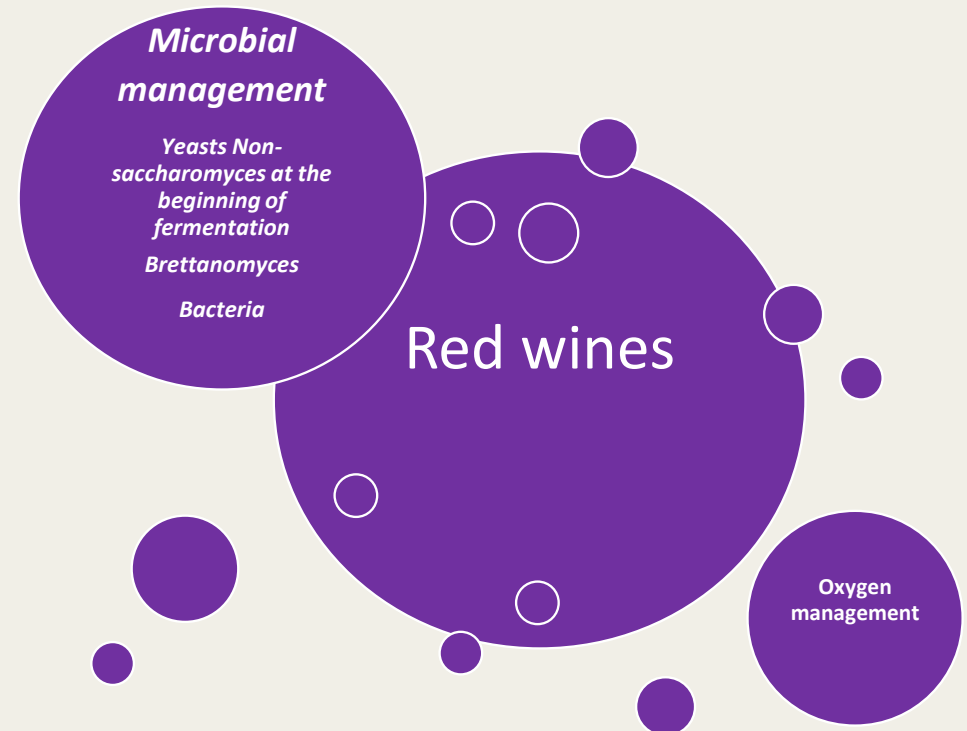
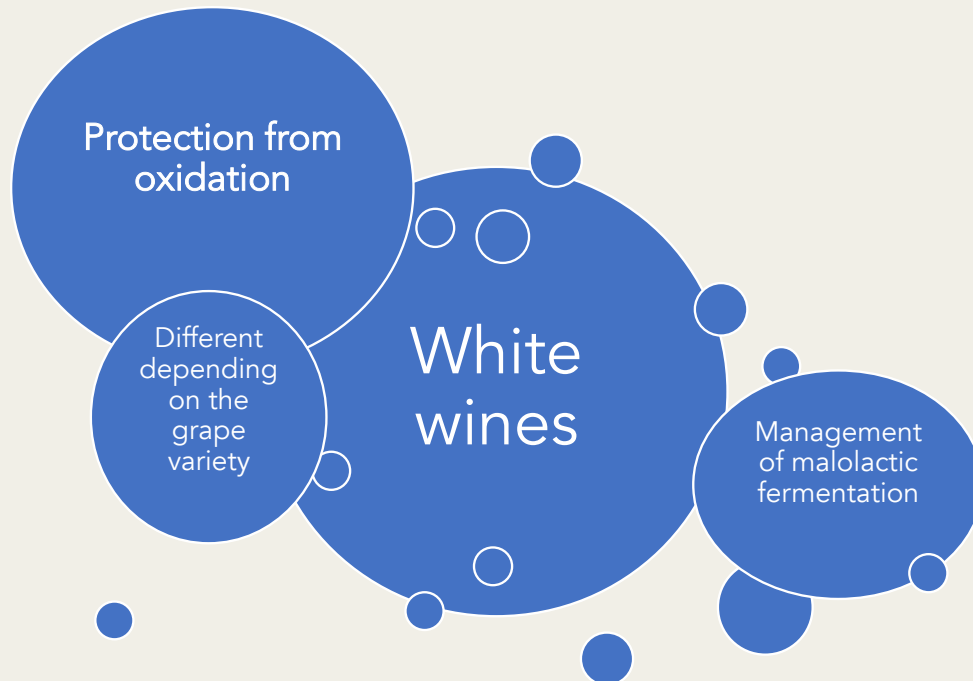


	Rgt Européen OCM Viti- Vini N° 479/2008	(1) Rgt Européen BIO (CE) 889/2008 (2019)
SO2 en mg/l	Total	Total
Vins rouges secs (< 2g/l sucre)	150	100
Vins rouges secs (≥ 2g/l et < 5g/l sucre)		170
Vins rouges (≥ 5g/l sucre) et demi-secs	200	150
Vins blancs/rosés secs (< 2g/l sucre)	200	150
Vins blancs/rosés secs (≥ 2g/l et < 5g/l sucre)		170
Vins blancs/rosés (≥ 5g/l sucre) et demi-secs	250	220
Vins blancs pour certaines AOP (dits "liquoreux")	400	370
Vins moelleux (>12 et < 45g/l sucre)	300	270
Vins doux (≥ 45g/l sucre)	200	170
Vin de liqueur (TAV > 15%)	200 (sucre ≥ 5g/l)	170 (sucre ≥ 5g/l)
Effervescents	235	205
Effervescents de qualité	185	155



VIGNERONS BIO
NOUVELLE AQUITAINE

Wines without SO2



VIGNERONS BIO
NOUVELLE AQUITAINE

Program to accompany the production of wines without SO2

Brettanomyces et tolérance au SO2

Characterization of Brettanomyces properties and tolerance to SO2

RESPECT 2017/2020

So2-free wines: Characterization and implementation of microbiological and physical tools to make so2-free wines, from winemaking to bottling

Vins de Bordeaux sans SO2 2017/2020

CIVB co-financing of the Respect project

Document and vidéos Vins sans SO2 2020/2021

FeADER Region Funding 1.2

2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023

CASDAR Levain Bio 2012/2015

Native yeasts and bacteria: diversity analysis, fermentation test and selection

WILDWINE 2012/2015

Characterization and selection of yeasts and bacteria for mixed sourdough production including non-Saccharomyces yeasts

Bioprotection 2016/2017

Evaluation d'outils microbiologiques pour vinifications réaliser des sans SO2

Vins Sans 2021/2023 Suite de RESPECT

So2-free wines: Livestock and gas management
Impact on the structure and aroma of wines
Liquorous wines

Projet Chitosan 2021/2023 Suite de RESPECT

Brettanomyces management
Management of malolactic fermentation in white wine without SO2

Les domaines de recherche :

Microbiologie

Vinification sans SO2

Diffusions



VIGNERONS BIO
NOUVELLE AQUITAINE

Research



An articulation of projects on three scales:

fundamental research: to better understand the mechanisms and develop analytical tools

controlled tests: To control the bays

Field:

- Needs land and feedback on existing practices
- Real-world tests
- Communication on trials/results

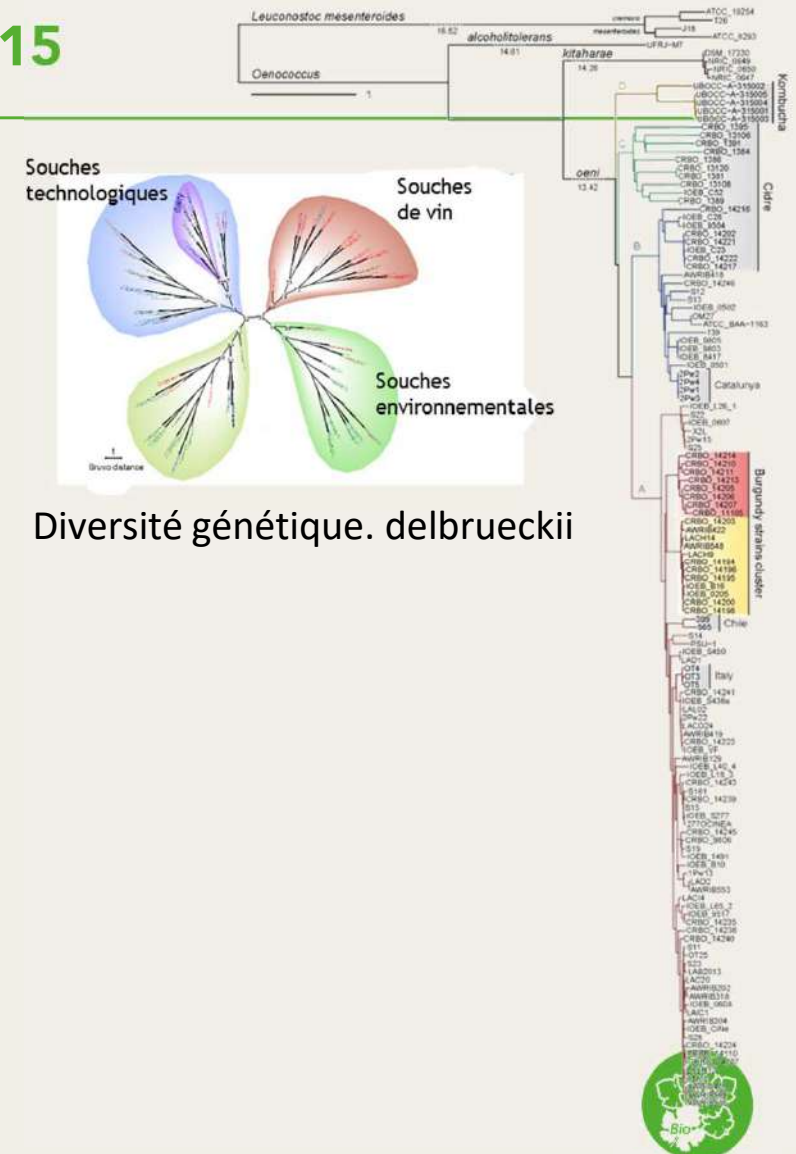


Projet CASDAR levain Bio et WILDWINE 2012/2015

- Understanding the mechanisms of early fermentation

- Characterization of yeast and bacteria populations that are a key element in the management of good fermentation and therefore of the production of wines without SO₂

- Development of analytical tools, especially on non-saccharomyces that will be used in so₂-free wine projects



Diversité génétique. *delbrueckii*

Diversité *Oenococcus oeni*

Projet Bioprotection 2016/2017

"Evaluation of the impact of so2-free winemaking tools, including "bio-protective" yeast-based preparations, Sacch- and/or Non-Saccharomyces, with the aim of making wines without SO2."

Impact on the occupation of microbiological space (yeasts - bacteria)

Impact on oxidation of musts and wines

Impact on alcoholic fermentation

Aromatic impact

Highlighting the importance of good fermentation management

Interest in early adding yeast with LSA or Tank foot

First results on bioprotection and are of interest in certain situations especially in cold pre fermentation phases on the management of acetic bacteria and oxidation of mutts

Rem: many winemakers realize and begin to master fermentation without SO2 on red wine

Different grape varieties:
Sauvignon/Sauvignon
Blanc/Merlots/Cabernet Franc

So2-free
modality

Modality
with SO2

Bioprotection
harvest

Bioprotection
Must

Different strains
of non-
saccharomyces

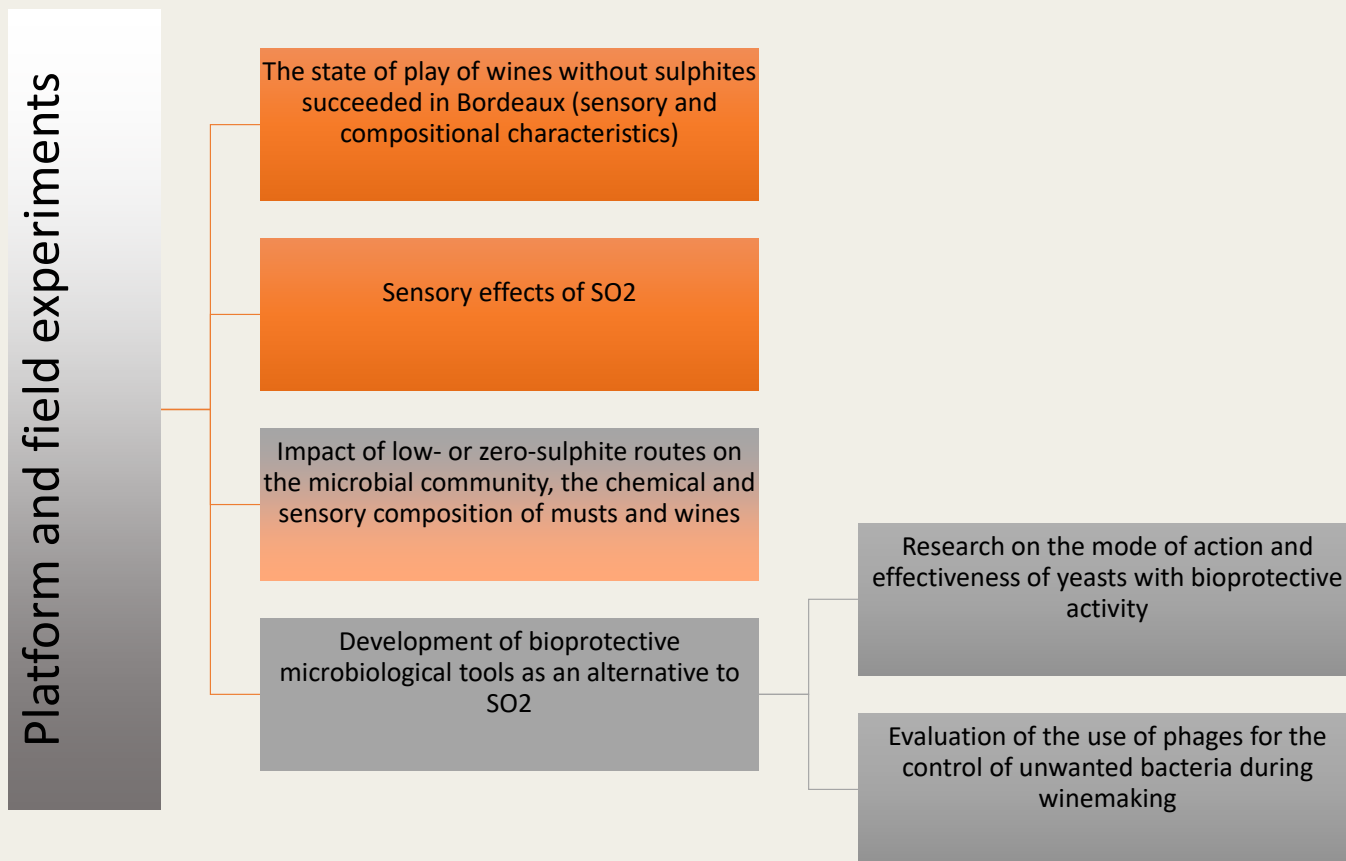
Early seeding in
saccharomyces

Leverage



VIGNERONS BIO
NOUVELLE AQUITAINE

Respect/Vin de Bordeaux without SO2 2018/2020



Respect/Vin de Bordeaux sans SO2 2018/2020

It aims to answer three questions:

- Do successful red wines from Bordeaux grape varieties and sulphite-free products have their own distinctiveness?
- What are the compositional and sensory consequences of the absence of SO2?
- What are the technical routes suitable for the successful development of these types of wines?



VIGNERONS BIO
NOUVELLE AQUITAINE

Evaluation of the quality of so2-free wines



CONTAINS SULPHITES

Commercial wines

52 vins

26 millésime 2015

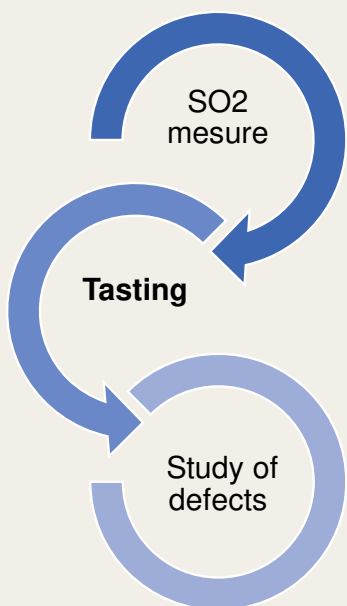
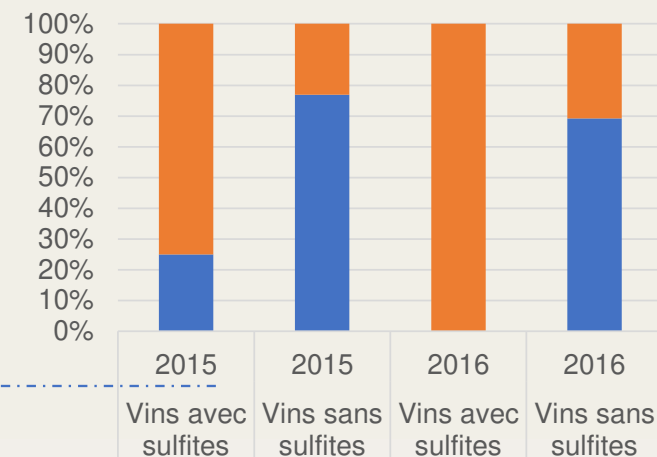
26 millésime 2016

20 vins

12 millésime 2015

8 millésime 2016

Proportions of wines with and without defects

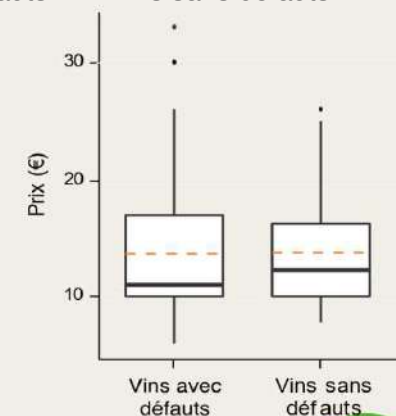


- 8 expert tasters
- Does the wine have a defect?
- If so, which one?
- Test for '2 presence/no defect'

(fréquence de partage entre dégustateurs > 60%)



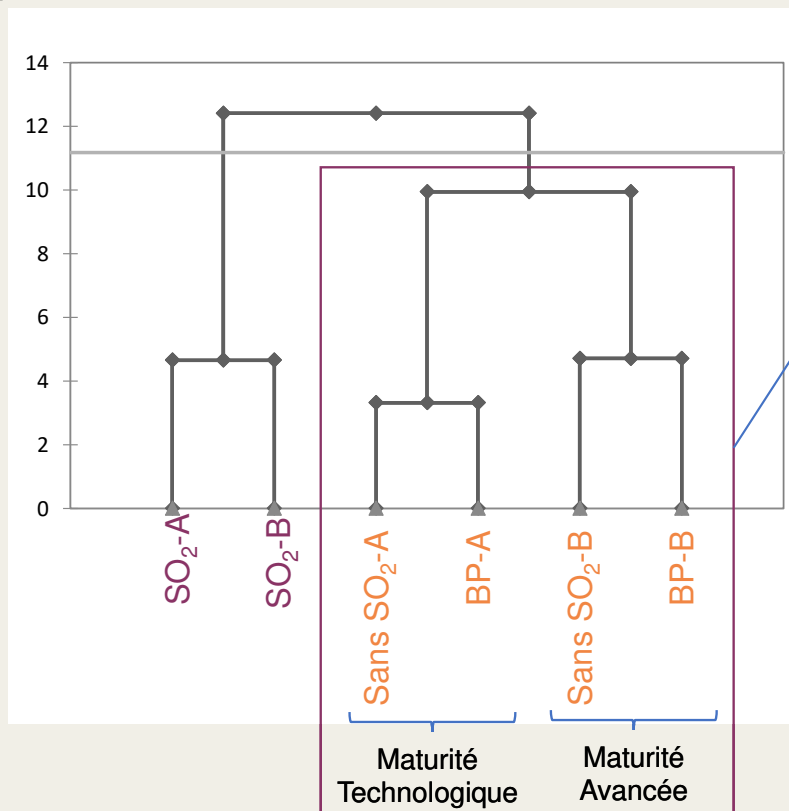
■ Vins avec défauts ■ Vins sans défauts



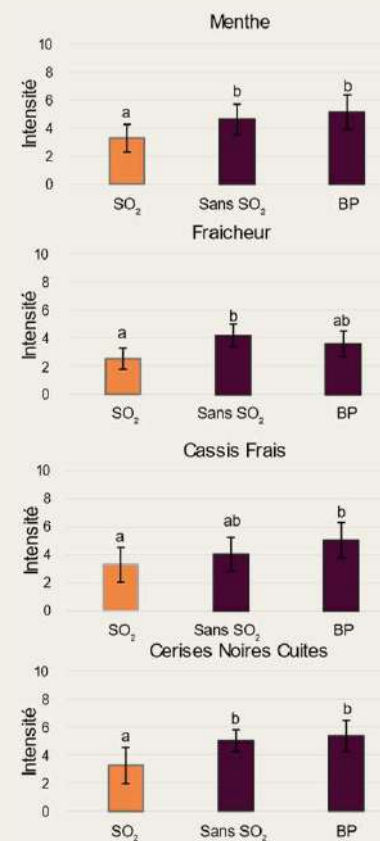
- Défauts majoritairement liés à l'oxydation et aux micro-organismes d'altération
- Pas de lien entre le prix et l'occurrence des défauts

Do successful red wines from Bordeaux grape varieties and sulphite-free products have their own distinctiveness?

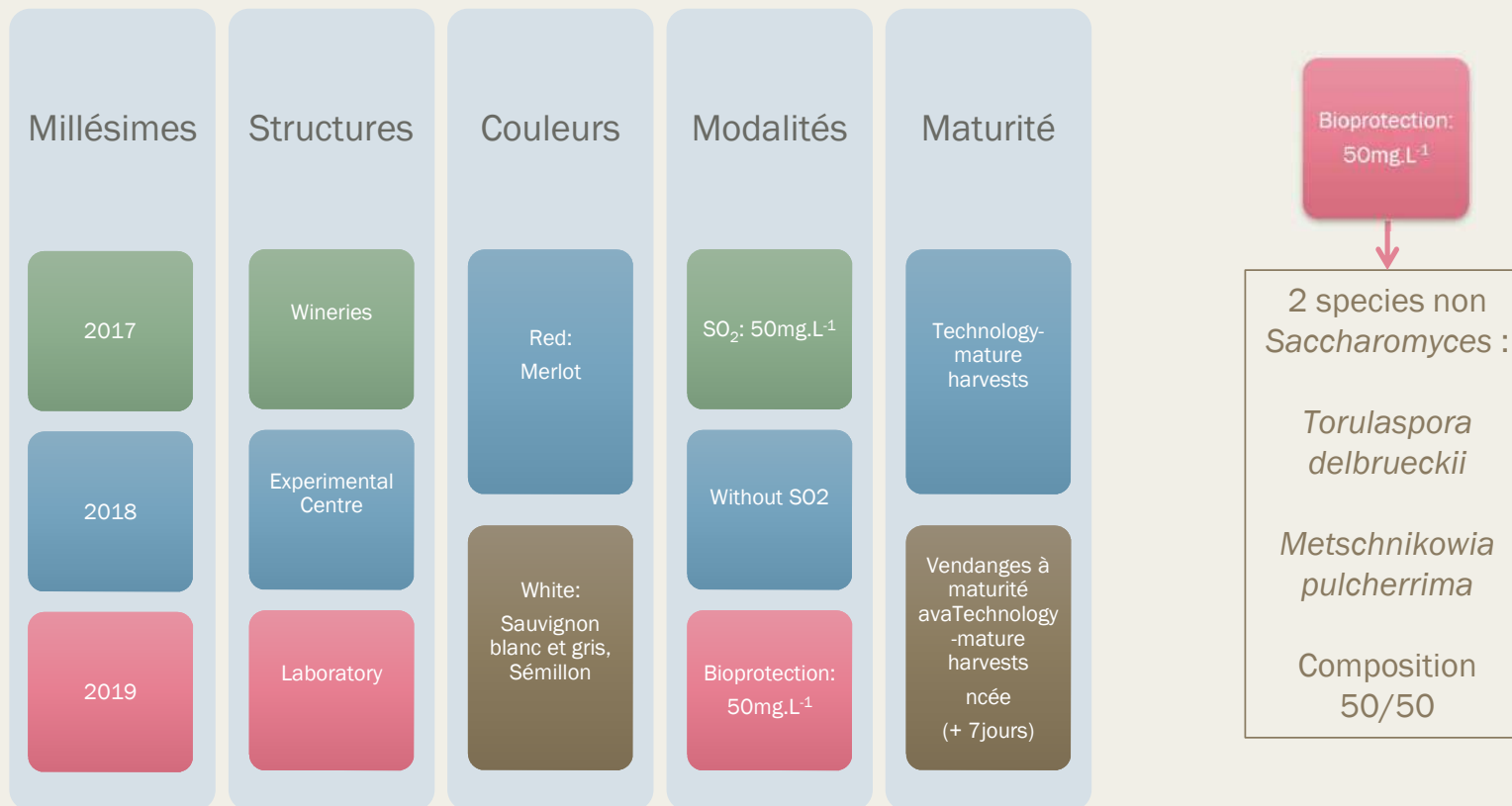
Sensory profile



4 descriptors discriminate 200 wines without sulphites
Mint - Fresh Cassis - Cooked Black Cherries
Freshness



Experimentation

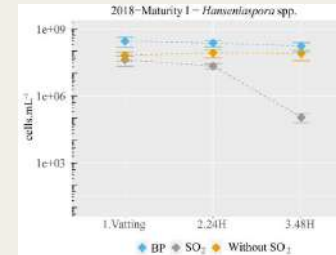


VIGNERONS BIO
NOUVELLE AQUITAINE

Summary of results

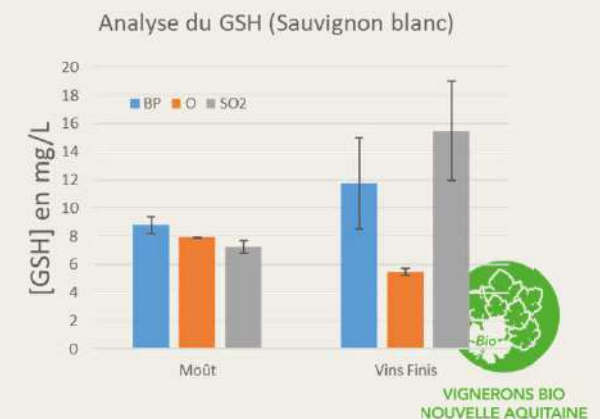
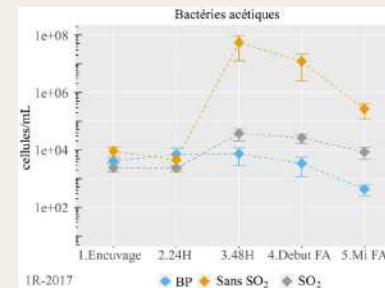
General

- Yeast diversity decreases during the preferred phase of red winemaking, especially in the absence of sulphur dioxide
- In spontaneous fermentation, the diversity of *S. cerevisiae* strains drops in the presence of sulphur dioxide
- Negative impact of sulphur dioxide on *Hanseniaspora uvarum*



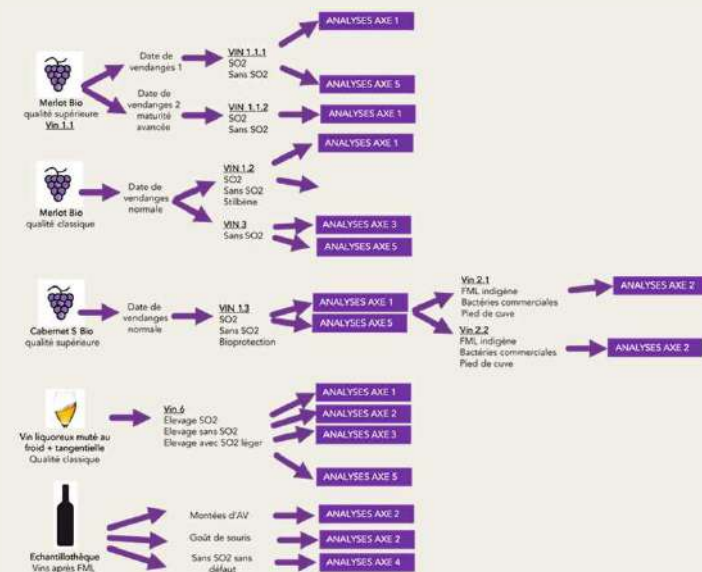
Bioprotection

- Bioprotection colonizes the environment and limits the growth of acetic bacteria
- Low impact of bioprotection on *Hanseniaspora uvarum*
- Bioprotection partially protects white musts from oxidation phenomena

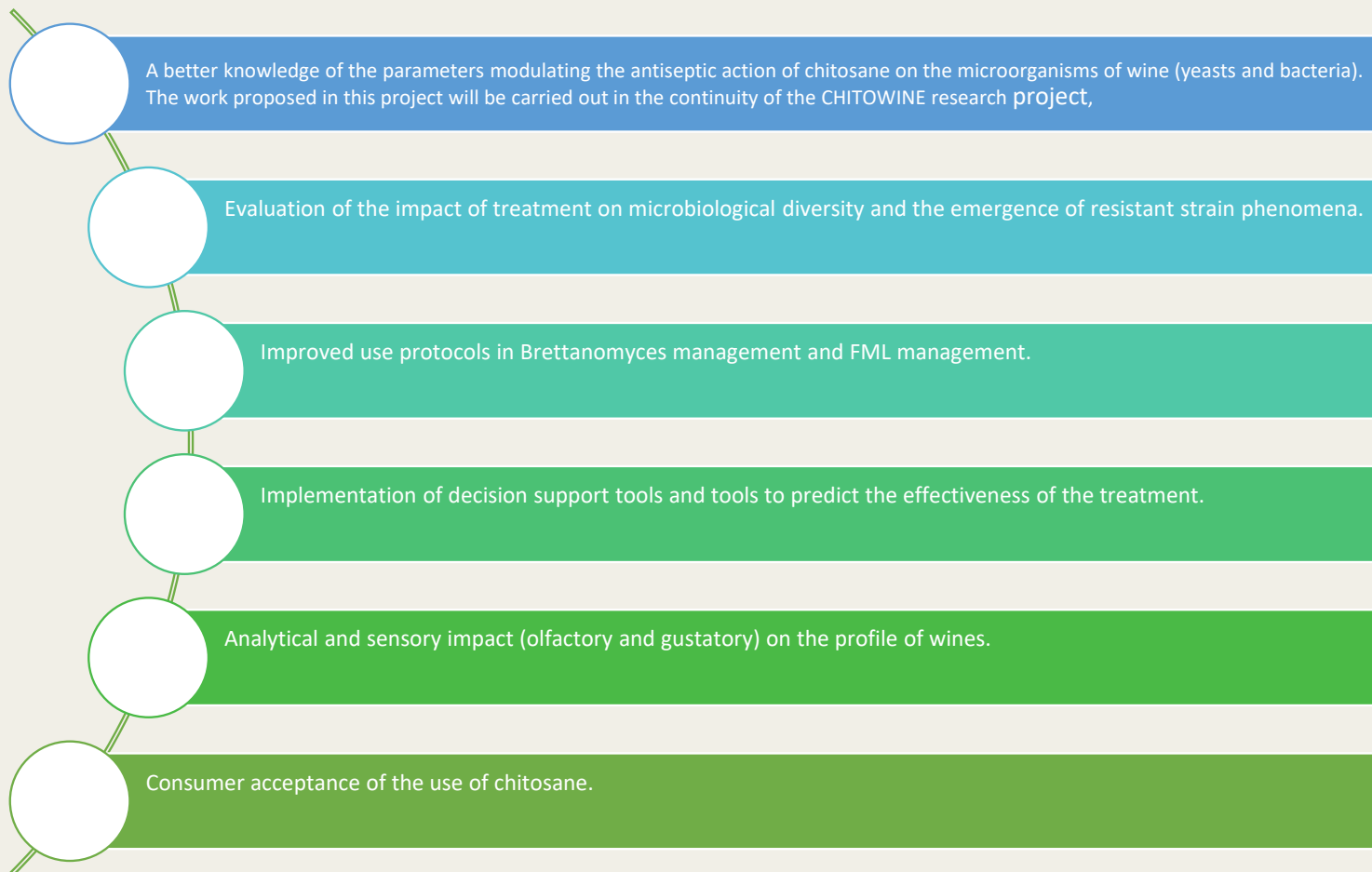


"Wines without" perspective and future work

AXE	Responsible	PROBLEMATIQUE OWNS
1. Vinification without SO2, microbiological impact	Isabelle Masneuf	Climate change context: vinifying grapes with advanced maturity without SO2
2. Bacteria management in livestock	Patrick Lucas	What impact does so2-free farming have on microbial populations? What impact does this have on volatile acidity? Work on mouse tastes (characterization, training of winemakers and winemakers, management tools...)
3. Management of farmed and bottling gases	Rémy Ghidossi	Better control of oxidative phenomena in breeding without SO2 by playing on the supply of gas. Influence of CO2-saturated farms
4. So2-free breeding, impact on phenolic compounds	Mickaël Jourdes	What influence of so2-free breeding on tannins and anthocyanins?
5. Breeding without SO2, impact on aromatic	Jean-Christophe Barbe	Alternatives to SO2 to fight defects. Analytic characterization of so2-free winemaking routes
6. Sweet wines without SO2	Jean-Christophe Barbe	Field application for the production of sweets without SO2/ Compilation of existing tools in liquorice to do without SO2 and implementation of a protocol for making sweets without SO2



« Vins sans » partie chitosan



VIGNERONS BIO
NOUVELLE AQUITAINE

And on the estate

Winemaking without SO2

In red wine

- Acquired in Red Vinification and increasingly developed
- Bio-protection as a tool for implementation in the early years: reinsurance tools
- Majority use of early seeding in LSA of reception or on the harvest

The main principles:

- Managing a good, fast and frank alcoholic fermentation
- Management of a good rapid and frank malolactic fermentation co-inoculation is practiced but is not majority

This includes

- Good temperature control
- Good management of the nitrogen nutrition of these wines
- Healthy and not too advanced harvests in maturity (technological and phenolic)
- Good oxygenation of fermenting juices

It is best not to start removing SO2 at the same time making native fermentations.
It is better to proceed in stages

In White Wine

- Many trials among winemakers still in progress
- Difficulty mastering oxidation especially on Sauvignon
- Difficulty managing malolactic fermentation



VIGNERONS BIO
NOUVELLE AQUITAINE

Aging without SO2

- Bottled often early before the heat returns in the spring in March
- Filtering wines before entering the winter
- Important CO2 management
- Development of the use of new tools: Chitosan

For wines without long-aged SO2

- O2 and CO2 gas control: two schools for gas management
- need for temperature regulation of conservation cellars (caves or barrels)
- Enhanced microbiological control
- Management of rackings/and Microbean populations

Bottling

- Tangential filtering
- gas management at bottling (O2/CO2)
- Bottling under Azote



VIGNERONS BIO
NOUVELLE AQUITAINE

Labeling of WINES WITHOUT ADDED SULPHITES ETIQUETAGE

Allegation

"Wine without added sulphites"

"It may appear on the label of any wine, as long as the operator can prove that no sulphite was used in the manufacture of the product."

However, if sulphite levels are $> 10\text{mg/l}$ (SO_2T), the word "contains sulphites" is mandatory."

- OIV -

Yes

$0 \text{ SO}_2 \text{ Vinification} + \text{SO}_2 \text{ Total} < 10 \text{ mg/L}$

Yes

$0 \text{ SO}_2 \text{ Vinification} + \text{SO}_2 \text{ Total} > 10 \text{ mg/L}$
Mention: "Contains sulphites" mandatory

No

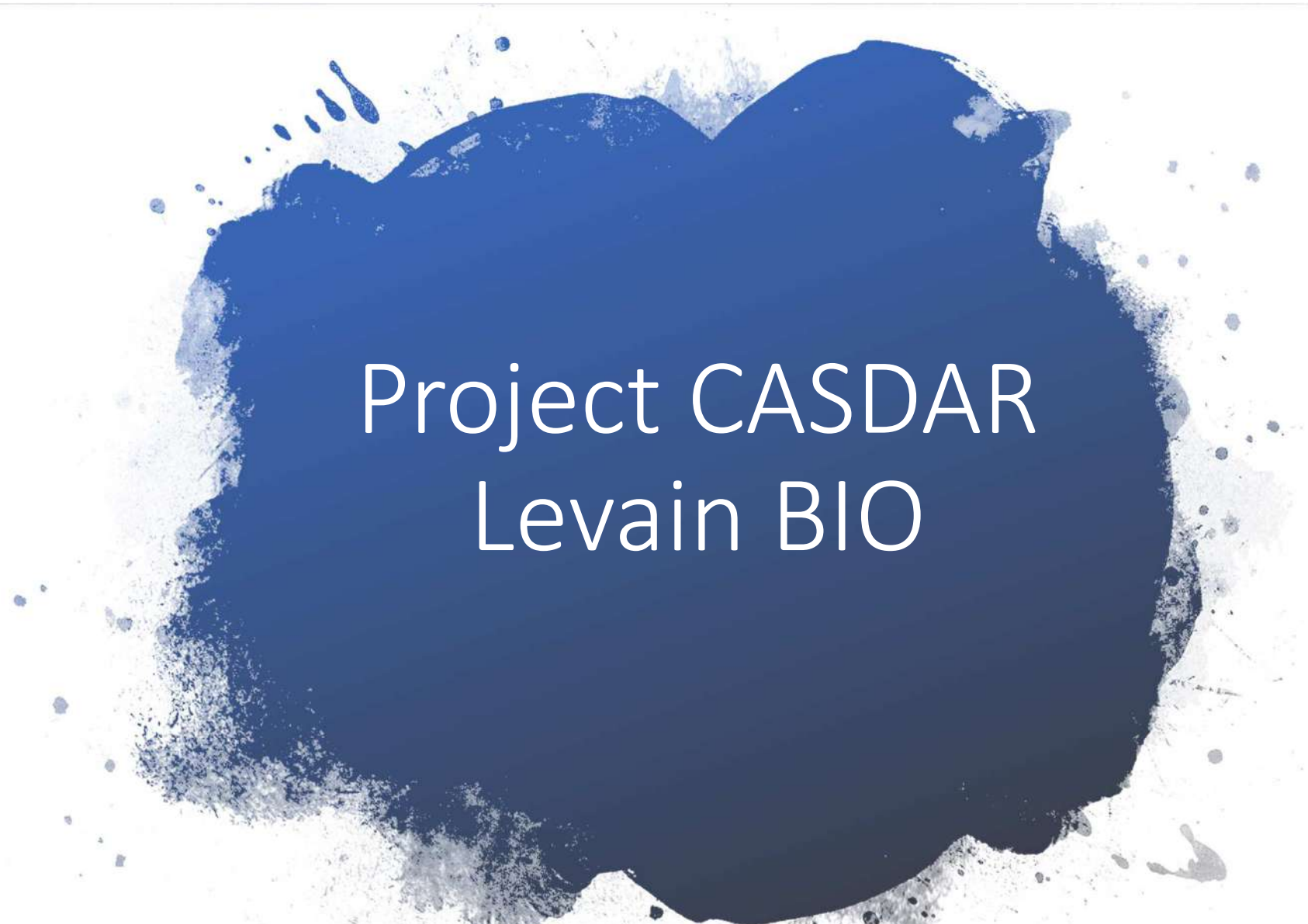
$\text{SO}_2 \text{ vinification but } \text{SO}_2 \text{ Total} < 10 \text{ mg/L}$

No

$\text{SO}_2 \text{ vinification } \text{SO}_2 \text{ Total} > 10 \text{ mg/L}$ Mention:
"Contains sulphites" mandatory

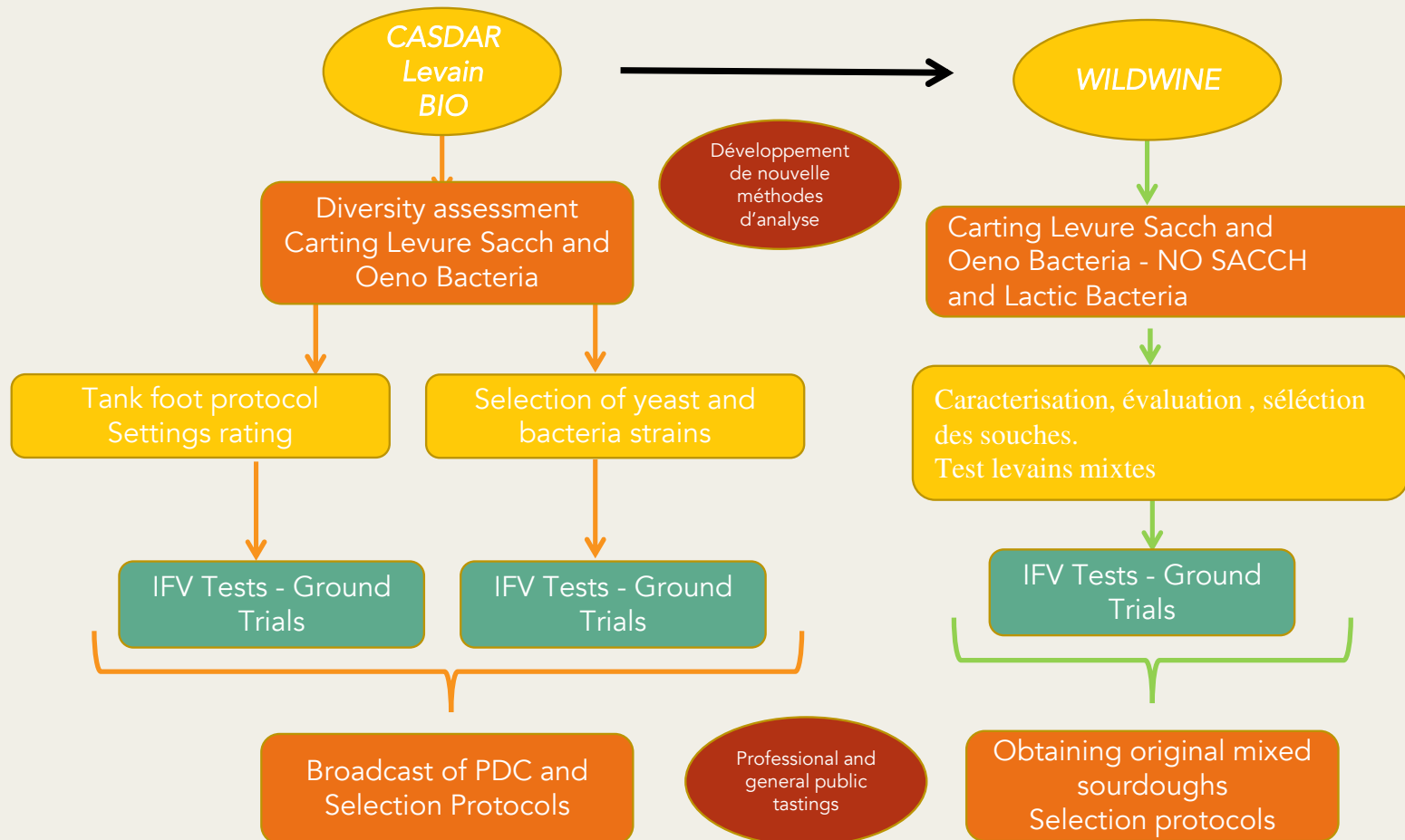
Rem : You have to do a specified analysis for wine without SO_2 like Frantz Paul.
The enzymatic analysis have to much uncertainty for low level of sulfite and the level detect will be higher than 10 mg/L

Indigenous fermentation



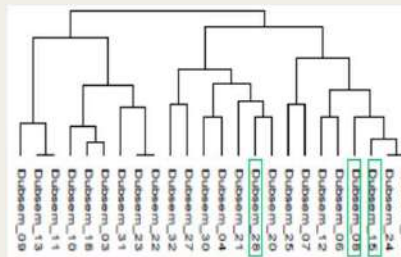
Project CASDAR Levain BIO

protocol



Selection of FA strains

method



ISV

IFV

Châteaux

In a castle:

- Diversity analysis
- Strain isolation

Synthetic Must Preselection

- Fermentary Kinetics
- Chemical analysis

Natural Must selection

- Fermentary Kinetics
- Chemical analysis
- H₂S test for yeasts
- Biogenic amines test for bacteria

Production of cream to test the strain in vat strain at the IFV

Production of cream to test the strain at the estate



VIGNERONS BIO
NOUVELLE AQUITAINE

CURRENT CONTEXT

problematic:

	benefits	Disadvantages
Commercial Yeast	-Quantitative and qualitative mastery -"pure guaranteed strain" -Quick and simple implementation	-Buying -Minimum dose to be respected
Spontaneous natives	-No purchase -Diversity of strains -Typicity	-Heavy implementation -Unknown native population -Possibility of unnecessary or harmful yeasts -Random success
PDC	-No purchase -Diversity of strains -Typicity	-Heavy implementation -Unknown native population -Possibility of unnecessary or harmful yeasts -Random success
Selected natives	-Physiological quality mastery -Better sourdough control	-Heavy implementation -Precaution to avoid contamination -Costly

————→ **Mastering tools**



VIGNERONS BIO
NOUVELLE AQUITAINE

CASDAR PROJECT « LEVAIN BIO »

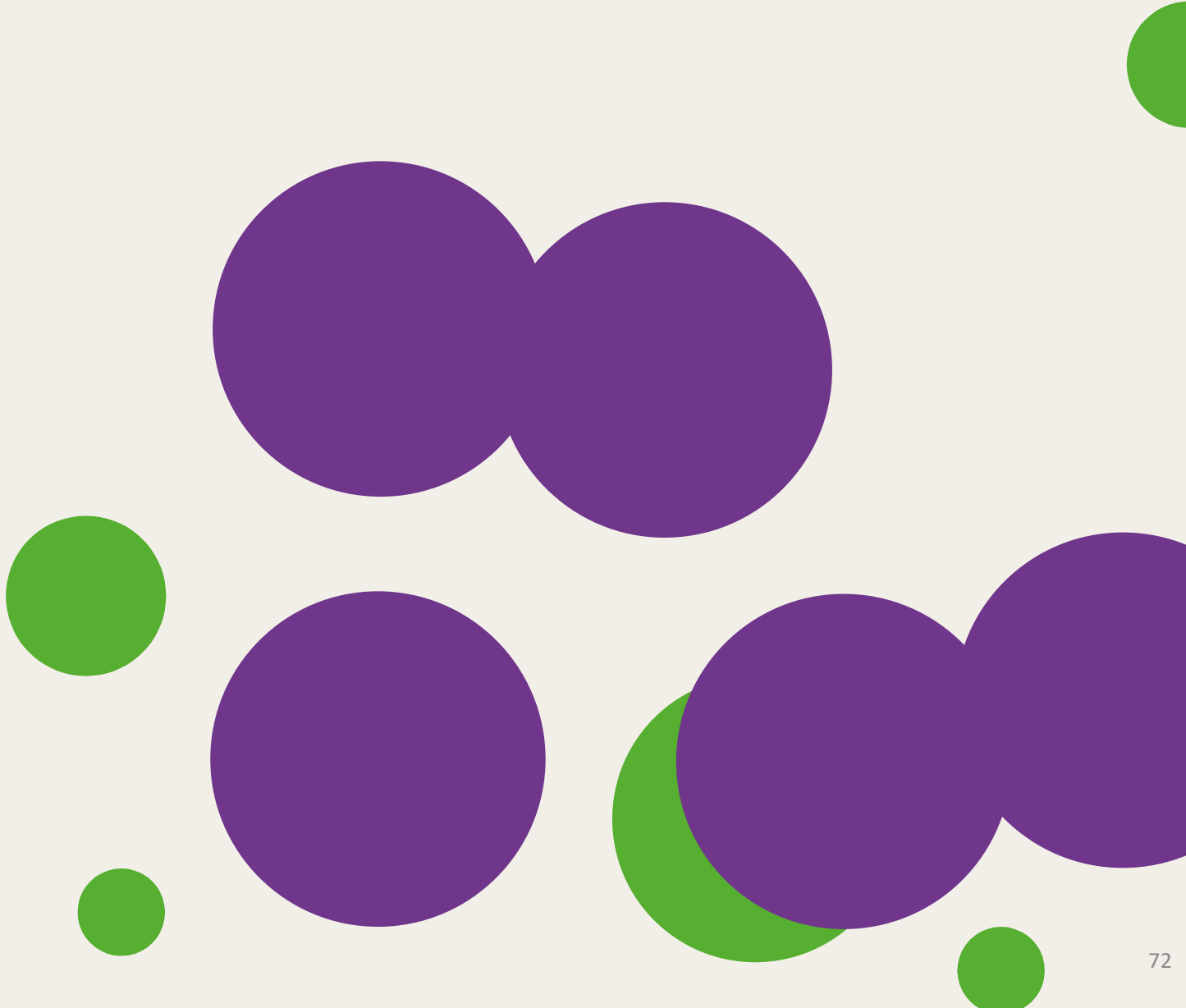


Improving the quality of organic wines and ciders obtained through the use of native yeasts and bacteria



Projet National

Diversity



VIGNERONS BIO
NOUVELLE AQUITAINE

Mapping the diversity of yeast and bacteria strains in Aquitaine

ACTION 1

Yeast

LIBOURNAIS

- 1-Château les Minauderies
- 2-Château Moulin de lagnet
- 3-Lycée Montagne
- 4-Château Bellevue
- 5-Château Dubourg
- 6-Château Franc Boudron
- 7-Château Lateyron
- 8-Château Brandeau
- 9-Châteaux des Rochers
- 10-Château Franc la fleur
- 11-Clos Puy Arnaud
- 12-Château Beynat

ENTRE DEUX MERS

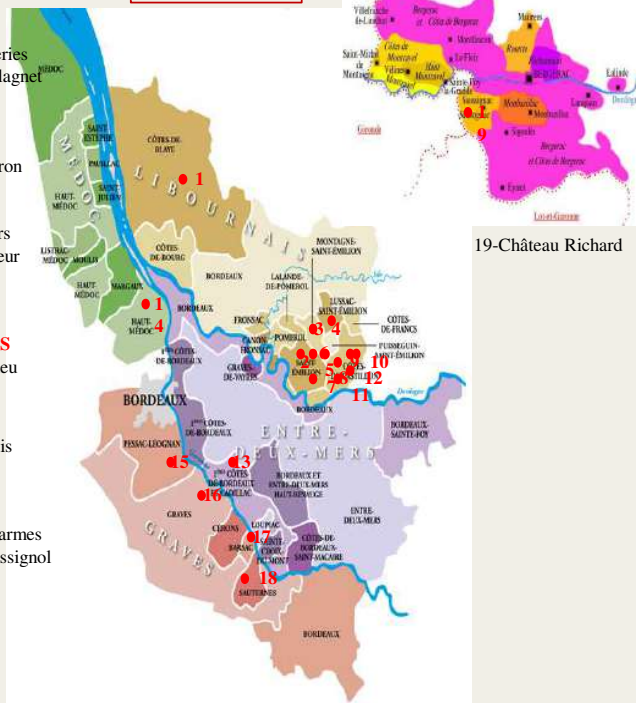
- 13-Château Du bourdieu

MEDOC

- 14-Closierie des Moussis

GRAVES

- 15-Château Baulos Charmes
- 16-Château Bichon Cassagnol
- 17-Château Climentis
- 18-Château Guiraud



19-Château Richard

Bacteria

LIBOURNAIS

- 1-Château de Piotere
- 2-Château Rioublanc
- 3-Château La croix de Roche
- 4-Château Annereaux
- 5-Châteaux Beauregard
- 6-Château Moulin de lagnet
- 7-Lycée Montagne
- 8-Château Bellevue
- 9-Château Laroque
- 10- Château d'Arcole
- 11-Château Beynat
- 12-Château Tour haut balette
- 13-Château Brandeau
- 14-Château La Peyrere OU LA CROIX

ENTRE DEUX MERS

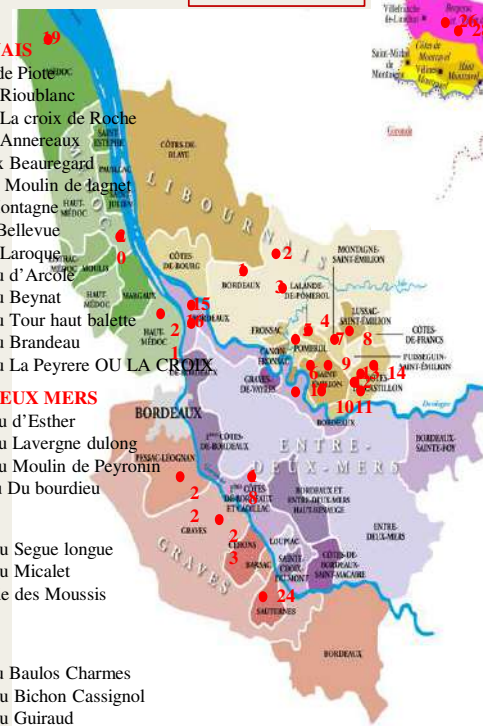
- 15- Château d'Esther
- 16-Château Lavergne dulong
- 17-Château Moulin de Peyronin
- 18-Château Du bourdieu

MEDOC

- 19-Château Segue longue
- 20-Château Micalet
- 21-Closierie des Moussis

GRAVES

- 22-Château Baulos Charmes
- 23-Château Bichon Cassagnol
- 24-Château Guiraud



- 25-Château Richard
- 26-Château Tuquet Monceau
- 27-Domaine ancienne cure
- 28-Château Grinou



VIGNERONS BIO
NOUVELLE AQUITAINE

ACTION 1

Genetic analysis and comparison of yeasts from regions and production sites :

PCR delta: Differentiating *Saccharomyces.cerevisiae* strains

Microsatellites: Genetic proximity of *Saccharomyces.cerevisiae* strains

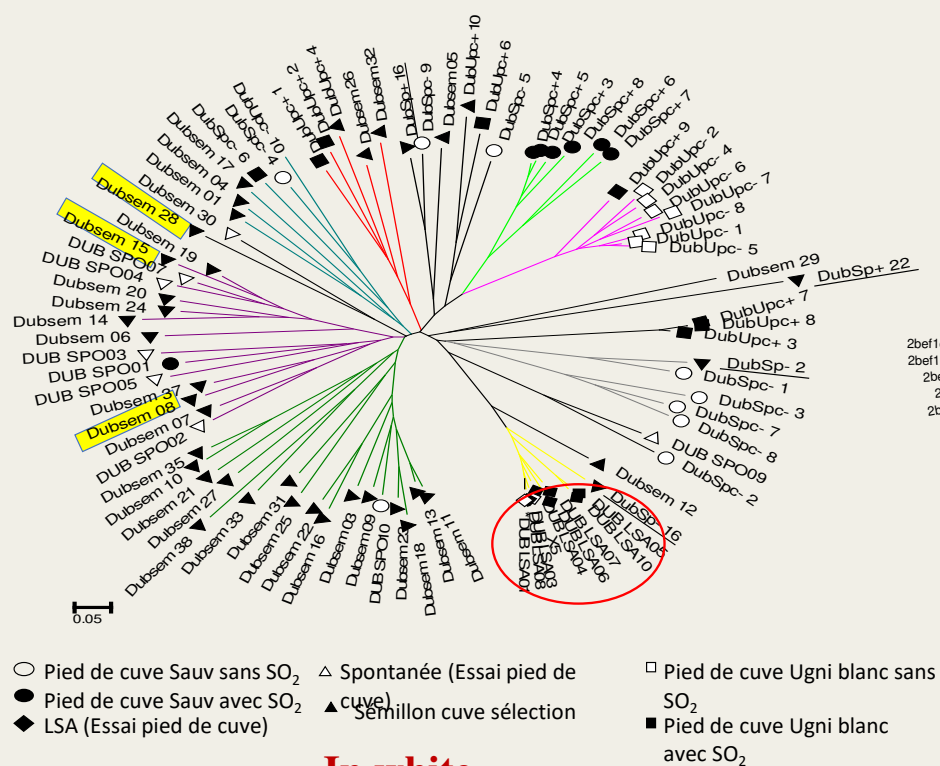
- 75% of the strains identified are of the species *S. cerevisiae* (287 clones)
- 5% to other yeasts of the genus *Saccharomyces*
- 20% to non-*Saccharomyces* species.



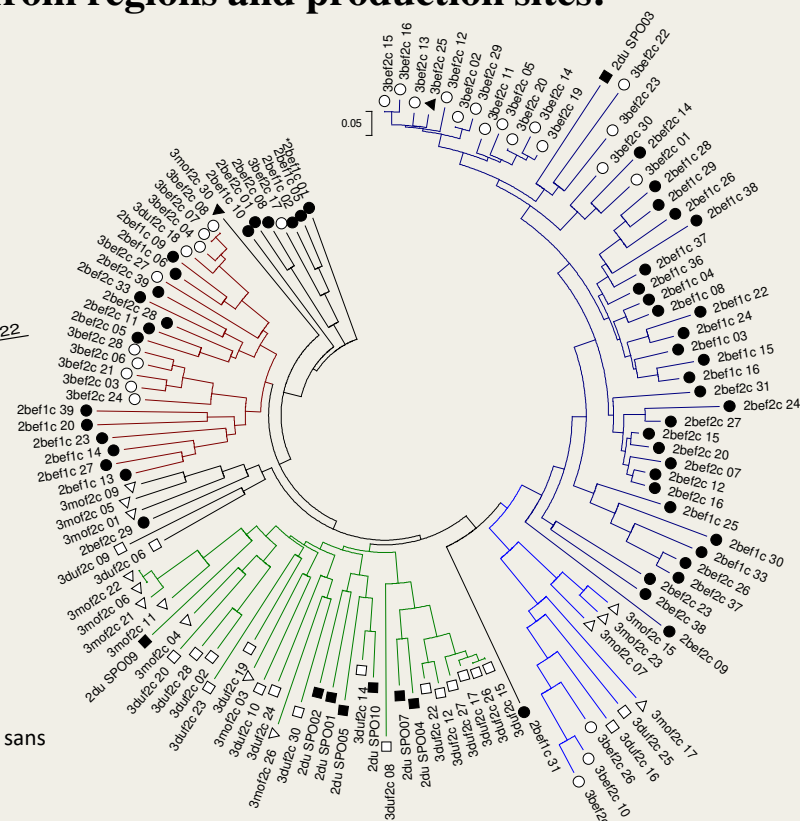
VIGNERONS BIO
NOUVELLE AQUITAINE

ACTION 1

Genetic analysis and comparison of yeasts from regions and production sites:



In white



In red

Isabelle Masneuf

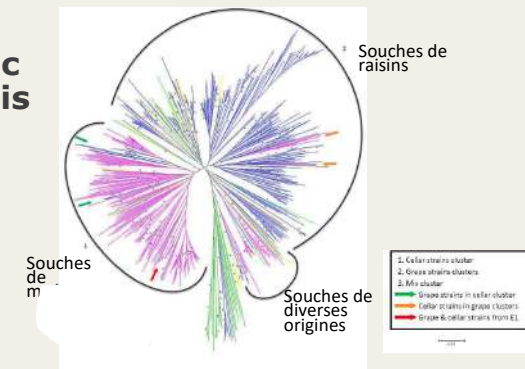
Saccharomyces cerevisiae Research: Biodiversity study



- **Sampling**

- New Aquitaine
- 26 farms
- 600 strains

- **Genetic analysis**



- **Very wide variety of strains (up 10,000)**
- **Separation of grape and chai populations**
- **No specific strain of region or estate**
- **But persistence of strains on several vintages in appellations and cellars**



VIGNERONS BIO
NOUVELLE AQUITAINE

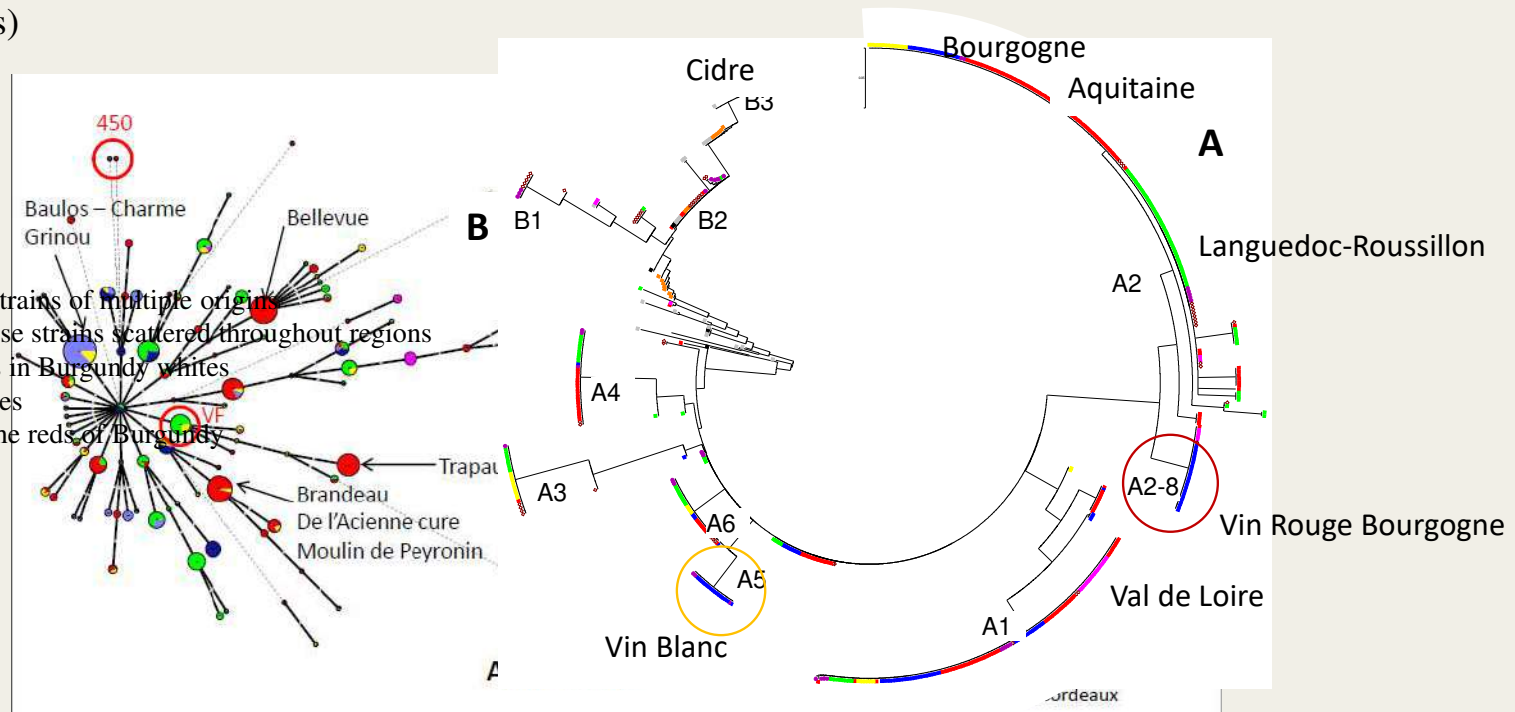
Genetic analysis and comparison of bacteria in regions and production sites

:

VNTR: Differentiates *Oenococcus oeni* strains

SNP: Genetic proximity of *Oenococcus oeni* strains (determine strains are specific, or not, of regions, farms or products)

In a region of strains of multiple origins
Genetically close strains scattered throughout regions
Specific strains in Burgundy whites
and champagnes
And partly in the reds of Burgundy

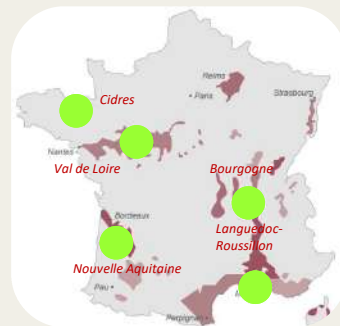


1072 colonies *Oenococcus oeni* and 200 strains *Oenococcus oeni*

Mariette El Khoury
Patrick Lucas



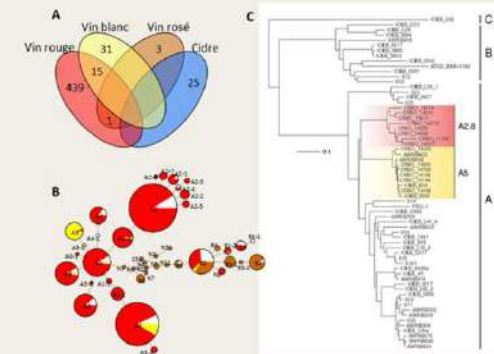
VIGNERONS BIO
NOUVELLE AQUITAINE



set of samples

5 regions
74 farms
235 wines and ciders
3000 bacteria

Genetic analysis



- **No regionally specific strains**
- **Nor exploitation**
- **But strains adapted to types of wines**
- **And strains persisting on farms**



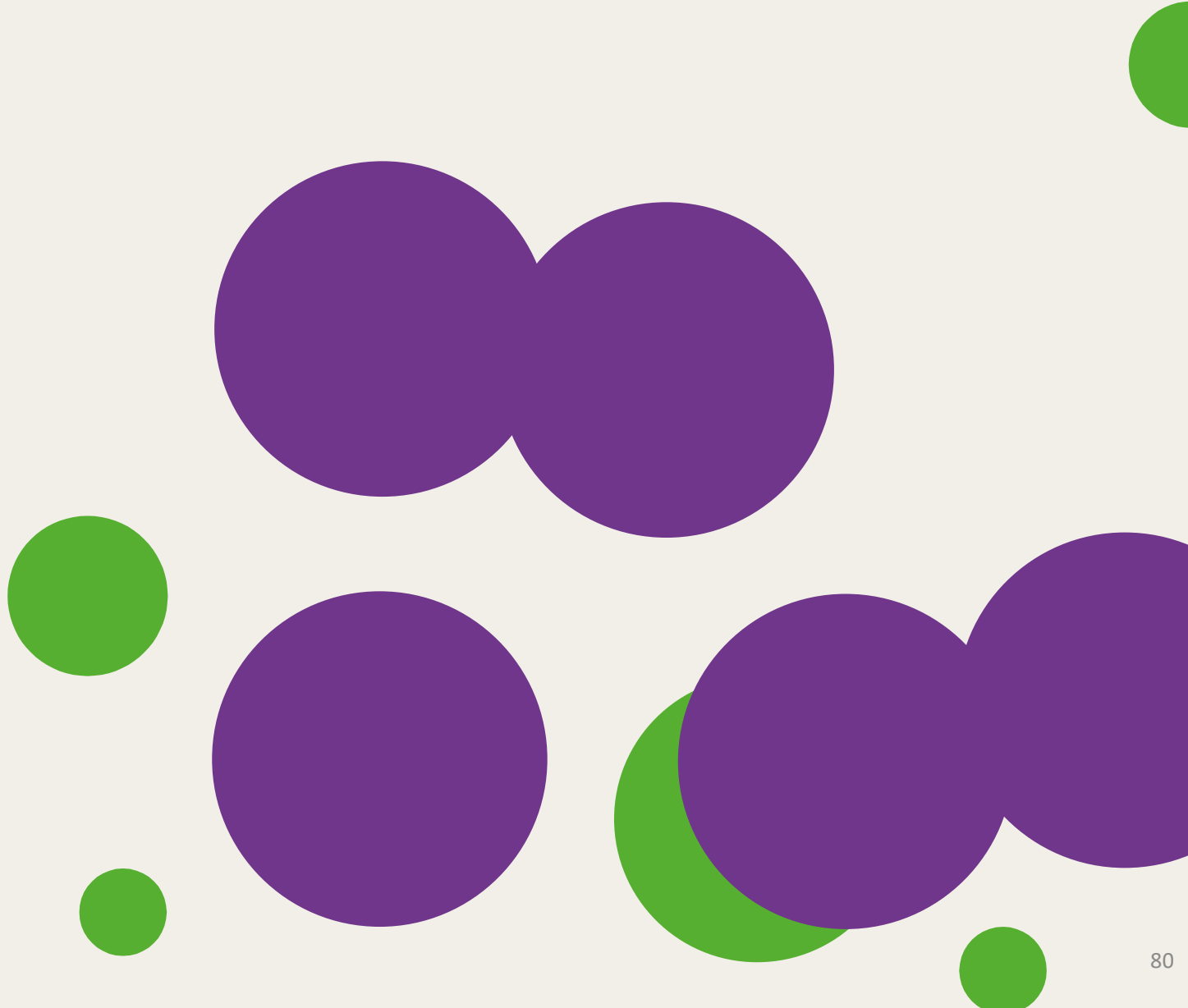
Conclusion

For each species, yeast strains and oenological bacteria:

- form a single genetic family (a single origin)
- their recent appearance and is related to human activity
- form genetic groups (sub-families) sometimes specific of products
- no specific groups of region (or exploitation), as they disperse
- no dominant strains, (no "invasion" of commercial strains)
- are very diverse (a few hundred or thousands of different strains per region)
- are rarely found in several regions
- without being "genetically specific" to a region, or a farm, they can sometimes appear "unique" to a region or exploitation (because they are very diverse)



Starter for Yeast



VIGNERONS BIO
NOUVELLE AQUITAINE

PDC

30L Or 50L

ISVV/IFV

Controlled
environments

2012

PDC: Merlot

Harvest: Merlot

Modality: -Liquid and Solid
-With SO2 and Sans SO2
-LSA Bio
-Spontaneous

2013

PDC: Sauvignon Blanc

Harvest: Sauvignon Blanc

Modality: -13C and 18C
-With O2 and No O2
-LSA F33
-Spontaneous

2014

PDC: Sauvignon Blanc

Harvest: Sauvignon Blanc

Modality: -T-18 degrees Celsius
-With O2 Or Without O2
-LSA F33
-Spontaneous

PDC: Merlot

Harvest: Merlot

Modality: -18C and 24C
-With O2 and No O2
-LSA F33
-Spontaneous

PDC: Merlot

Harvest: Merlot

Modality: -T-24degrees Celsius
-With O2 and No O2
-LSA F33
-Spontaneous

2,5hL

Estate

Ground

2012

PDC: Ugni Blanc and Sauvignon blanc

Harvest: Sauvignon gris

Modality: -With SO2 and Sans SO2
-LSA Bio
-Spontaneous



2013

No ground test

2014

PDC: Sauvignon Blanc

Harvest: Sauvignon Blanc

Modality: -LSA X5
-PDC

PDC: Merlot

Harvest: Merlot

Modality: -LSA F33
-PDC

PDC: Merlot

Harvest: Merlot

Modality: -
Spontaneous
-PDC

PDC: Merlot

Harvest: Merlot

Modality: -Be15
-Mixe

Conclusion

- Solid/Liquid - No difference → Choice - Liquid because it's more convenient to manage
- Picking grapes with maturity, healthy and not too acidic
- SO₂= No difference → But better control of microorganisms and promotes the development of *Saccharomyces.cerevisiae*
- Température → 18oC for whites
24oC for the Reds
- O₂ = No answer

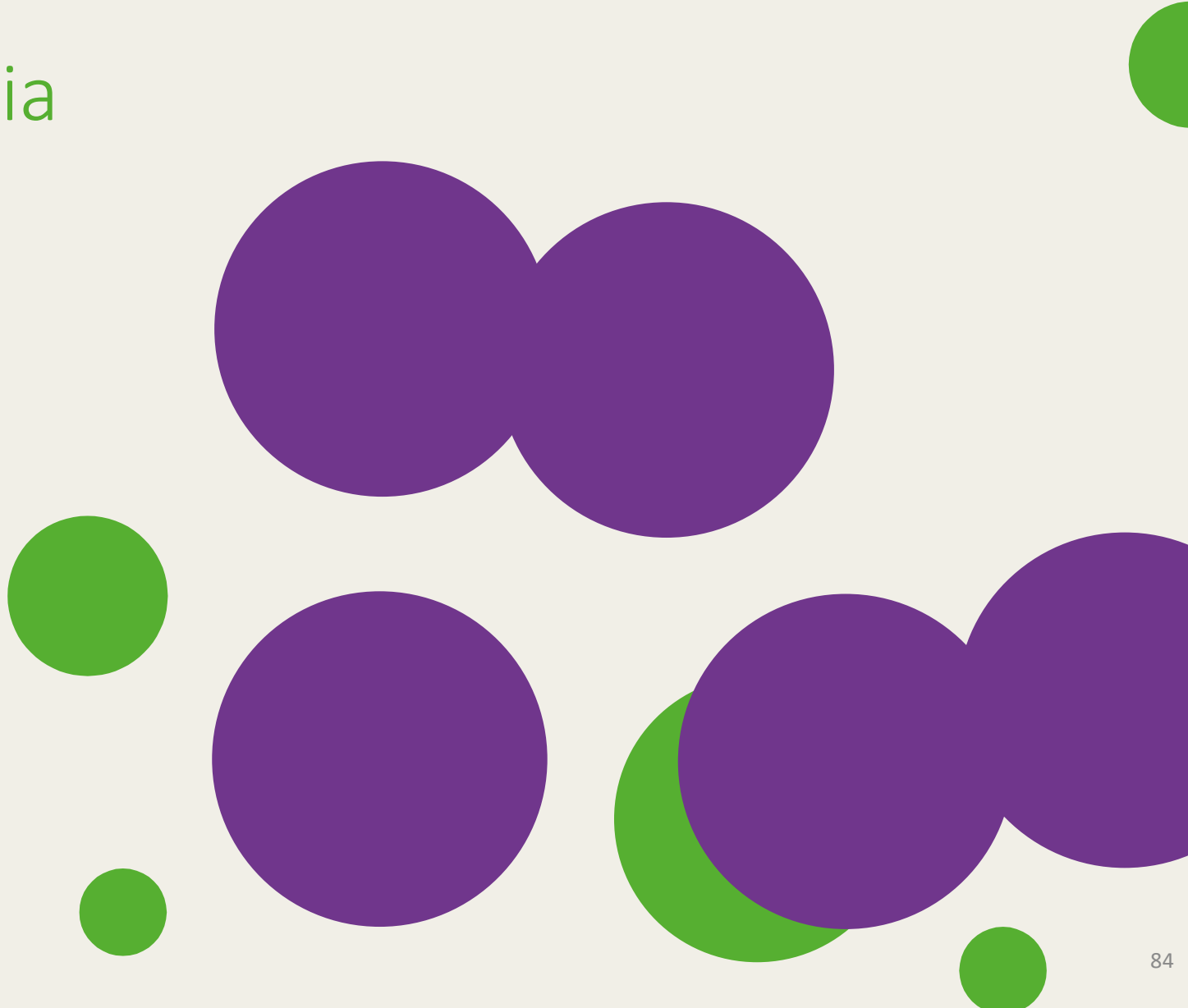
PDC Protocol



VIGNERONS BIO
NOUVELLE AQUITAINE

VIGNERONS BIO
NOUVELLE AQUITAINE

Starter for Bacteria



VIGNERONS BIO
NOUVELLE AQUITAINE

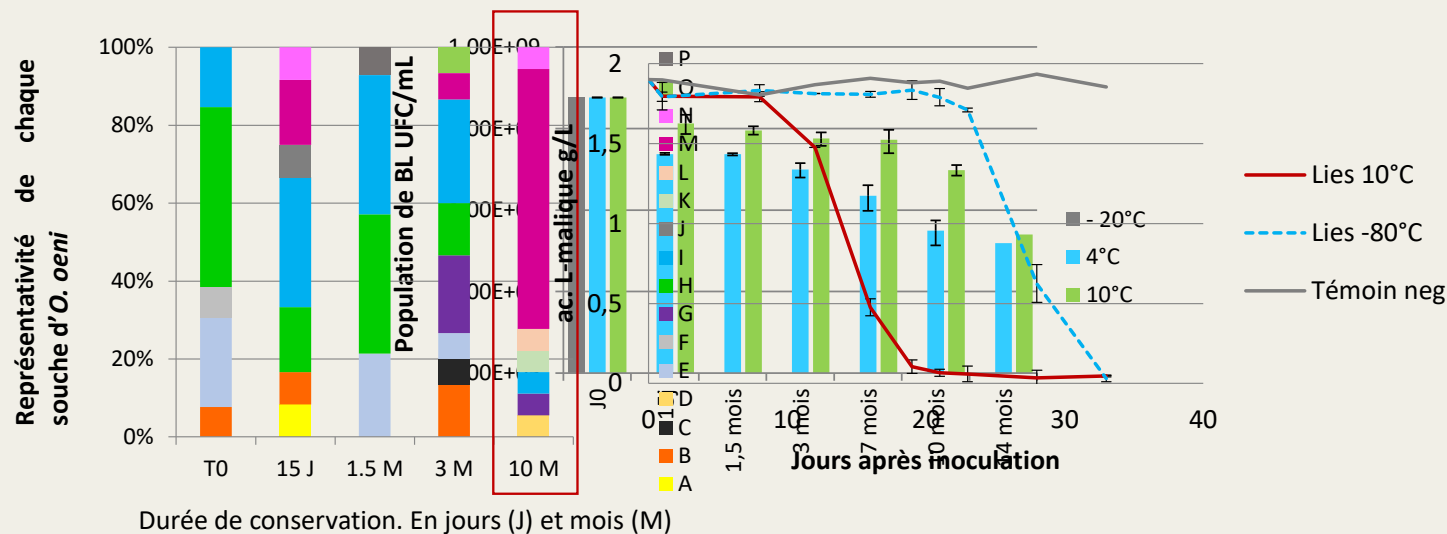
Develop FML tank foot preparation and control protocols

Conservation of lees

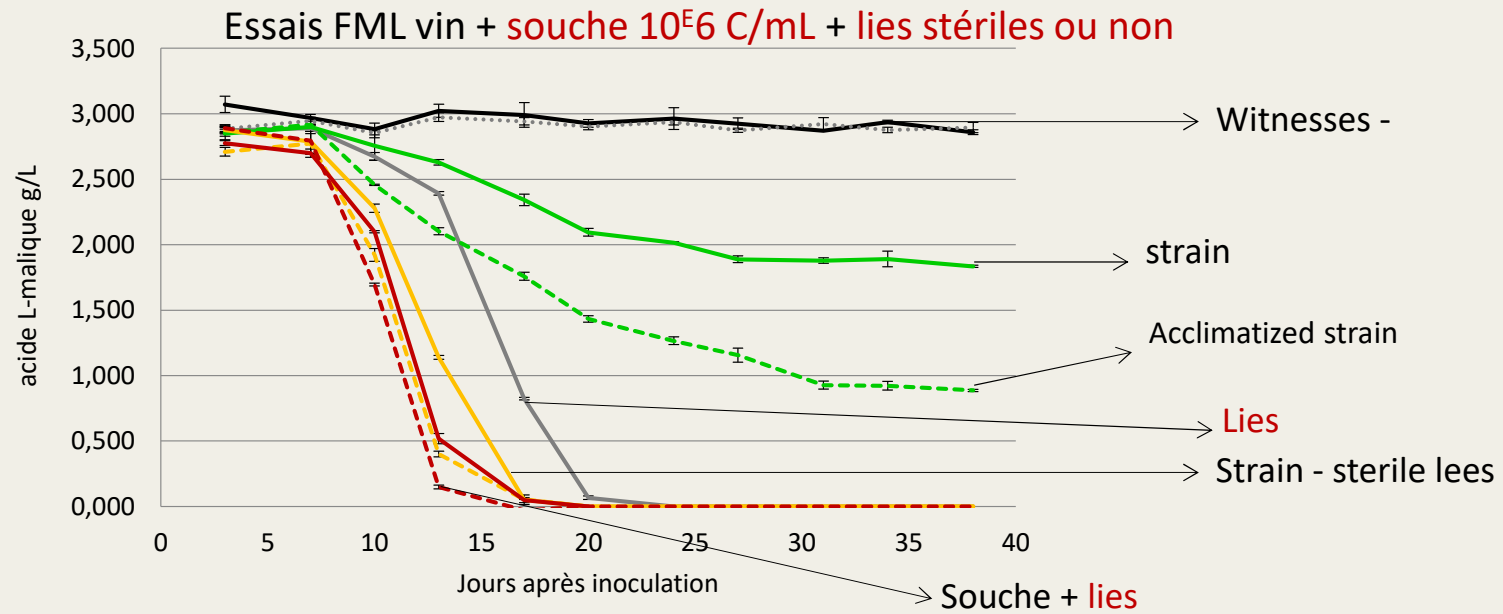
- Gradual disappearance of majority strains during conservation
- Detection of new strains

Keep the lees to use as PDC in year N+1

-Better survival of bacteria on lees at 10oC



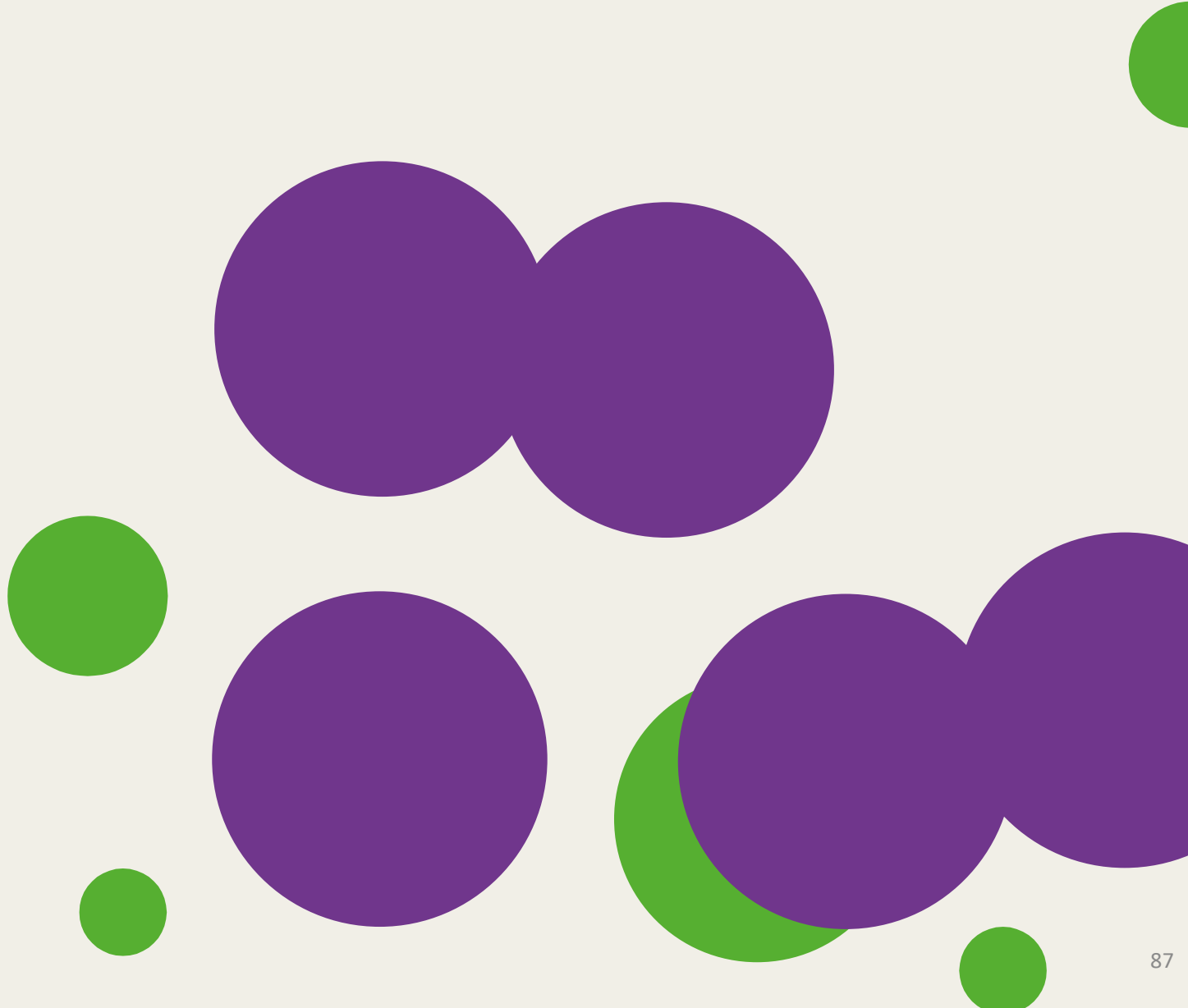
Relevance of FML's lees



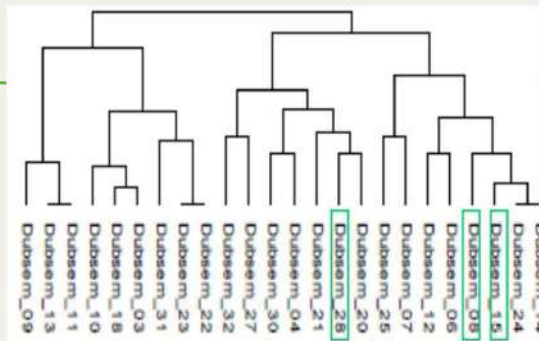
*FML only bad strain probably because of wine

*Strain - lees (sterile or not): FML - 15 days

Selection



VIGNERONS BIO
NOUVELLE AQUITAINE



SELECTION

STRAIN/CHATEAUX

Synthetic Must Preselection

- Fermentary Kinetics
- Chemical analysis

ISVV

Natural Must selection

- Fermentary Kinetics
- Chemical analysis
- H2S test for yeasts
- Biogenic amines test for bacteria

IFV

Production of cream to test the strain
in vats at the IFV

Châteaux

Production of cream to test the strain
at the property



VIGNERONS BIO
NOUVELLE AQUITAINE

Tools for winegrowers



Sélection de Levures Indigènes

Processus et Étapes Clés

Principe et Intérêt

Principe

Identification et Sélection

Identification et Sélection

La sélection de levures indigènes nécessite un milieu dures influençant entre la sélection et les coûts à la production. Cependant se débiter garanti par forcément l'obtention d'une souche possédant des caractéristiques fermentaires souhaitées.

	Avantages	Inconvénients
USA	- Méthode quantitative et qualitative - résultats par gramme à l'aise - Mise en œuvre rapide et simple	- Coût
Indigènes quantifiables	- Pas d'écart - Diversité de souches - Typique	- Mise en œuvre longue - Population microbienne incertaine - Possibilité de venime (inhibiteurs ou inhibiteurs) - Succès aléatoire
FDC	- Pas d'écart - Diversité de souches - Typique	- Mise en œuvre longue - Population microbienne incertaine - Possibilité de levures pathogènes ou toxiques - Risque d'arrêt de fermentation
Indigènes non quantifiables	- Méthode de clonage de la fermentation Alcoolique - Meilleur contrôle des levures - Souche potentiellement plus puissante	- Sélection difficile et aléatoire - Précaution pour éviter contamination - Coûteux

Le succès à la fermentation indigène est en plus un travail et demande pour certains professionnels, un ajustement de coûts. Cependant indigène est souvent de difficile à faire et les levures indigènes, souvent souvent se voient de roulement des fermentations à être envisagées.

La sélection qui s'offre à nous est la sélection des levures sur leur domaine.

- Sélection des levures naturellement présentes dans ou à proximité des usines comme levain
- Cette sélection permet de maîtriser et d'assurer une fermentation alcoolique complète.

Identification et différenciation des souches.

Séparation parmi les levures isolées par des méthodes de biologie moléculaire (Electrophorèse sur Champ Pulsé, PCR In situ analyse de marqueurs Microsatellites).

Conclusion, sur le matériel ou le processus de culture souche d'une souche présente dans une souche

Comparaison des levures entre elles et sélection des levures les plus représentatives pour les tests

- il est important de vérifier l'absence de souche contaminante
- les levures sélectionnées utilisées au domaine, parmi les levures sélectionnées.

Le pré-traitement se réalise au stade de la fermentation, depuis l'ajout d'un 1022 pour le développement et l'éclatement des levures sur milieu nutritif gelé.

Le pré-traitement se réalise au stade de la fermentation, depuis l'ajout d'un 1022 pour le développement et l'éclatement des levures sur milieu nutritif gelé.

Le pré-traitement se réalise au stade de la fermentation, depuis l'ajout d'un 1022 pour le développement et l'éclatement des levures sur milieu nutritif gelé.

Le pré-traitement se réalise au stade de la fermentation, depuis l'ajout d'un 1022 pour le développement et l'éclatement des levures sur milieu nutritif gelé.

[illegible]



WILDWINE

Stade de la nouaison



Aureobasidium
Cryptococcus
Rhodotorula
Rhodosporidium...

Stade de la véraison



Aureobasidium
Cryptococcus
Rhodotorula
Rhodosporidium
Candida
Sporobolomyces...

Stade de la récolte



Aureobasidium
Cryptococcus
Rhodotorula
....
Candida
Pichia
Hanseniaspora
Metschnikowia
Torulaspora
Saccharomyces

Variable selon la maturité, les conditions du millésime

Population totale /Baie

Brettanomyces...

Evolution fungal community on cluster

Evolution of yeast population during Alcoholic fermentation

There is little tool at the time to discriminate against strains of the same species:

Methods that are difficult to implement, not very reproducible

No methods available to analyze genetic relationships in NS

Development of molecular markers for NS: The example of *Torulaspora delbrueckii*

Genome sequence available (Gordon et al, 2009)

Development of 8 microsatellite markers on 6/8 chromosomes

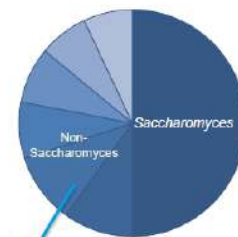
Application to a collection of strains from around the world and various substrates

Début de fermentation

Fin de fermentation

Population 10^4 à 10^6 cell/mL

Population 10^7 - 10^8 cell/mL



Hanseniaspora
Candida
Metschnikowia

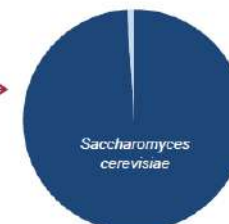
Majoritaires

Pichia
Torulaspora
Schizosaccharomyces
Issatchenkia
Brettanomyces...

Minoritaires

Diminution des populations des Non-Saccharomyces

Sensibilité au SO_2
Compétition substrats
Faible résistance à l'éthanol
Manque d'oxygène
Température élevée
Molécules toxiques



Saccharomyces cerevisiae
Hanseniaspora
Candida
Brettanomyces...

2 situations :

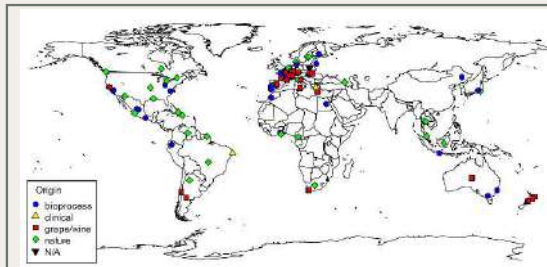
FA spontanée ou inoculée (LSA)



VIGNERONS BIO
NOUVELLE AQUITAINE

Non-Saccharomyces Yeasts Research: Biodiversity study

- *Torulaspora delbrueckii*
- *Hanseniaspora uvarum*
- *Metschnikovia pulcherima*
- *Candida zemplinina* ...

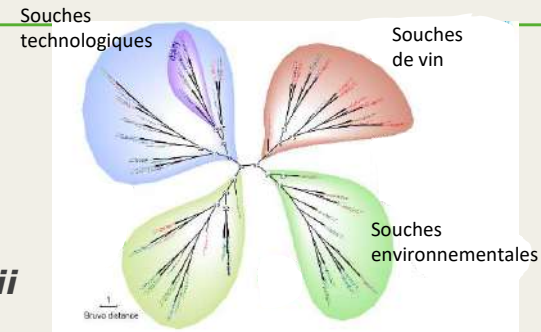


set of samples

- Depending on the species
- Global, regional, farms

Genetic diversity

T. delbrueckii



Grouping according to human activities
'Oenological' group - 1900years

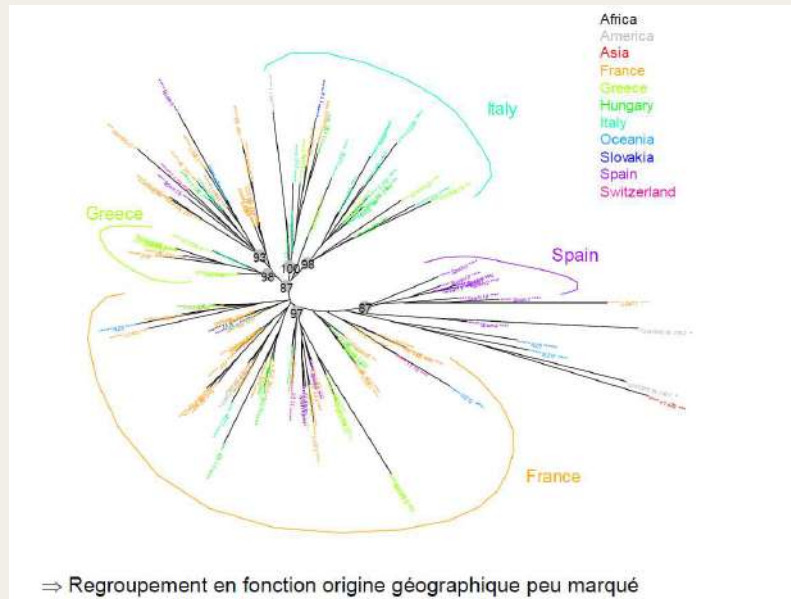
Technology properties

- Fermentary performance below *S.cerevisiae* slow FA and incomplete
- T. delbrueckii*: No AV production, H₂S: interesting application for the production of sweet wines
- Organoleptic potential



VIGNERONS BIO
NOUVELLE AQUITAINE

Candida Zemplanina



- * No substrate grouping
- * Grouping by low geographical origin
- * No 'genetic' signature of the winery

Technology properties

- Fermentary performance below *S.cerevisiae* Slow FA and incomplete
- Production of unwanted metabolites (AV , H₂S....)
- Low ethanol/sugar yield
- Fructophilia
- No industrial application possible



VIGNERONS BIO
NOUVELLE AQUITAINE

protocol



Saint Emillion

$5 \cdot 10^5$ cell/ml

T=0

$3 \cdot 10^5$ cell/ml

10^5 cell/ml

10^4 cell/ml

10^4 cell/ml

Hanseniaspora uvarum

Candida zemplinina

Metschnikovia pullcherima

Trichosporon delbrueckii

Sauternes

$5 \cdot 10^5$ cell/ml



T=0

$2 \cdot 10^5$ cell/ml

$2 \cdot 10^5$ cell/ml

10^4 cell/ml

10^4 cell/ml

« Technological culture »

T=0

$5 \cdot 10^5$ cell/ml

S.cerevisiae

$5 \cdot 10^5$ cell/ml after 24 h

Same blend but without
H.uvarum and *C.zemplinina*

Non-Saccharomyces

only *T.delbrueckii*

at 10^7 cell/ml , t=0

« Pure culture »

$2 \cdot 10^6$ cell/ml

S.cerevisiae (Industrial and selected)

$2 \cdot 10^6$ cell/ml

Protocols

	AT IFV mircovinification	Estate	
	The different modalities		
Château Bellevue	Sc F33 Sc Be15 Sc Be15 + <i>Torulaspora delbrueckii</i> / <i>Metschnikowia pulcherrima</i>	« pied de cuve » /Spontaneous Fermentation Sc Be15 SC Be15 + <i>Torulaspora delbrueckii</i> / <i>Metschnikowia pulcherrima</i>	
Château Guiraud	Sc ST Sc ST + Td « Alpha » Sc 86 Sc 86 + Td 63	Indegenious fermentation Sc ST Sc43 Sc86	ST Sc ST+ Td Alpha Sc86 Sc86 + Td 63



VIGNERONS BIO
NOUVELLE AQUITAINE

Saint-Emillion



19/05/2015
VIGNERONS BIO
NOUVELLE AQUITAINE

WILDWINE

Château Bellevue



Merlot

Terms:

Spontaneous fermentation with « Pied De Cuve" x 2

S.cerevisiae selected Be 15 x2

S.cerevisiae selected Be 15 - *Torulaspora delbrueckii*/ *Metschnikowia pulcherrima* selected x2

- Harvest by hand
- Vinification 2 hl vat
- Temperature control (20-22 degrees Celsius to D-1000 and then 25 degrees Celsius).

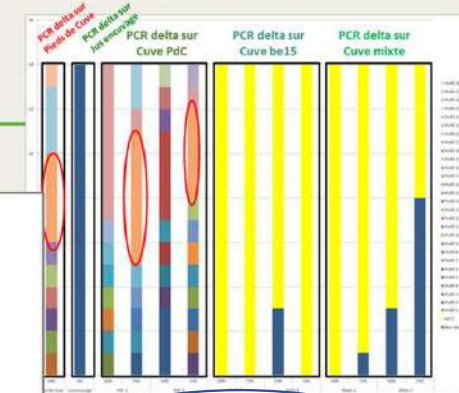
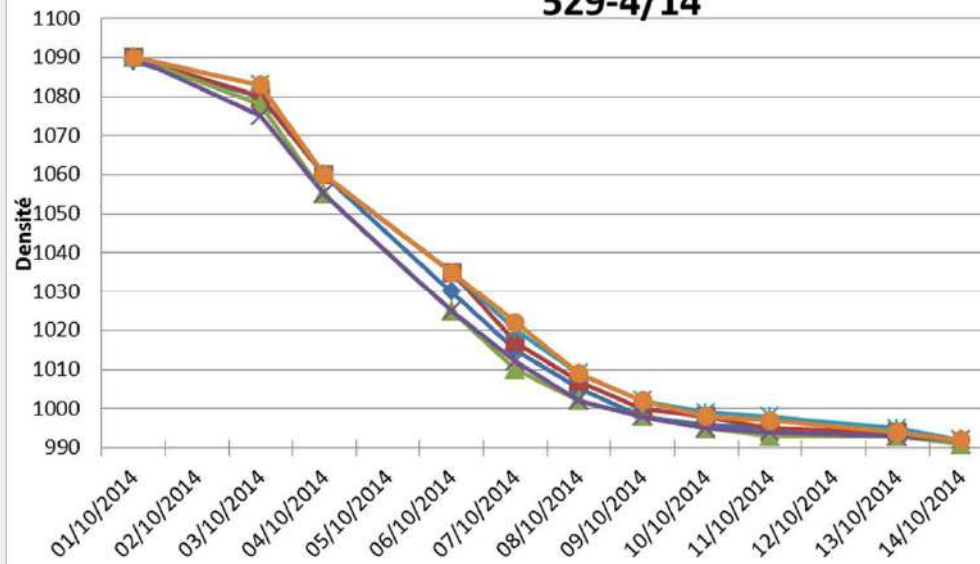


VIGNERONS BIO
NOUVELLE AQUITAINE

Nature de l'analyse	Modalités					
	PDC 1	PDC 2	Be 15 (1)	Be 15 (2)	Mix 1	Mix 2
TAV %vol	13	12.95	12.80	12.95	13.00	12.85
Acidité totale g/L H ₂ SO ₄	4.2	4.2	4.8	4.8	4.8	4.8
Acidité volatile g/L H ₂ SO ₄ (1)	0.27	0.26	0.24	0.23	0.24	0.24
pH	3.37	3.38	3.29	3.31	3.30	3.31
Glycéro l (g/L)	7.8	7.60	7.20	7.40	7.20	7.00



Suivi densité Essai Levain Bio Pôle Bordeaux Aquitaine - 2014 529-4/14

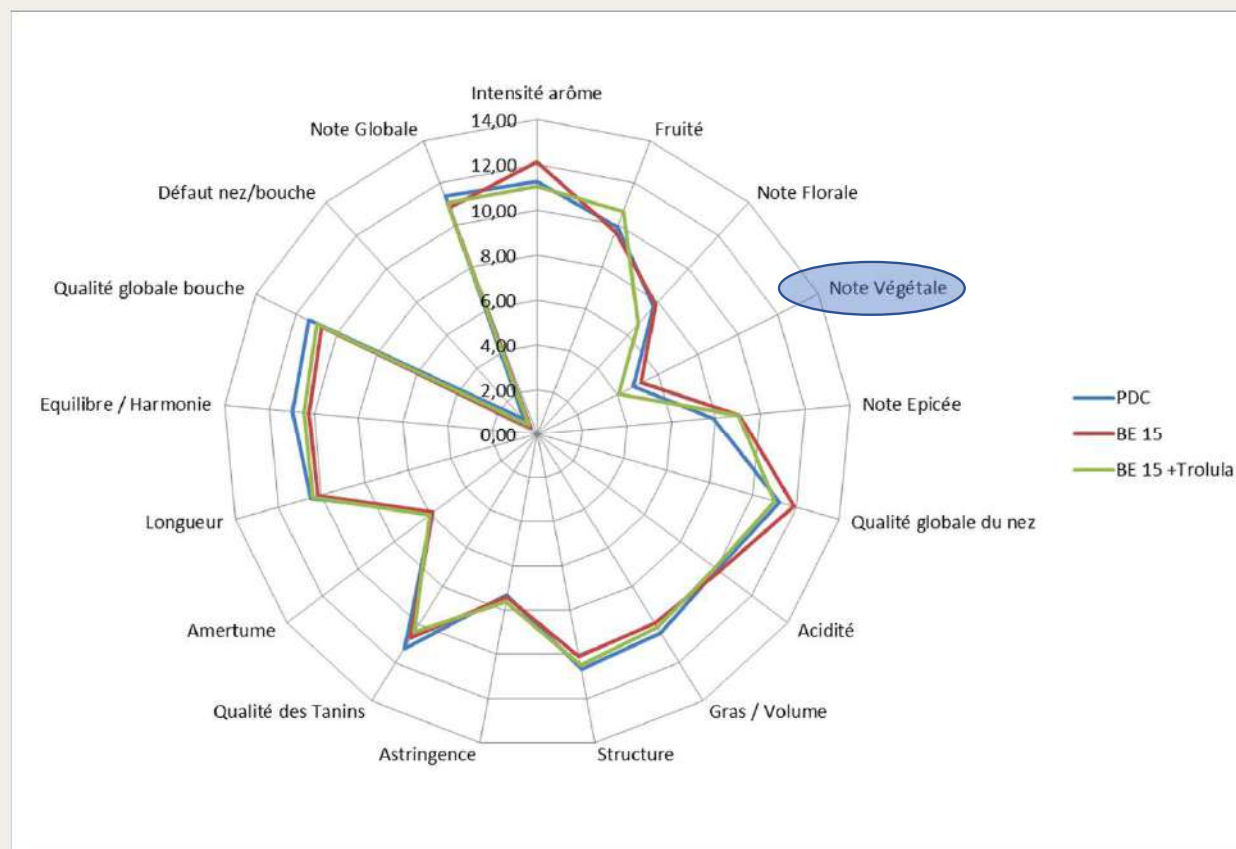


Implementation of
Positive *S.cerevisiae*

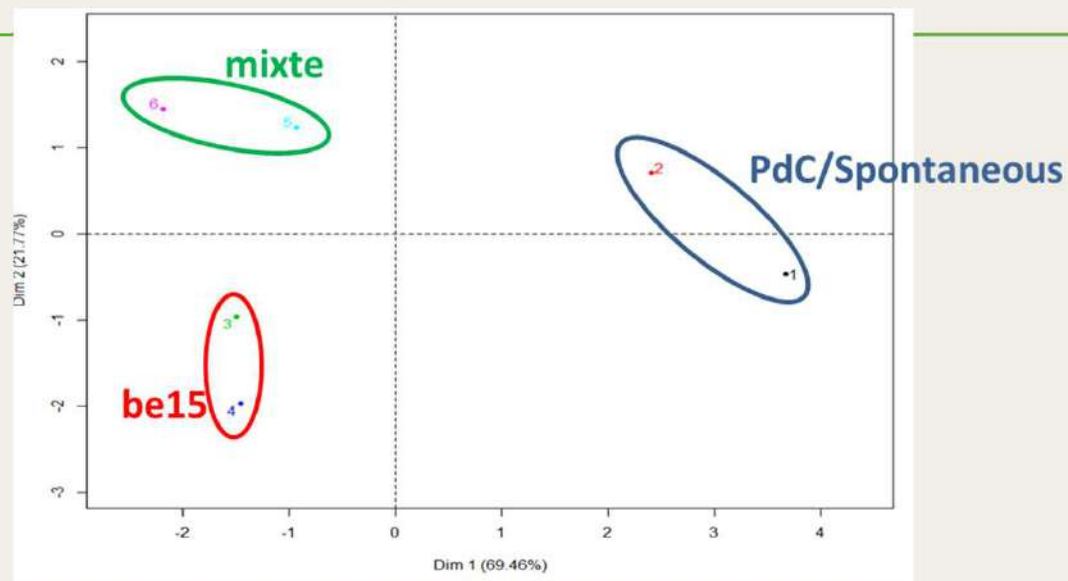


Tasting: IFV (professional tasters)

	PDC	BE 15	BE 15 +Trolula
Vegetal Aromas	4,78	5,17	4,06



Esters analysis



Test de Kruskal-Wallis							
	2-phényléthanol	Acétate d'isoamyle	Acétate de 2-phényléthyl	Décanoate déthyle	Hexanoate déthyle	Octanoate déthyle	Butanoate déthyle
PdC vs be15	>	>	>	NS	NS	NS	NS
PdC vs mixte	>	NS	>	NS	<	NS	NS
Be15 vs mixte	NS	<	NS	NS	NS	NS	NS

Tasting at the Paris Agriculture Show



VIGNERONS BIO
NOUVELLE AQUITAINE



Aquitaine
Blanc Sémillon

H O F O

Votre âge :

Modalités	Echelle de plaisir	Descripteurs (2/3 mots)
035	☹️ ————— 😊	
036	☹️ ————— 😊	



Même questionnaire

Château Bellevue Rouge modality IFV SALON DE L'AGRICULTURE

No significant differences.

Average :

CBR1 = 3.17 (+/-1.28)

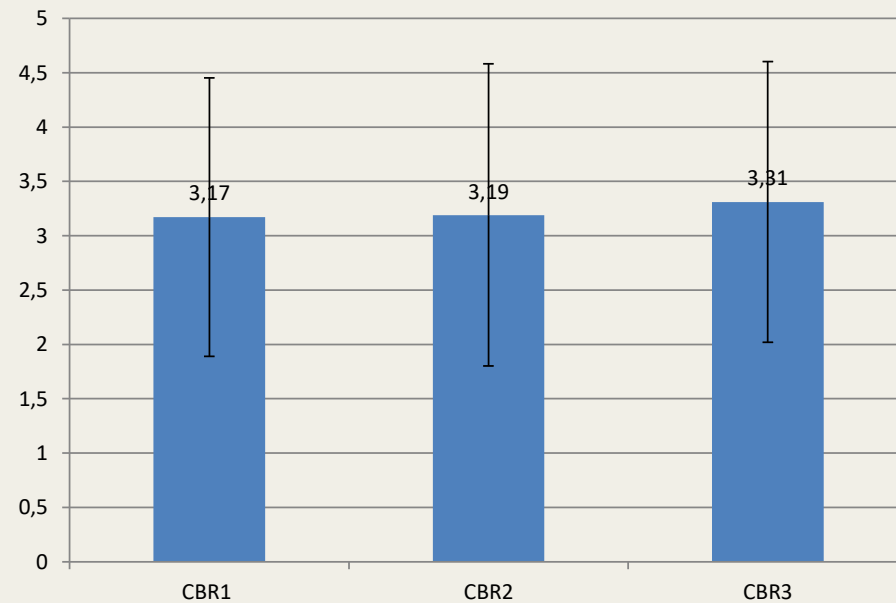
CBR2= 3.19 (+/- 1.39)

CBR3= 3.31 (+/- 1.29)

CBR1: LSA F33

CBR2: Souche Be 15

CBR3: Souche Be 15+ Torulaspora



VIGNERONS BIO
NOUVELLE AQUITAINE

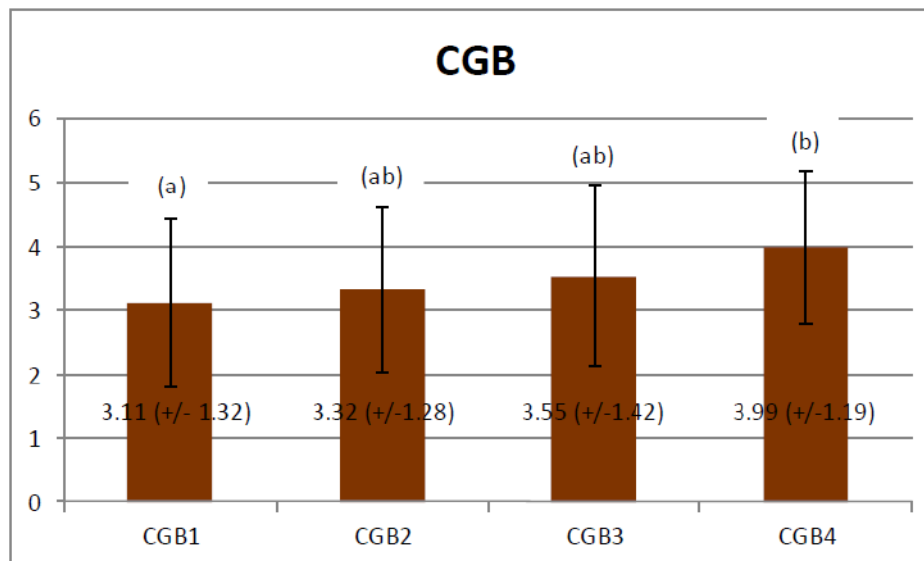
Tasting of ifv modalities at Château Guiraud

CGB 1 : LSA ST

CGB 2 : LSA ST+ *Torulaspora*

CGB 3 : Select Yeast (Sc 86)

CGB 4 : Select Yeast (Sc 86) + *Torulaspora*



The analysis shows a significant difference between modality with a preference for CGB4

Thanks for your attention



VIGNERONS BIO
NOUVELLE AQUITAINE

