Brettanomyces, or Management of a Contaminant Micro-organism

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New and useful information emanating from scientific research becomes available to the wine production industry every year. The contaminating yeast strain *Brettanomyces bruxellensis* has been the subject of in-depth studies for the last decade, and we now understand much more about its metabolism and the factors that encourage its development. The fact that this particular micro-organism has no negative health effects has regretfully meant that research funds are limited, with preference being given to studies of other micro-organisms.

Companies such as Bio-Rad have developed automatic analysers for detecting the DNA of these yeasts, and the research team at Microflora (experts in wine microbiology) have developed a unique database from which a calibration curve can be extracted and precisely quantified.

The Chamber of Agriculture with its seven OenoCentres plays a major role in accompanying and supporting wine production in the Gironde. Responsible for three million hectolitres, our group is logically at the forefront of risk prevention in general and the study of the risk of *Brettanomyces* in particular.

So why are we convinced that this problem must be vigorously and rapidly dealt with?

Brettanomyces produces contaminant molecules when its population reaches a sufficient density; these are volatile phenols. Present in numbers below the level of perception they partly mask the natural fruit aromas of the grape. Above this level they are identifiable by more or less unpleasant “off” smells, far removed from the fruit desired by the winemaker and his customers, the final consumer.

The objective of prevention is therefore to contain populations present in low quantities without affecting the integrity of the wine.

Analyses of bottled wine show that only 70% is totally exempt of contamination.

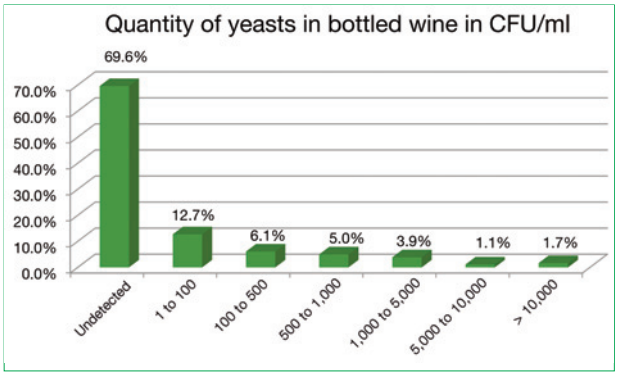

Figure 1: Quantities of contaminant yeast populations in bottled wine

Several types of contaminant yeasts can be found in grape must before the onset of alcoholic fermentation, but *Brettanomyces* is practically the only one that can survive in the ethanol levels found in wine.

What’s more, Professor Aline Lonvaud-Funel, expert in microbiology at the University of Bordeaux 2, analyzed wines from the beginning of the XXth century and found live *Brettanomyces* in these hundred year old wines.

So all bottled wine containing *Brettanomyces* cells that is not drunk whilst young runs the almost certain risk of showing volatile phenol characteristics when consumed.

Another example shows that a winery’s entire vintage production can rapidly attain high levels of volatile phenol pollution when good winemaking practices are not respected or fully mastered.

All the winery in µg/l	Minus one tank in µg/l
1.113 out of 9 tanks	653 out of 8 tanks

Figure 2: Average concentration in volatile phenols of 9 tanks two months after the beginning of harvest

By disregarding the most contaminated tank (which was at record levels), the average of the remaining 8 tanks still reached a level equivalent to 1.5 times the level of perception. It is clear that the task of winemaking here started with a large handicap.

Equally, proponents of spontaneous fermentations or approximate yeast dosages also run a certain risk.

Tanks	ABV	VA	G/F	Duration of AF	Brett
C1	13.50	0.24	0.0	20	
C2	13.64	0.23	0.0	20	
C3	13.82	0.24	0.0	20	
C4	12.54	0.20	0.3	11	
C5	13.99	0.26	8.2	5	1,100
C6	13.39	0.21	6.3	5	

Figure 3: Characteristics of wines from the same winery following spontaneous fermentation in 2009

For the 2009 vintage, this winery practicing spontaneous fermentation was spared the development of *Brettanomyces*. Luckily for them, since the transformation of sugars proved laborious on some tanks.

Tanks	ABV	VA	G/F	Date	Brett	Date	VP
C1	13.58	0.37	1.7	oct, 30			
C2	12.90	0.35	0.3	oct, 26	5,500,000	oct, 27	1,522
C3	13.04	0.18	0.0	oct, 26			
C4	13.37	0.33	0.1	oct, 26	5,400,000	oct, 27	2,539
C5	13.04	0.31	0.0	oct, 26	6,000,000	oct, 27	2,080
C6	13.09	0.21	0.3	oct, 30			

Figure 4: Characteristics of wines from the same winery following spontaneous fermentation in 2010

In 2010 however, the same winery practicing spontaneous fermentation was subject to heavy *Brettanomyces* contamination and certain tanks rapidly attained phenolic volatility.

Allowing indigenous populations of micro-organisms to initiate fermentation can give satisfactory results, but there is no guarantee of this. With no control the risks are considerable, and the producer must therefore assume full responsibility for such a choice, both philosophically and commercially.

At the other extreme, ignorance of the techniques of *Brettanomyces* management can lead to preventive or curative actions which are exaggerated and unnecessary.

	Tank A	Tank B
Number of *Brettanomyces*	90/ml	72/ml
Total E4P+E4G	192 µg/l	123 µg/l
Total/level of perception	45%	29%

Figure 5: Analyses of a wine before barrel ageing

The population of *Brettanomyces* yeast here is perfectly acceptable and manageable to achieve a satisfactory end result. In addition, the level of volatile phenols is far below the level of perception.

Nevertheless, in spite of this, the recommendation made to the producer was to finely filter before putting the wine in barrel. This quite unnecessary precaution would no doubt have stripped this young wine of its qualities. Eliminating all microbe populations long before bottling would have constituted a far greater risk of recontamination by disturbing the natural balance. Good management of the maturing process is quite sufficient to contain low populations without impoverishing the wine by unnecessary intervention.

It is for this reason that we consider it essential to regularly test for the presence of *Brettanomyces* as an aid to effective decision making. But the moment to carry out a microbiological analysis should not be chosen at random.

We have been looking at the phase of fermentation and the maceration which follows it. What level of population is acceptable at this stage? And what action should be envisaged for a tank full of a heterogeneous mixture of skins, pulp and juice?

It rapidly became apparent to us that the use of quantitive PCR before the end of alcoholic fermentation was too complicated. We consider it preferable to control fermentation in order to block the development of contaminating micro-organisms, beginning with the successful introduction of a selected yeast strain chosen for its ability to adapt to the conditions in situ.

	All grape varieties								
	Strain A	**Strain C**	**Strain D**	**Strain F**	**Strain G**	**Strain I**	**Strain J**	**Strain K**	**Strain L**
	Under 12.0								
No of tanks		1	3	1					
Average VA		0.17	0.12	0.16					
Average G/F		0.0	0.1	0.0					
Average duration		9	9	12					
	12.1 to 12.5								
No of tanks		1	4	2			3	1	
Average VA		0.19	0.15	0.14			0.19	0.11	
Average G/F		0.0	0.2	0.1			0.1	0.0	
Average duration		9	12	10			13	9	
	12.6 to 13.0								
No of tanks	2	7	5	3	3	1	6		1
Average VA	0.15	0.23	0.18	0.22	0.10	0.07	0.25		0.16
Average G/F	0.0	0.1	0.1	0.3	0.1	0.4	0.1		0.1
Average duration	13	9	12	**ND**	7	15	16		13
	13.1 to 13.5								
No of tanks	2	12	9	3	3	1	10		6
Average VA	0.07	0.27	0.19	0.25	0.11	0.20	0.27		0.24
Average G/F	0.0	0.1	0.2	0.6	0.1	0.1	0.2		0.1
Average duration	16	13	14	**ND**	17	9	17		14
	13.6 to 14.0								
No of tanks	2	23	12	5	6	6	2	1	2
Average VA	0.13	0.31	0.26	0.35	0.23	0.23	0.30	0.26	0.36
Average G/F	0.4	0.2	0.6	3.3	0.1	0.1	0.3	0.1	0.4
Average duration	15	14	24	**ND**	18	11	**ND**	14	15
	14.1 to 14.5								
No of tanks	1	26	7		3	3		9	4
Average VA	0.14	0.32	0.27		0.26	0.33		0.24	0.46
Average G/F	0.9	0.1	0.2		0.2	0.1		0.1	0.2
Average duration	17	13	28		9	**ND**		9	21
	14.6 to 15.0								
No of tanks	7	20	2		2			4	2
Average VA	0.19	0.36	0.32		0.36			0.27	0.51
Average G/F	0.1	0.4	0.6		1.4			0.1	0.3
Average duration	15	18	**ND**		28			12	30
	Over 15.1								
No of tanks	5	4						1	
Average VA	0.23	0.31						0.26	
Average G/F	0.1	1.0						0.1	
Average duration	17	24						16	

Figure 6: Relative performances of different selected yeast strains (evaluated from the study of 249 tanks)

This table demonstrates the variable results obtained from the different strains faced with the task of fermentation. Strain A shows itself to be well adapted to fermentation even when the must is heavy in sugars, whilst Strain F rapidly appears to be in difficulty even at average potential degrees.

Whilst it is essential to choose the right strain of yeast, it is just as important to give it the best possible conditions for fermentation. This begins with a correctly managed rehydration phase, given a little 'extra' by means of the addition of protective agents which help the yeast to resist the alcohol that it will encounter at the end of fermentation.

	Merlot		**Cabernet Sauvignon**	
	With	**Without**	**With**	**Without**
No of tanks	137	25	94	14
Percentage	85	15	87	13
Average ABV	14.27	13.97	13.28	13.33
Average VA	0.29	0.29	0.21	0.24
Average G/F	0.2	1.0	0.2	0.8
Average duration of AF	15	26	15	19

Figure 7: Impact of the addition of protective agents in the rehydration water of yeasts on their fermentation performance (evaluated from the study of 270 tanks)

First of all, we note that 85% of the tanks studied had used added protective agents. This is most encouraging and illustrates an increasing awareness of their usefulness.

The averages also clearly demonstrate the superior fermentation performances obtained by yeasts prepared with protective agents. The ensuing residual sugar levels are noticeably lower.

And finally, the timing of inoculation also has an influence on the success of the operation. Ideally yeast should be added to the first grapes going into the tank. Added too late, the indigenous populations have time to develop and the introduced yeast is prevented from developing, or develops poorly. In other words, it's simply a waste of money.

Tanks	ABV	VA	G/F	Duration of AF	Strain
C1	14.18	0.29	0.0	18	C
C2	14.73	0.11	0.0	17	A
C3	14.24	0.14	0.9	17	A
C4	14.60	0.41	0.0	13	C
C5	14.66	0.37	0.0	14	C
C6	15.18	0.16	0.0	18	A
C7	14.90	0.16	0.0	17	A
C8	15.23	0.17	0.0	16	C
C9	14.76	0.43	0.0	11	C
C10	15.04	0.18	0.1	15	A
C11	14.98	0.20	0.0	15	A
C12	15.78	0.25	0.1	14	A
C13	15.45	0.25	0.1	14	A
C14	13.47	ND	0.0	18	A
C15	14.02	0.06	0.6	19	A
C16	14.17	0.21	0.1	18	C

Figure 8: Results of alcoholic fermentation in a winery in 2009, with yeasts added at 13 g/hl with Strains A and C, rehydrated with protective agents, introduced into the tank with the first bin of grapes

Good cellar practices are the guarantee of correct fermentation control and the avoidance of the development of *Brettanomyces*, even in the presence of high sugar levels.

Tanks	ABV	VA	G/F	Date
C1	13.70	0.11	0.0	oct, 21
C2	15.00	0.13	0.0	oct, 25
C3	14.10	0.16	0.0	oct, 25
C4	14.76	0.14	0.0	oct, 25
C5	15.30	0.21	0.0	oct, 27
C6	15.24	0.14	0.0	oct, 27
C7	14.36	0.16	0.0	oct, 27
C8	15.24	0.18	0.0	oct, 30
C9	14.74	0.16	0.0	oct, 30
C10	15.03	0.20	0.3	nov, 04
C11	13.37	0.11	0.0	nov, 15
C12	12.73	0.11	0.0	nov, 15
C13	13.18	0.10	0.0	nov, 15
C14	13.48	0.11	0.0	nov, 15

Figure 9: Results of alcoholic fermentations of the same winery in 2010, yeasts added at 12 g/hl with Strain A rehydrated with protective agent, added to the tank with the first bin of grapes

The respect of these basic rules enabled total fermentation of high concentrations of sugars whilst producing little volatile acidity, and with perfectly controlled costs.

The phase of post fermentation maceration is also a vulnerable moment for the development of *Brettanomyces*, due to the absence of SO_2. It is therefore important to 'occupy the space' with the inoculation of a malolactic culture: the lactic bacteria thus present inhibiting the multiplication of undesirable micro-organisms. Once malolactic fermentation is complete and sulphites have been added microbiological stability is easier to maintain.

Before and during the wine maturing phase, microbiological analysis by quantitative PCR allows us to monitor the wine's *Brettanomyces* population and evaluate the risk to which it is exposed. Depending on the results, various different actions may be envisaged:

- Population is zero to low, and conditions are favourable to maintaining this level (pH, temperature): no intervention is necessary; maintain sufficient levels of active SO_2 until the next analysis.

- The concentration is equal to or exceeds 1,000 CFU/ml: it is preferable to take action to reduce the population to a controllable level, either:

- By racking: we have noted significant drops in population levels following a carefully executed racking. The wine must, of course, be protected from air during this process.
- By heat treatment: possible only if the population is not too high as the success rate of this procedure is not as good as one is led to think. Residual populations may therefore remain too great and require a second intervention, meaning also a second traumatism for the wine.
- By filtration: whatever the original population (on condition that the operator is informed of the level of course) filtration - particularly with a filter press - proves extraordinarily effective.

Brett by QPCR in CFU/ml	Filter press	Heat treatment
Before treatment	110,000	150,000
After treatment	440	9,300
Effectiveness	99.6%	93.8%
Taste	+	-

Figure 10: Comparative results of the effectiveness of filtration by filter press and of heat treatment on the same wine in the same winery in 2010

In this trial *Brettanomyces* in the tank treated by filter press descended to a level sufficiently acceptable for the wine to embark on its maturing period, whilst the tank treated by heat treatment still showed unacceptably high levels of yeasts after a lapse of time which should have been sufficient to kill all the affected cells. North American technicians, who consider heat treatment to be perfectly effective, were shocked by these results which they attributed to a lack of hygiene on the part of the operator.

Nonetheless, of the two batches matured separately and regularly blind tasted, the one that had been filtered was always preferred. So, for an equivalent cost, filtration offers numerous advantages compared to other methods.

Once the maturing phase is complete, the wine needs to be prepared in the appropriate manner for bottling. At this point it is absolutely essential to entirely eliminate all *Brettanomyces* to prevent development in the bottle if the wine is not to be consumed rapidly.

Filtration remains the most reliable method to eliminate a residual population before bottling, but it is important that the wine should not clog the filter as this causes pressure to increase with a resultant degradation of the wine's structure. As clogging is the only cause of wine impoverishment during filtration it is necessary to establish a protocol which allows the wine to be filtered at the required grade without increase in pressure.

To do this we need to incorporate two lots of data:

1. The grade of filtration that the wine needs: this is evaluated via microbiological analyses using epifluorescence microscopy and quantitative PCR.

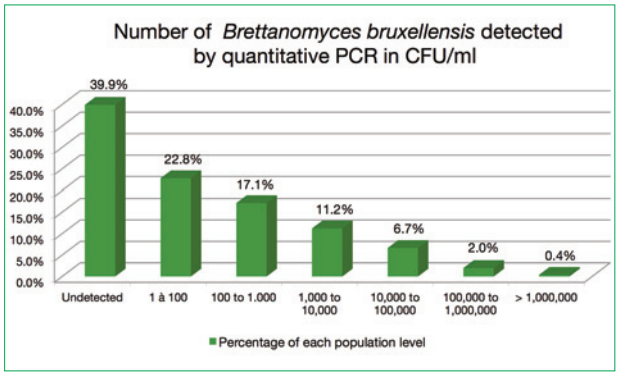

Figure 11: Populations of Brettanomyces measured by quantitative PCR before bottling

We note that only 40% of the wines analyzed are exempt of contaminating yeasts at this stage. All the other wines require treatment to eliminate populations of varying densities.

2. The grade of filtration that the wine is able to accept without clogging the filter: for this we use the CFLA (Coefficient of Filterability Lamothe-Abiet) developed by the company Lamothe-Abiet; this data allows a precise and reliable evaluation of the level of filterability of the wine in question.

Figure 12: CFLA apparatus

Currently we carry out 360 measurements annually by CFLA, and this continues to evolve.

These two elements (what grade of filter can my wine pass through, and what grade of filtration does it need?) are then taken into account when establishing the pre-bottling procedure; the objective being zero *Brettanomyces* in the bottle.

The validation by a quantitative PCR after bottling to confirm that the operation has been correctly carried out is the final element offering proof to the client that the risk of development of volatile phenol characteristics in his bottles has been eliminated.

What parameters need to be controlled during the maturing period to protect the wine from *Brettanomyces* ?

- Firstly, the level of active SO_2 is a barometer for the protection of wine against microbial attack. It evolves over time and must be adjusted by regular, reasoned doses of sulphites. When it drops below a level sufficient to inhibit *Brettanomyces* then these can multiply and develop out of all control of the winemaker.

- pH acts directly on the active SO_2 and when low proves to be an effective and easy method of inhibiting *Brettanomyces*, but makes protection difficult, if not impossible, when high, with a consequent risk of contamination. Assiduous work in the vineyard will, in the space of just a few years, produce well-balanced grapes with greater acidity at maturity resulting in wines that are more resistant to microbial attack.
- We are developing methods of measuring levels of dissolved oxygen throughout the winemaking process, since 1 mg of dissolved oxygen per litre of wine "consumes" 2.5 mg of SO_2. Dissolved oxygen levels can have an extremely detrimental effect on the future quality of wine when optimum winemaking techniques are not applied.

How to determine when your wine should be analyzed?

The oenologists from the Chamber of Agriculture and the OenoCentres have access to an interactive and progressive programme of aid to decision making which indicates when a particular batch of wine is in need of microbiological testing.

Wine Maturing - June

Risk limit for analysis: 3

Batch Identification	A	B	C	D	E	F	G	H
Previous risk noted	1	2	1	1	1	1	1	1
pH	3.66	3.65	3.63	3.59	3.59	3.57	3.68	3.64
Maturing Conditions								
In tank - non micro-oxygenated		o						
In tank - micro-oxygenated								
In new barrels	o		o		o	o	o	o
In used, disinfected barrels				o		o		o
In used, washed barrels								
Racking and other methods of airing								
Racking with airing (lees taken out)	o		o	o	o	o	o	o
Racking without airing (lees taken out)		o						
Cliqueur Oxygen injection (or airing without removing the lees)								
Clarifying treatments								
Filtration carried out ?								
Type of filtration								
Fining carried out ?								
Maximum temperatures in the winery								
Week 1	17	18	17	17	17	18	17	17
Week 2	17	18	17	17	17	18	17	17
Week 3	17	18	17	17	17	18	17	17
Week 4	17	18	17	17	17	18	17	17
Levels of active SO_2								
First 15 days	0.6	0.6	0.56	0.63	0.69	0.91	0.64	0.76
Second 15 days	0.6	0.6	0.56	0.63	0.69	0.91	0.64	0.76
Estimated risk	2	1	3	1	2	2	2	2
Microbiological count necessary	NON	NON	OUI	NON	NON	NON	NON	NON
Populations								
Brett population (cells/ml)			0.E+00					
Phenol characteristics								
Detectable at tasting								
Dosage of Ethyl phenols (µg/l)								
Risk noted for the following month	2	1	1	1	2	2	2	2

Figure 13: Extract from Brett'less®, a programme of aid to decision making relating to microbiological analysis

The data fed into Brett'less® during the winemaking period constantly updates the evaluation of risk and generates alerts which remain in place until subsequent analyses are satisfactory. Brett'less® is therefore an analytic reasoning tool and an aid to management of intervention on the wines.

In 2010 and again in 2011 we trialled a vinification strategy of non-addition of SO_2 at the moment of vatting in several pilot wineries. In the first instance the idea was to see if it was possible, without risk, to forgo this first dose of sulphites and to only add the first SO_2 following malolactic fermentation.

After having defined a protocol designed to compensate for the lack of the antiseptic and antioxidant qualities of SO_2 we proceeded with vinification right up to pre-maturing blending.

Quantitative PCRs carried out on these pilot wineries confirmed that the respect of fundamental winemaking rules in the absence of SO_2 effectively prevented the development of *Brettanomyces*.

Minimum population CFU/ml	Maximum population CFU/ml
85	350

Figure 14: Populations in blended wines before maturing (site 1)

Minimum population CFU/ml	Maximum population CFU/ml
41	170

Figure 15: Populations in blended wines before maturing (site 2)

Population CFU/ml
2,400

Figure 16: Population in blended wine before maturing (site 3)

The populations of *Brettanomyces* identified before barrel ageing or maturing in tank are perfectly tolerable. And, in the case of site 3, the transfer into barrels which followed the analysis acted in the same way as racking, and significantly reduced the population. Furthermore, the wine concerned had a very low level of pH which easily allowed the level of SO_2 to be maintained, thus creating an inhibiting effect on the contaminating yeasts. Once again, the best decisions are made only after reflection and reasoning in order to intervene uniquely when strictly necessary.

Also, a pleasant surprise the first year was confirmed in the second. The malolactic fermentations in all of the participating wineries in this experiment were rapid and passed without incident, when previously one of them had until then experienced problems in this area.

We can suppose that previously the SO_2 added before alcoholic fermentation created an inhibitory effect on the lactic bacteria, whether selected or indigenous, generating an additional risk of development of *Brettanomyces* by interfering with the cultures responsible for malolactic fermentation.

In the same way, certain tanks to which sulphites were added to compensate for less than perfect sanitary conditions during mechanical harvest showed signs of reduction during post fermentation maceration. The other tanks to which no SO_2 had been added showed, on the contrary, superbly fruity characters. This difference is almost certainly undetectable when all the tanks in a winery are treated in the same way.

One of the factors crucial to ensuring that microbiological analysis is a pertinent source of information and an effective management tool is the importance of the sample being representative of the batch tested. It is therefore primordial to respect the sampling procedure.

As an illustration, here are two examples of bad practice:

Population CFU/ml	Volatile phenols µg/l
Absence	Absence

Figure 17: Results of analyses in the month of January

At the beginning of the maturing phase, the analyses are perfect and show no contamination.

Population CFU/ml	Volatile phenols µg/l
Absence	700

Figure 18: Results of analyses from the same winery in the month of July

Six months later the wine shows volatile phenols, but still no *Brettanomyces*.

After these laboratory results had been challenged by the client (a perfectly reasonable thing to do), they were verified and confirmed as being correct. It was subsequent questioning of the sampling method that threw light on these apparently paradoxical results.

It transpired that the sample had been taken from the barrel from the bung hole using a pipette of around 30 cm in length. This meant that the sample came from the middle of the container, far from the bottom where the lees were situated.

But the maximum density of micro-organisms is obviously to be found at the lowest point, in the lees, whether in barrel or in tank.

In this particular case the yeasts present in the barrel were not detected in the January analysis since they were situated way below the level sampled. They then had plenty of time to propagate at the bottom of the barrel and constitute a sufficiently large population to create volatile phenols.

By July these had diffused throughout the wine and were detectable at analysis. The *Brettanomyces* however remained undetected since the sample had still been taken too high.

Similarly, 2 tanks in a different winery were analyzed after fermentation: the first because phenols were already detectable at tasting, and the second because its malolactic fermentation was stuck at a few hundreds of mg of malic acid/l from being finished.

Population CFU/ml
4.3 Millions

Figure 19: Results of quantitative PCR from the aromatically tainted tank

Population CFU/ml
1.9 Millions

Figure 20: Results of quantitative PCR from the stuck fermentation tank

In both cases the sample had been taken from the bottom of the tank after purging. The results of the analyses revealed, quite correctly, the highest density of population.

These rather alarming results led to a testing of the other tanks in the winery.

Population CFU/ml	Volatile phenols µg/l
From 1,100 to 15,000	From 33 to 3,943

Figure 21: Results of analyses of the other tanks

Some of these population levels could justify non-intervention. But such a difference in the two sets of results seemed curious to us.

Questioning of the operators involved revealed that the samples for the second lot of analyses were taken via the tap situated at 1.50 m from the bottom of the tanks. The true populations situated in the lees were therefore much higher, and justified an urgent filtration of all the wines in the winery.

So, the basic rules which must be respected when sampling for microbiological analysis are:

- Sample from the bottom of the container, at the lowest accessible point
- Purge taps thoroughly (several litres) before collecting the wine to analyze to avoid contamination by a non-representative liquid
- Use only new material or disinfected with alcohol then thoroughly drained to avoid sources of non-representative contamination
- In the case of heat treated wine or one to which sulphites have recently been added, wait at least two weeks before sampling. A test too near to treatment is likely to detect living cells which are in the course of dying, and so overestimate the population and resultant residual risk. The treatment must be allowed to act and accomplish its task before any new analyses are undertaken.

To conclude, regular microbiological analysis is today a precious tool for those who seek a still more reasoned approach to the treatment of wine.

Management of microbial populations varies and should be adapted to each stage in the life of the wine. The tolerance levels depend on the individual context.

Brett'less® is an invaluable aid to reasoned decision making concerning *Brettanomyces*. It permits intervention only when necessary and in an appropriate manner.

Quantitative PCR is a method of analysis which facilitates the management of *Brettanomyces*. It is the only method of microbiological analysis that specifically quantifies all the living cells of *Brettanomyces*, and uniquely its living cells. It has by far the lowest level of detection as it is sensitive to 2 cells/ml. Tests have shown that it systematically detects *Brettanomyces* in the samples where it is present and not in those where it is not present. That might sound obvious, but in fact it isn't the case for other more classic methods of microbiological analysis commonly used.

This is why we have been associated with this technique since its inception in oenological practice and contribute actively to its continued development. In just a few years we have become the major prescriber of this technique in the world – proof of the faith that we have in it, and we fully expect that it will continue to progress. The figures are proof of the growing awareness of the value of microbiology in the search for improved wine quality and the ever greater satisfaction of our clients, the consumers.

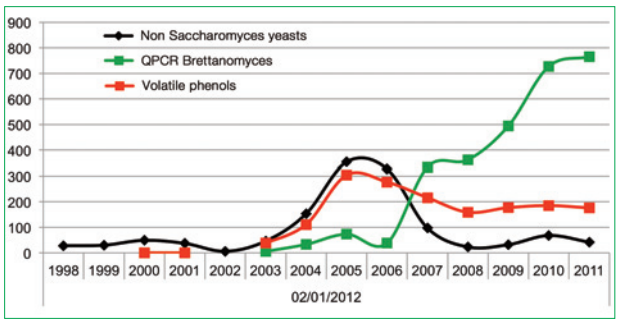

Figure 22: Evolution in the number of analyses over time

Finally, let us keep in mind these quotations from two talented and thoughtful winemakers:

- "Nothing more resembles a wine affected by reduction or *Brettanomyces* than another wine affected by reduction or *Brettanomyces*, whatever the distance between the vineyards or the aromatic profile of the grape varieties". (Philippe MATHIAS, Clos de l'Anhel)
- "Man is a creator in the heart of the system. For, by its very essence, wine is not natural, it is a human invention" (André Ostertag, Revue du Vin de France n°548)

Christophe Coupez
Chamber of Agriculture of the Gironde
Director of the OenoCentres Pauillac

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