BRETTANOMYCES BRUXELLENSIS OCCURRENCE, GROWTH, AND EFFECT ON WINE FLAVOR

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Brettanomyces bruxellensis can make some red wines smell like a horse blanket that has been stored in a metal Band-Aids® box. Any, or a combination of all of these flavors and aromas can be found in affected wines harboring this yeast. Dekkera/Brettanomyces has been known to inhabit many specialized environments including tree sap, dairies, breweries, and most notably the winery environment.¹

There are currently five accepted species of Dekkera/Brettanomyces: D. (B.) bruxellensis, B. custersianus, D. anomalus, B. naardenensis, and B. nanus.³ These genera were re-classified from multiple isolates after having been given various synonyms throughout the history of mycology. Conserved gene sequence analysis and traditional biochemical tests were recently used to re-classify this group.³

What is the difference between Brettanomyces and Dekkera? To answer this question, one should know a little about yeast sex.

Dekkera, which makes spores when times get tough, is the “perfect” sexual form of Brettanomyces, mycologically speaking. Thus, the reader will often see the name “Dekkera/Brettanomyces” in the literature to describe this duality.

Let’s just focus on the term Brettanomyces, since only a few fortunate people have been able to get the Dekkera form from a wine.¹ Brettanomyces bruxellensis has supposedly been isolated from a 10-year-old wine! Not surprising if you are a microbiologist, but winemakers bristle at the thought.

Amazingly, during the time that they are in barrels or bottles, Brettanomyces can ferment small amounts of sugar if given a small amount of oxygen. Supposedly, Brettanomyces grows actively in wine for a relatively short time, although, to our knowledge no basic research has been made evident to tests this hypothesis directly. “Brett” cannot actively grow forever.

However, there is a hypothesis that Brettanomyces (like many other microorganisms) can remain in a viable but non-culturable (VNC) state for long periods of time. In this state, cells that cannot be observed by standard culture methods continue to metabolize nutrients from their surroundings and are thus still considered alive. This is quite common for specialized microbes that are considered human pathogens and has recently been illustrated in wine bacteria.⁵

That being said, what about the B. bruxellensis isolated from 10-year old wine? Quite likely, they were either spores or they were VNC cells that were in a sort of environmental “stasis.” Either physiological state will theoretically remain viable for many years under the gentle storage conditions of a fine red wine. They emerge when cultured on rich media as normal colony forming units. That is, when they are signaled by good nutrition and suitable environmental conditions.

What is “Brett” doing in my wine?

Until recently, saying the word “Brett” in the company of winemakers was equivalent to making disparaging remarks about their parentage. Now, it seems winemakers are willing to discuss the issue less defensively. After all, there are some very highly regarded red wines, which are excellent examples of “Brettiness” by virtue of their specific aromas and flavors. Winemakers are learning to recognize these complex aromas and understand how they are formed in wine. With this new understanding of the ecology of Brett and the formation of off-flavors, it is hoped that winemakers will be better able to control formation of these flavors in their wines.

Most wineries have had experience trying to control Brettanomyces in their cellars. Several wineries we have worked with swore us to secrecy about their “contamination problem.” Some said that they had been “working with Brett” to make more complex wines, but now, it was just an overwhelming problem in some of their reds. One project in particular identified two Brettanomyces bruxellensis strains indigenous to the winery that alternated in three vintages."¹"³
Recently, the atmosphere surrounding Brettanomyces in the wine industry has undergone a change. Wine writers have been chiding California winemakers for being too “hygienic.” Some have gone so far as to say that Brett odors and flavors in wine are more desirable than not, as evidenced by the exorbitant amounts of money paid for bottles exhibiting the “classic character of a standard Bordeaux.” The debate has begun and winemakers we have worked with are not excited by the potential loss of fruitiness and varietal aromas to an easily amended issue.

### What is the history of Brettanomyces?

Brettanomyces species have a long anecdotal history that connects them to various foods. Thus, industry reports offer multiple names (and discoverers) for the same organism over the last 110 years.\(^1\) There are copious reviews in the literature describing the infection of various beverages, specifically, beer and wine containing a haze of yeast in bottles or finishing vessels and a malodorous quality (for red wines especially).\(^1\)

One author described the patented use of Brettanomyces for secondary fermentation of specialty beers. The name Brettanomyces was officially given to the “Torula-like” yeast that was characterized by N. Hiltje Clausen, in 1904, as that responsible for production of the “real type of English beers.”\(^2\)

Clausen’s story goes like this: British applications of a pure Saccharomyces yeast starter culture system completely ruined the flavor of English stock beers. Brewing chemists around the world wondered why the British could not get their act together and produce the same good quality stock ales with the new, improved single-yeast system.

Clausen proved experimentally that Brettanomyces was a secondary fermenter in cask, and thus, responsible for production of the typical English stock ale character. The beer is ironically described as “wine-like.” Interestingly, home brewers can purchase Brettanomyces bruxellensis (a.k.a. B. lambicus) strains from yeast starter companies for that same characteristic “vinous” expression of true English stock ales and Belgian lambics.

There are very few positive descriptors given to the individual compounds derived from Brettanomyces bruxellensis in red wine. Some of the wine-like qualities of specialty beers are most often described in red wines as “medicinal,” “animal,” “sweaty sock,” “barnyard,” “smoky,” “metallic,” “Bandaid®,” and “spicy.”\(^1,12\)

However, some descriptors like “clove,” “smoke,” and “spice” are extremely desirable in the right background of intense or forward “floral” and “fruity” aromas typical of Cabernet Sauvignon, Merlot or Cabernet Franc. In wines of lesser intensity (with less body and extraction), the “brettiness” can be overpowering, and indeed ruinous, to the final product’s sale value.\(^12,17\)

Here is another nebulous issue: Few people are able to smell every aspect of the typical Brett defects in a red wine. Some sense the purely “medicinal” odor of 4-ethylphenol and little of the “green apple” and “wet goat” aromas of valeric and isovaleric acids, each produced to various degrees.\(^1,13,17\) Still others sense an odor of “animal” and “barnyard.”\(^1,13\)

Few people experience the “mousy” or “bread crust” like aromas which are produced by the oxidation of volatile acetyl tetrahydropyridines in the mouth.\(^14,24\) It is important to note that the mousiness of a wine is probably less related to Brett than to other bacterial inhabitants but that it is possible.

Paul Henschke of the Australian Wine Research Institute adds: “In all our years of working with mousy wines, we have only been able to smell the [mousy] off-flavor in one catastrophic case, the red wine was incredibly volatile and off-scale for volatile phenols and other microbiological palate strippers as well.”

None of the above attributes could be considered positive in and of themselves. However, as stated previously, a complex and full wine background can be enhanced by small amounts of any singularly noxious odor if the context is correct.

This leads one to believe stories told by winemakers when they describe their many encounters with Brett. “It just seemed like the strain I had was doing good things to my wines and then maybe I got another strain last year, or something, and the whole lot began to stink.” Are there good or bad strains of B. bruxellensis?

After visiting with several winemakers at a “Mega-Brett” seminar sponsored by Vinquiry in Santa Rosa, CA, in 2001, it appeared that the industry has decided to explore the “good Brett vs. bad Brett” hypothesis.
Our lab and others are currently trying to characterize strains from around the world with several technologies, to see if there might be strains which give a wine desirable Brett aromas (smoky, spicy) aromas or simply bad ones.

**How does Brettanomyces do its business?**

Like any good survivor, Brettanomyces eats what it finds. As one might expect, the more selective the environment, the easier it is for a tolerant organism to compete successfully with its rivals for limited resources.

With Brettanomyces bruxellensis, small amounts of fermentable sugars (0.1 g/L) and high concentrations of ethanol (up to 14%) can act as fuel. A little bit of oxygen is also very good for growing Brett in wine. Brett can produce 2.0 g/L of acetic acid before growth is severely affected. This is why it is so difficult to predict what will happen to an unfiltered wine in the bottle, which may be complex at bottling, and horrendous one year later!

A major noxious odorant associated with “Brett” is 4-ethylphenol. There are at least 10 new odorant compounds which can deliver similar “plastic” aromas including 4-ethylguaiacol, a “smoky,” “spicy” odorant in some wines.

Production of 4-ethylphenol from p-coumaric acid is completed in a short series of steps. p-coumarate is degraded to 4-vinylphenol, a compound with a high odor threshold. It is not uncommon, even for Saccharomyces cerevisiae, to produce 4-vinylphenol during active fermentation, just not to the same extent as Brett. The final step, transforming the 4-vinylphenol form to the 4-ethylphenol form is unique (at least thus far in science) to Brettanomyces. This conversion is believed to occur continuously over several months, though nobody really knows the complete physiological picture (for review and reactions see Boulton, R., B. et al., 1996).

Observations in wineries and laboratories have shown that growth conditions, the concentrations of various cofactors, the numbers of yeast, and temperature in the contaminated vessel have a great deal to do with off-odor production.

There are two major hypotheses to explain why B. bruxellensis decarboxylates the 4-vinylphenol to the 4-ethylphenol form. One theory states that Brettanomyces derives some energy from the transformation in the form of a small electron gradient and thus formation of a small amount of useful ATP. We like this theory since Oenococcus oeni does the same thing with the conversion of malic to lactic acid and can derive the same benefit. One might theorize that a H+ ion gradient might develop and any excess may be “dumped” to gain ATP.

The second theory is that Brett is detoxifying the p-coumarate via decarboxylation and reduction to 4-vinylphenol. After all, if S. cerevisiae does it, why not Brettanomyces bruxellensis? There is nothing in the literature yet to support either claim. Thus, this question of “why” is something which needs to be looked into. The bottom line is that more basic physiological work needs to be done on as many strains as we can obtain through the system.

**Where is Dekkera/Brettanomyces found?**

Brett is ubiquitous. That is to say, anywhere in the world that you look for it, if you look hard enough, you will find it. In the vineyards, in water, in soil, in the grape must, in the cold room, fermentation tanks, on concrete floors, and in barrels.

Brettanomyces bruxellensis gets established in a winery when cellar hygiene is not under control, when SO2 is not used appropriately, when barrels or infected containers are neglected and allowed to absorb oxygen from the air. Brettanomyces are unique in that they utilize the “Custer’s effect,” a means of completing alcoholic fermentation in the presence of a small amount of oxygen.

We must not forget the fungistatic effect of SO2 on Brett. Maintaining 80ppm total SO2 (< pH 3.5) will keep Brett from growing. Also, temperature is critical. Growth is much faster above 20ºC and essentially stops below 8ºC. Filtration can physically eliminate Brett. Part of the larger problem of Brett wine is that some winemakers are following fashion and bottling wines without filtration.

Brett can be monitored, but not controlled. Barrels that are not monitored and topped up frequently are susceptible to blooms of Brettanomyces. As far as what to do about this, winemakers may want to consider what has happened with the European livestock industry.
When you go to Europe now, you may be asked to walk through a disinfectant shoe bath to prevent the spread of viruses and other potentially harmful organisms that you might have dragged in. The same should go for the winery, especially in the cellar. Think of where that visitor's feet have been and then ask him to bag his/her shoes.

When a hose is used to clean the floor of juice or spilled wine, aerosolized microbes travel through the air and onto the wall, barrel, or transfer hose next to it. They also travel when winemakers visit sites heavily contaminated without disinfecting their shoes and changing clothes before working with wine.

We are not suggesting that wineries should become clean rooms. However, a little advanced thought and precaution may help to lessen the incidence of contamination. One day, work in the winery, the next day, work anywhere you like, but do not enter the cellar after handling other equipment or visiting another winery or grapes without disinfecting their shoes and changing clothes before working with wine.

We are not suggesting that wineries should become clean rooms. However, a little advanced thought and precaution may help to lessen the incidence of contamination. One day, work in the winery, the next day, work anywhere you like, but do not enter the cellar after handling other equipment or visiting another winery or grapes without breaking the cycle for possible entry of organisms into the winemaking areas. Brett will come in on grapes and be killed by good vinification and sanitation practices. There is no need to introduce yeast from your friend’s winery to yours.

There are indications that Brett comes into the winery with the grapes. The fact that it is present in low numbers makes it difficult to isolate, but given enough time, a few are enough to grow to numbers large enough to cause problems. Likely more common is entry with wine or barrels from other wineries.

The most important issue is that Brett seems to bide its time waiting for a good time to emerge. A small population of Brett might be waiting in a barrel or somewhere else in the winery environment, waiting for the right conditions to grow!

If you are dealing with Brettanomyces infections in one or two barrels, isolate them and use different dedicated equipment (if possible) to work with them. If you can, filter the wine in the barrels and clean the barrels prior to dosing with sulfur. You may want to store the filtered wine in something else for a while and perhaps consider using it (after it is verified stable) for blending.

The work of Mark Sefton et al. has shown that oak barrels, which are infected with B. bruxellensis can not be effectively sterilized. Neither careful washing and rinsing with sulfited water, nor shaving and firing, or ozone treatment can sterilize a barrel. Due to the large internal volume and porous nature of the oak, not all Brett can be removed or killed.

NASA shares a problem similar to that of winemakers: when there is an incident in the middle of a mission (or vintage), it can be disastrous and unrecoverable. For this reason, engineers at NASA got together and developed the Hazard Analysis and Critical Control Points (HACCP) program. This is really a philosophy of checking and re-checking valuable points in a system to make sure that there is a recoverable solution, should something fail. Thus, once an infected barrel is discovered, it must be considered contaminated throughout its useful life in the winery. Wine inside the barrel should be filtered, sulfited, and tested for the absence of Brett prior to blending away or bottling. Taking a chance to blend away bad aromas is not the solution.

As winemakers struggle with weird contamination patterns, even in the presence of good cellar hygiene, locating the sources of contamination will be too late to be of use in most cases. Use of sterile plastic pipettes in lieu of a single thief would help to greatly reduce the chance for Brettanomycoses inoculation.

Sampling equipment can be sterilized by boiling for 15 minutes. It can also be sterilized with 70% ethanol. Paul Henschke adds: "...non-'sporing' bugs like Brettanomyces can be killed by hot water exposure for 10 minutes or 70% ethanol for 5 minutes (Fitting a condom to the thief is extremely effective for controlling Dekk [sic] infection!!)"

Brett-free wine (filtered, pasteurized, or treated with Velcorin®) for topping barrels will help in the same regard. Keeping barrels topped-up with no splashing will delay the growth of indigenous "Brett" populations by restricting that extra oxygen they like. The best way to exclude O₂ is to bung tightly and leave the barrel alone!!

That brings up another point: to micro-oxygenate or not? Well, Dr. Ralph Kunkee (UC Davis) and a few other microbiologists who know Brettanomyces would advise strongly against it unless you had a very good monitoring program for things such as 4-ethylphenol, 4-ethylguaiacol, and plating on specialized media. Wine testing laboratories usually provide this for a moderate fee.
Unfortunately not all Brett aromas and off-flavors can be detected or monitored in the same way as 4-ethylphenol and plating is often too slow. Therefore, new methods need to be developed to prevent Brettanomyces growth or to detect them in a viable state early in the wine’s storage.\(^{23}\)

In our experience, we could not correlate the concentration of 4-ethylphenol directly to numbers of viable B. bruxellensis. Nor could we rely solely upon the inability to detect 4-ethylphenol to verify the absence of Brett in a survey of local wines. We do however favor this method as a proactive measure to monitor the alterations an active population of Brett can incur on a barrel over time.

**Effect of Brettanomyces on wine aromas and flavors**

Primary effects observed in wines infected by Brett include a loss of fruitiness and an increase in the overall complexity of the wine in the short term. In the long run, increases in noxious aromas such as Band-Aid®, creosote, burnt rubber, and a general loss of fruity and floral aromas is apparent.

In a study at a prominent California winery, three vintages were followed for development of brettiness and the alterations of other components in wine. Most significantly, the aroma and flavor compounds associated with varietal impact suffered the greatest conversion to noxious compounds.

Cinnamic acids (such as p-coumaric) can add to the floral and “honey-like” aromas of wine. Since Brett converts these compounds to stay viable in the bottle for a long time, it makes sense that those delicate aromas would go away with prolonged Brett activity. Gas chromatography-olfactometry (GCO) showed just this effect at the winery mentioned previously. Medicinal and metallic aromas and percepts replaced the floral ones.\(^{1}\)

There are different degrees or types of brettiness in wines. Not all Brett-defects are noticeable to all people. Some people have an anosmia (inability to smell) to the “animal” and “barnyard” attributes that are common to affected wines, some are more sensitive to the “plastic” aromas (such as 4-ethylphenol). Both groups of chemical compounds have very different odor thresholds and most people do not have the receptors to detect them all.\(^{1,21}\)

It is also important to remember that several compounds determined to be in Brett-affected wines were shown to have similar “plastic” aromas yet very different detection thresholds in taste panels.\(^{1}\) The odor threshold of 4-ethylphenol in water is approximately 50 ng/L. That of 4-ethylguaiacol is 500 ng/L. In concert with other odorants with similar thresholds, there is an additive effect for the taster in a wine. Brett can be a very complex phenomenon in rich wines.

“Mousiness” is another attribute blamed on Brettanomyces bruxellensis and on some Lactobacillus bacteria. A “mousy” wine is detected as an aftertaste, not as an odor. “Mousiness” is created by isomers of 2-acetyl-tetrahydropyridine and 2-acetyl-1-pyrroline, which are oxidized in the taster's mouth and experienced retro-nasally.\(^{14,24}\) These compounds are commonly associated with bread crust, stale popcorn, and mouse urine.

The odor threshold for the 2-acetyl-tetrahydropyridines is approximately 1.6 ng/L! Moreover, Dr. Paul Henschke and coworkers have shown that all Brettanomyces and Dekkera species tested can produce mousy off-flavor as well as acetyltetrahydropyridines.\(^{24}\)

**What does the future hold for Brett?**

Within the next five years, a molecular detection method will be developed that will yield enumeration results in one day. Our laboratory has continued to develop methods for this purpose. Currently, work is being done on probes that are specific to Brettanomyces bruxellensis gene sequences having a claimed specificity of 100%.\(^{22}\)

There are problems with detection of any organism when using DNA methodologies. One problem is that DNA is relatively stable (and so are some types of RNA). If these molecules are detected and no viable cells of B. bruxellensis are actively changing your wine, then you have a false positive result. Meaning, Brett looks to be there, happily altering the fruitiness factor and it really isn’t.

DNA technologies are so sensitive that false positives are a real issue facing developers of molecular detection methods. However, traditional plating methods can yield false negatives just as easily (e.g. non-growing Brett don’t make colonies on a plate but they do metabolize cinnamic acids?).

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Another big issue is who will pay for it? When one considers that a barrel of wine may be worth $4,000 or more, a $50 bi-monthly test to detect and enumerate Brettanomyces prior to any perceivable off-odor and flavor problems might be worth just as much. The cost of the equipment to do such work would be great at first, but detection of Brett in barrels prior to sensory threshold via chemical and molecular methods might pay off in just a few years! Look for such services at commercial wine labs soon.

The wine industry has been slow to adopt molecular typing and detection methods. Wine producers haven’t had to because microbes, which survive in wine are not pathogenic. The food industry has borne the brunt of the expense in development of standard protocols and equipment. Now we have a golden opportunity to use these methods if we can overcome the cultural inhibition and cost limitation in wine that prevent their everyday use.

Finally, we think someone needs to look at the ability of the enzymes responsible for conversion of precursors into unpleasant odors in a test tube. What really does happen when Brettanomyces bruxellensis yeast cells rupture and release all of their contents into the wine? Can the enzymes that were once contained within the cell now continue to alter the wine in the same negative way? For how long does this happen? That idea sends chills up your spine if you are a winemaker.

Conclusion: Perception is everything

Several winemakers have proposed theories for why reactions might occur in a wine contaminated by Brett. Others have proposed solutions that would take a lot of time to put into effect, if only for the stigma attached to them, for the management of Brett in the winery. Among these controversial topics are the proposed use of commercial beverage sterilants and flash pasteurization.

Topics discussed here are meant to further the general knowledge of Brettanomyces and as a review of current literature regarding general problems associated with Brettanomyces infections in wineries. Brettanomyces is still a red-hot issue compared to other problems in viticulture and the economics of running a winery. So where is the funding for research?

Some in the industry have stepped away from the issue entirely and proclaimed a natural symbiotic relationship between good wine and Brett that winemakers should embrace.

Some people that we have spoken to regarding the “good Brett” idea, believe it to be an excuse for producing a faulty wine. After all, if a wine was made under conditions that promoted a specific good Brettanomyces bruxellensis strain, any of the other “bad” strains may compete, so where would any sense of control over production be implemented? Perhaps in strain selection and in selective filtering.

One wine buyer told us that he was disappointed in the winemakers and wine writers that promote “Brettiness” in wine. He had believed until now, that the era of making what he called “dirty wine” was over. We have a few more years of very basic research ahead of us before one can completely claim a manageable impact from Brett. It seems that the wine industry has selected Brett and a few other specific organisms based on their abilities to compete in tiny niches.

But we are scientists, so we would say that. If it were possible to have that sort of microbiological control in a dynamic biological system, I think winemakers would welcome discussion of it. Until then, we think Brettanomyces will continue to consume time in debates over research funding. That is why wineries are doing their own basic work in the discoveries of Brettanomyces bruxellensis behavior.

By the way, if anyone has a strain or two that they want to send us, please do so! We are building a database of characteristics and molecular markers so that when someone discovers a specific subtype of B. bruxellensis that produces only positive attributes in a red wine, we can have some information about it. The most important thing we have learned in working with Brett is to keep consumer perception in mind.

Several years ago, one bottle of red wine was purchased in California specifically to take back to the lab and plate for Brettanomyces bruxellensis. The “stink” was strong with this one. One could barely bring it to one’s mouth! We had seen similar wines from France that were over five-years-old that produced odors which were much worse, but this was the most “French character” we had ever seen in a wine from California or New York.
I could only afford to buy one bottle ($25) and I could not justify the expense for a case of such a faulty wine to bring back for a wines course, but now I wish I had. The lesson that it teaches is important. The winemaker’s tasting notes stated that this was their finest representation of a Bordeaux blend. They heralded its arrival just in time for the Millennium. Recently the winery sold its last bottle for a lot more than $25. Just something to consider when you are staring at the ceiling late at night wondering what Brett is doing to your wine.

On the other hand, wines have been received for analyses in our laboratory which exhibited “some” Brett aromas which were certainly in line with specific regional characteristics and which had been rejected by wine buyers apparently for their Brett character. The cost of rejection for a wine due to over sensitivity to Brett is not only damaging to profit margins for a winery, but its reputation as well.

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