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To: **Grape and Wine Producers**

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1. *Brettanomyces* and the Vineyard?

According to leading authorities (anyone who has guessed correctly more than once), we may be in the midst of a shift in both our knowledge and attitudes towards *Brettanomyces bruxellensis.* One of the most controversial topics in the wine industry, Brett has played the role of the "spoilage yeast." Brett's earthy, animal-associated aromas are often negative, but opinions differ widely due, in part, to the varying mix of wine aromas/flavors, the subjectivity of sensory and hedonic perception, ethnic origin, and significant differences among Brett strains. Some winemakers, wine critics, and consumers believe that Brett can add a positive note of complexity. Traditionally, the concern for *Brettanomyces* was the exclusive providence and concern of the winery, but perhaps no longer.

a. Empirical Observations. One day in August 1992, I found myself on a USAID mission traveling on one of Romania's state- of-the-art medieval roads to visit one of the country's most respected vineyard managers. He had a lot to say, including that all of his vineyards were administered based on lunar phases (biodynamic farming, although that term was not used). The high-calcareous soils provided the highest quality, while the clay soils of some of his plots produced astringent, sometimes coarse, wines. In addition, he said something that I did not fully appreciate at the time, that over the last two seasons he had needed to segregate several blocks of Merlot because they regularly developed *Brettanomyces* in the wine. The fruit was not compromised: no rot, very "clean."

The link between the vineyard and Brett was also highlighted during the 2012 Technical Study Tour that I led to Bordeaux. At Château Luchey-Halde, our host, Jean-Philippe Roby, informed me that his new cellar building, with all-new everything, had several vineyard blocks that produced Brett-impacted wine during the first vintage. A visit to Ch. Palmer was also enlightening. They farm some of their vineyards biodynamically and believe that helps control Brett in their cellar. As yet another example, a California vineyard sold all of its fruit last season to three different wineries, and each one reported having *Brettanomyces* in their wines for the first time!

b. Our *Brettanomyces* Research.   In the late 1990s, my lab team was intrigued by the wide and varied reaction winemakers around the world had toward Brett. Some seemed to follow the mantra of the eighteenth-century Irish philosopher George Barkeley, *Esse est percipi* (To be is to be perceived). They could not perceive a Brett character, so it was not there. Other winemakers seemed much more sensitive to any sensory characteristics they associated with Brett, good or bad.

We conducted a number of studies evaluating 24 genetically-characterized Brett stains obtained from around the world (McMahon et al. 1999, Mansfield et al. 2002, Fugelsang and Zoecklein 2003). We evaluated differences in the ability of Brett strains to grow, produce various metabolites, and to produce hydrolytic enzymes to support their growth. In one of those efforts, replicated sterile Pinot noir wines post-alcoholic fermentation were individually inoculated with one of eight strains of *Brettanomyces bruxellensis.* Population changes were monitored for 23 months, or until cell densities declined to very low levels. The variations among strains, including growth rates, population densities, and metabolite production, were dramatic. These examinations demonstrated several important *Brettanomyces* features:
- Different strains have very different growth patterns.
- Strains can decline in cell population, and then bloom again, a phenomenon we now call viable but not culturable (VNC). This called into question the validity of the traditional plate culture method used to determine the concentration of viable *Brettanomyces.*
- The correlation between primary metabolites and viable cell density was not nearly as strong as that between total cell populations (living plus dead cells).
- There are very large differences among strains with regard to metabolites produced and, therefore, impact on wine.
- Various strains have the ability to produce enzymes that can hydrolyze, or break down, complex molecules to provide the carbon source to support growth.

These findings have been supported by other researchers, including Curtin et al. (2005), and support the concept of regionality. Strains from various parts of the world are different and react differently!

c. Managing *Brettanomyces* in the Winery. Brett management strategies in the winery have at times involved protocols that are somewhat draconian and perhaps not consistent with fine winecrafting. Excessive use of sulfur dioxide is frequently the immediate choice of action. This is a bit like the story of

the housewife and the vacuum salesman: "Ma'am, this unit will cut your workload in half." "Great," she says, "I'll take two."

Not only does too much sulfur dioxide "harden" a red wine's mouthfeel, but at least one study showed that a fairly high percentage of the Brett strains isolated from around the world are tolerant of 30 mg/L SO_2 at pH 3.4 (a level previously thought to assure death; Froudiere and Larue 1988). On the other hand, if sulfur dioxide is added in multiple doses that are too small, winemakers might unintentionally be selecting for SO_2-resistant strains. Brett is opportunistic yeast. Like a hospital- type disease organism, its dominance may actually be promoted by some of our sanitation attempts (Smith 2013). Timing and magnitude of SO_2 additions, therefore, may be important.

The traditional methods of control have included these:
- co-fermentation with MLF (to be able to add sulfur sooner, rather than later)
- sulfur dioxide
- dropping the pH
- sterile filtration
- chitosan-based commercial addition products
- dimethyl dicarbonate (DMDC) known commercially as Velcorin
- sorbic acid/ potassium sorbate
- fining to lower yeast populations
- thermal processing (approximately 40°C)
- avoiding oxygen exposure by limiting splash racking, etc.
- lees-free storage
- keeping the cellar as cold as possible
- keeping pomace away from fermentors
- various sanitation methods, including ozone, hot water, ultrasound, etc.

Overall, our winery management, at times, is a bit reminiscent of the Monte Carlo fallacy. Although some think of this fallacy as a strategy, it is simply uninformed guesses. As an example, a roulette wheel with half-red and half-black positions has a 50% chance of landing on red. So, if we turn the wheel six times and it lands on red, we may be tempted to place the next bet on black; after all, back is due, correct?

Brett can and does occur in white wines, although it is much more common in reds. Red wines are higher in grape phenolic compounds and are generally higher in pH, both of which can encourage Brett development. Elevated pH levels in red wines lower the effectiveness of sulfur dioxide. The phenolic content is important because these compounds are the precursors for the volatile phenols largely responsible for Brett off-odors. For example, 4-ethylphenol (4-EP), a principal "marker" metabolite, is produced from *p*-coumaric acid via several Brett-produced enzymes.

d. Measuring and Monitoring *Brettanomyces*. The key to *Brettanomyces* management is monitoring. Sensory evaluation may be important but, based on the results of Fugelsang and Zoecklein (2003) and others, marker compounds such as 4-EP are produced in excessive quantities by many strains only after the accumulative cell population is very high. Culturing, genetic marker testing, and the analysis of metabolites are choices via contract lab services. For the winery and vineyard, perhaps the easiest option is to use Sniff Brett. This is a commercially-available growth medium that contains the precursors for 4-EP development. Samples of fruit, must, or wine are added and, within one or two days, if Brett is present, the pungent odor of 4-EP will be noted.

The sensory impacts of Brett vary widely. To help winemakers through this labyrinth, Dr. Linda Bisson and her colleagues at UC-Davis have developed a *Brettanomyces* aroma wheel. One review (Joseph 2013) demonstrated that in a defined medium, 17stains produced positive aroma components, while five produced negative attributes. The positive compounds include esters, higher alcohols, and terpenes, which can contribute to woody and fruit aromas (Joseph 2013). Some common and mostly-negative metabolites and their sensory ranges are listed below. The positive characteristics of Brett can be much more evidenced if the volatile phenols and isovaleric acid perceptions are limited (Bisson 2013).

- 4-Ethylphenol (4- EP)   120-1200 ng/L – Band-Aids, plastic
- 4-Ethylguaiacol (4-EG)   70-150 ng/L – smoky, spice, burnt beans, medicinal
- Isovaleric Acid   +/- 1200 ng/L – rancid, vomit, barnyard

Combinations of these and other metabolites provide the typical sweaty horse, leather, horse blanket-type odors. There is a large matrix effect impacted by cultivar, notably the phenolic composition, which affects the impact of metabolites. For example, a low phenol variety such as Tempranillo, with a 4-EP level of 125 µg/L, might have the same Brett impact as a Cabernet Sauvignon with 420 µg/L of 4-EP. Additionally, there is a synergistic effect on detection level. For example, 4-EP + 4-EG might be detected at 426 µg/L, while 4-EP alone may be around 620 µg/L.

e. More Brett Now? With rising winemaking standards, why has Brett remained a problem? Indeed, some believe Brett incidences are on the rise. How could that be? There may be several possible reasons, including the following:

- Climate change – Rising temperatures may increase ripeness, resulting in higher pH and phenolic content in the fruit. With increased alcohol, there may be a tendency for a greater concentration of residual sugar remaining post-alcoholic fermentation. Are rampant Brett infections in the winery a function of large inoculums on the fruit?
- Minimalistic winemaking – Limited use of sulfur dioxide at crush and during aging may increase Brett growth.
- Phenolic management – The trend towards relatively-highly extracted wines may increase the concentration of certain grape phenols which are precursors to volatile phenols produced by Brett.

- Storage *sur lie* – Such storage may increase the total nitrogen content, helping to support growth.
- Excessive nitrogen in the fermentor – The tendency to use supplemental addition products, even when unnecessary, may result in excessive residual nitrogen which can support growth.
- Adaptability of Brett strains – *Brettanomyces* is much more tolerant to changes in temperature and pH than *Saccharomyces*, and has a more energy-efficient metabolism (Bisson 2013).
- Very large regional differences among Brett strains.
- Perhaps we are simply more aware of Brett than in the past.

f. Microbial Ecology and the Nutrient Desert. Like a hospital-type disease organism, *Brettanomyces* dominance may actually be promoted by some sanitation practices, as suggested by Smith (2013). French research implies that one of our greatest allies against Brett may be native organisms. In wines where the natural microbial balance was eliminated by pasteurization, Brett growth was much more rapid and developed significantly greater population densities.

The concept of microbial balance is gaining attention as we realize that ,despite the addition of cultured yeasts to a red must, a substantial portion of a fermentation can actually be conducted by other, native organisms (Bokulich et al. 2012). As outlined by Smith (2013), there is a substantial difference in microbial populations among different wines produced at the same facility. It may well be that microbial ecology may impact Brett growth**.**

With regard to Brett, Stern (2011) expressed the situation very well: "The life cycle of Brett in juice and wine in conjunction with other fermentative yeast and bacteria is a part of a complex series of interactions where competition, equilibrium and collaboration form a dynamic ecosystem that winemakers need to understand." Changing conditions, including those in the winery such as the amount of alcohol, oxygen, acidity, sulfur dioxide, temperature, etc., may impact the microbial ecology, with each variable helping to select the most resistant and adaptable organisms.

As suggested by Murat (2011), there are several winemaking stages that may be significant for increased Brett growth and development, including cold soak, and at the end of the lag growth phase and before malolactic fermentation. At these stages the molecular free sulfur dioxide is low. As such, cold soak should only be conducted on "clean fruit." The reason co-fermentation has gained support is to eliminate or reduce the time between the completion of alcoholic and malolactic fermentation, allowing for the addition of sulfur dioxide sooner.

After alcoholic fermentation finishes, the *S. cerevisiae* population decreases. If, by this stage, there is no carbon source and nutrient supplies are exhausted, there is a greater likelihood that the wine will be stable with regard to Brett growth. If these conditions are not met, an opportunity for Brett growth remains.

Creating a nutrient desert to help minimize *Brettanomyces* growth has been preached by leading wine educators such as Lisa Van de Water for years!

Brett lacks the genetic capacity to synthesize many of the micronutrients required for growth, a reason why blooms or excessive growth can follow alcoholic fermentation. If there is an excess of YAN (yeast assimilable nitrogen) in the fruit or must, there will be an excess remaining in the wine post-alcoholic fermentation. This can help Brett, if present, to flourish. This is one reason why I have encouraged our winemakers to have the Virginia Tech Enology Service Lab measure YAN at or just before harvest.

g. Where Does the Vineyard Come In? The problem of combining suitable plant nitrogen nutrition, for vine balance with optimum concentrations of natural berry nitrogen, remains largely unsolved. Last season in Virginia, we generally experienced fairly high YAN levels around the state, due in part to the higher than usual spring rains. Adding nitrogen was, by and large, not needed and, indeed, not desired. Winemakers universally appear to take the approach that if a little is good, more is better, and add exogenous nitrogen to the fermentor. This may be an important factor aiding the growth and development of Brett in wines.

Whether or not *Brettanomyces* is commonly present in the vineyard, and present in a concentration that would be a problem, remains controversial (Van de Water 2010). Thanks to recent advances in media and methods of detection, Brett has been detected in vineyards and on grapes. Several have suggested that fruit does represent a source of potential winery contamination (Agnolucci et al. 2007, Renouf et al. 2007).

Brettanomyces is known to be present on plant material and transferred easily by insects. A grape berry naturally caries between 10^4 and 10^6 microbial cells. The mix of organisms can vary depending on stage of maturity, variety, region, season, and condition of the fruit (Guerra 2010). The majority of these organisms are from a few major species. *Brettanomyces* is usually in limited numbers, a reason why it has not been associated strongly with the vineyard (Guerra 2010). David A. Mills and Nicholas A. Bokulich of UC-Davis have shown that grape varieties from various wine-growing regions carry distinctive patterns of fungi and bacteria. The discovery of different patterns of microbial communities from one region to another has broad implications for both vineyard disease susceptibility/management and perhaps the concept of *terroir*.

With compromised fruit, there is a possibility for this yeast to come into the winery with the broad-spectrum saprophytes on the fruit. *Brettanomyces* has been associated with *Botrytis* and sour rot. It seems logical any fruit breakdown, including from Spotted Wing *Drosophila*, can release both sugar and nutrients to aid growth. Guerra (2010) noted that the physical state of the fruit, as well as whether any

anti-*Botrytis* spays have been applied, could impact the *Brettanomyces* populations. Vineyard sanitation also has a role to play, including simple things such as the use of clean lugs. Certainly, activities such moving wet pomace to the vineyard could increase the inoculum levels.

The argument that Brett cannot come from the vineyard in high-enough concentrations is a standard mantra. The one truism that should always be kept in mind, however, is this: it is what you learn after you know it all that really counts! Regardless, an understanding of the diversity of this yeast and methods of control will remain essential for consistent fine winecrafting.

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2. Research Review – Effect of Foliar and Soil Nitrogen and Sulfur Applications on *Petit Manseng* Grape Wine Composition

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The effect of foliar nitrogen and sulfur applications on Petit Manseng vine nitrogen status and grape composition was studied during the 2011 and 2012 seasons. Vines were planted in 2008 in Dobson, North Carolina (elevation 2,000 feet), using 101-14 MGT rootstock, cordon-trained, and spur-pruned on a vertically shoot positioned (VSP) trellis. Four treatments were applied each season with six replicates of six vines each and included
(1) control – no nitrogen or sulfur applications
(2) nitrogen at 30 kg/ha (calcium nitrate) applied to soil just after flowering
(3) 15 kg/ha of urea nitrogen in two foliar applications prior to véraison
(4) 15 kg/ha of nitrogen (as urea) and 5 kg of micronized sulfur (microthiol) in two applications prior to véraison.

Foliar treatments were applied twice, at post-berry set and immediately pre-véraison. The site is a region IV (UCD heat summation system). Rainfall during the last thirty days prior to harvest in 2011 was 84 mm, with 156 mm of precipitation in 2012. Soil-applied nitrogen fertilization prior to bloom has been shown to increase cluster weight through more and larger berries/clusters (Spayd et al. 1993). In this study, N was applied post-bloom at a relatively low rate to a vineyard of low nitrogen status.

Applications had little to no impact on components of yield and pruning weights. The canopy density did not change as a function of treatment applications as evidenced by point quadrat analysis. In 2011, °Brix and titratable acidity did not differ among treatments. In 2012, foliar nitrogen plus sulfur-treated vines had fruit with higher °Brix (24.6± 0.03) compared to the control treatment (23.8± 0.02). Slight pH elevation (0.09 units) was demonstrated for both nitrogen and nitrogen plus sulfur treatments, compared to the control treatment. The slight pH elevation may have been due to the smaller berry size in these treatments.

Free alpha-amino acids (FAN) and ammonia, collectively referred to as yeast assimilable nitrogen (YAN), may impact wine aroma and flavor (Keller 2005) since amino acids are precursors of some aroma and flavor compounds (Rapp and Versini 1991). Table 1 shows that YAN levels in foliar nitrogen plus sulfur-treated vines were almost 4 times higher than YAN concentrations in the control treatment in 2011. In 2012, the foliar nitrogen treatments displayed differences in YAN levels.

Table 1 Effect of soil and foliar applications of nitrogen and sulfur on juice nitrogen levels of 2011 and 2012 Petit Manseng berries.

Concentration (mg/L)		Control[a]	Soil Nitrogen	Foliar Nitrogen	Foliar Nitrogen/ Sulfur
2011	Ammonia- N	4.0c[c]	5.0c	9.0 b	16.0a
	Arginine	45c	50c	100b	193a
	YAN[b]	94b	148b	179b	346a
2012	Ammonia-N	10.0b	21.3ab	32.2a	16.5b
	Arginine	124b	174b	378a	136b
	YAN	98b	138b	221a	104b

[a]Control: no treatment; Soil Nitrogen: nitrogen 30 kg/ha after flowering, Foliar Nitrogen: nitrogen 15 kg/ha (2 applications prior to véraison); Foliar Nitrogen and Sulfur: nitrogen 15 kg/ha and sulfur 5 kg/ha.
[b]YAN = Yeast assimilable nitrogen
[c]Different letters within rows indicate significant differences at $p \leq 0.05$

Foliar nitrogen application resulted in increased total glycosides in juice compared to other treatments. This increase suggests an enhancement in the pool of flavor and aroma precursors in these treatments, since these non-volatile bound conjugates may undergo hydrolysis, resulting in free volatiles. Free volatiles may contribute directly to aroma and flavor.

Previous *Enology Notes* have reported the use of several electronic nose systems in our research. In this case, canonical plots of bagged clusters in the field demonstrated that a conducting polymer-based electronic nose could distinguish among treatments (Figure 1). Treatment differences are indicated in the plots by non-intersecting circles. The separation of treatments suggests the volatile compositions of the berries were different.

Figure 1 Canonical plots of volatile differences in Petit Manseng field clusters in 2012 from four treatments as detected by conducting polymer-based electronic nose. Trt. 1: control, no treatment; Trt. 2: soil nitrogen 30 kg/ha after flowering; Trt. 3: foliar nitrogen 15 kg/ha, 2 applications prior to véraison; Trt. 4: foliar nitrogen 15 kg/ha and sulfur 5 kg/ha, 2 applications prior to véraison. Significant differences at $p \leq 0.05$ are indicated by non-intersecting circles.
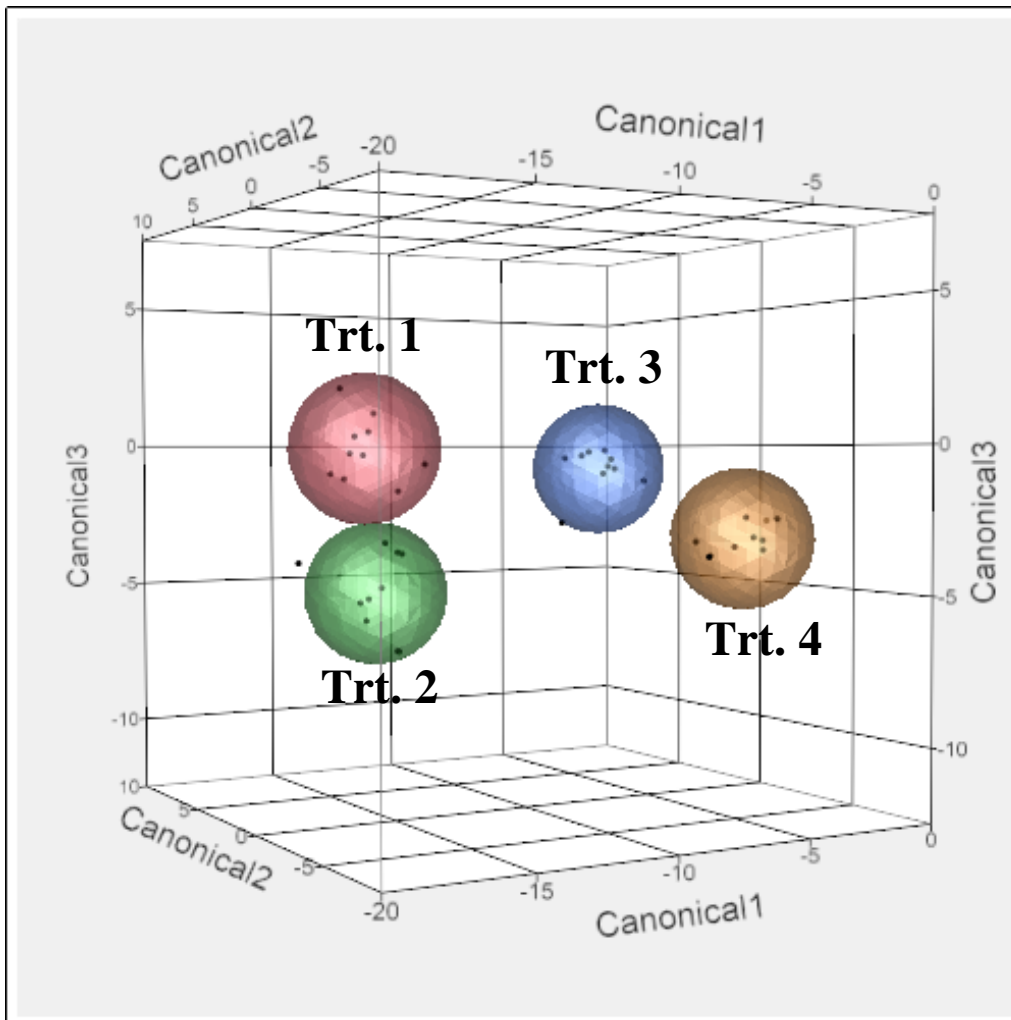
At maturity (at 24 ±1 Brix), fruit from the six replicates of the four treatments were hand-harvested, crushed and destemmed, and wine was produced using standard winemaking practices. A trained panel of eight members generated 24 descriptors for the experimental wines. Spider plots were created to demonstrate the mean intensities of aroma attributes (Figure 2), and flavor-by-mouth and texture/mouthfeel (Figure 3).

The foliar nitrogen plus sulfur treatment had greater melon and grapefruit aromas versus other treatments, while foliar nitrogen alone demonstrated greater pear, honey and pineapple aromas. The soil nitrogen treatment had greater peach and green apple aromas versus other treatments. The control wine had decreased peach, pear, pineapple, melon, lime, lemon, floral and grapefruit compared to the other wines.

Some differences in flavor were noted. Foliar nitrogen plus sulfur treatment had increased astringency and grapefruit flavors, while the foliar nitrogen treatment had increased grapefruit flavors and increased viscosity. The astringency found in the foliar nitrogen plus sulfur treatment was at a low enough level that panelists did not deem it undesirable. The soil nitrogen treatment had increased apple and sweet flavors while the control treatment had increased levels of sour, bitter and pear flavors.

Figure 2 Mean intensities of aroma attributes in Petit Manseng wines in 2011 from four treatments. 1: control, no treatment; 2: soil nitrogen 30 kg/ha after flowering; 3: foliar nitrogen 15 kg/ha, two applications prior to véraison; and 4: foliar nitrogen 15 kg/ha and sulfur 5 kg/ha.
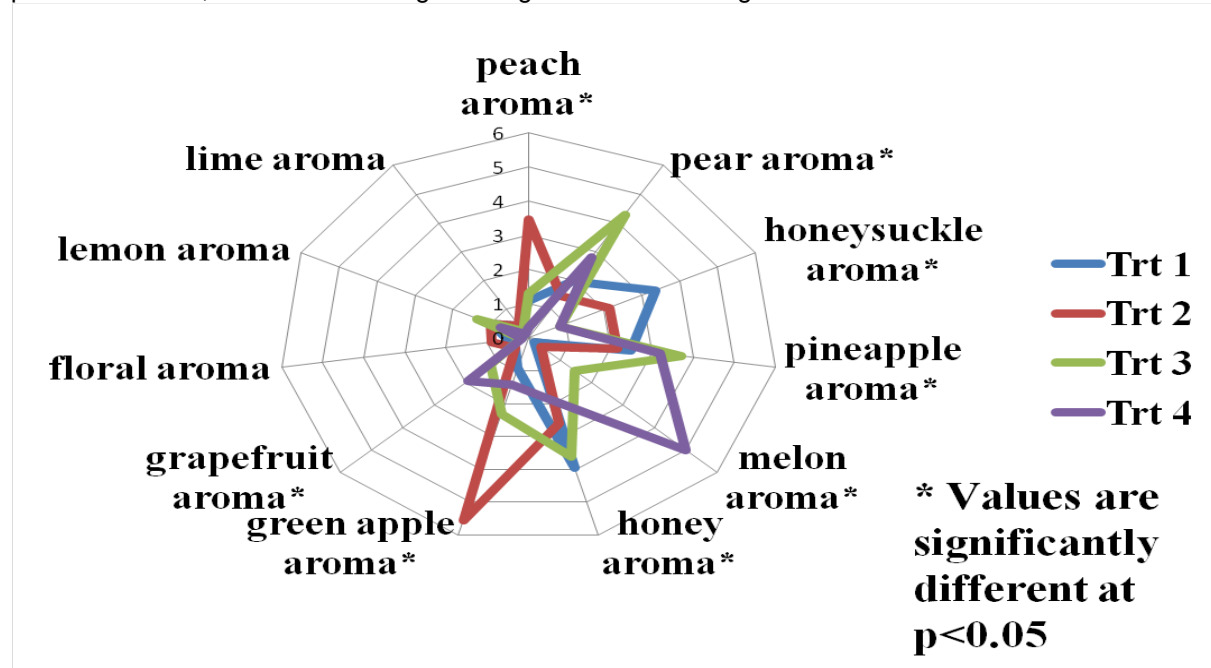
Figure 3 Mean intensities of flavor-by-mouth and texture/mouthfeel attributes in Petit Manseng wines in 2011 from four treatments. 1: control, no treatment; 2: soil nitrogen 30 kg/ha after flowering; 3: foliar nitrogen 15 kg/ha, two applications prior to véraison; and 4: foliar nitrogen 15 kg/ha and sulfur 5 kg/ha.
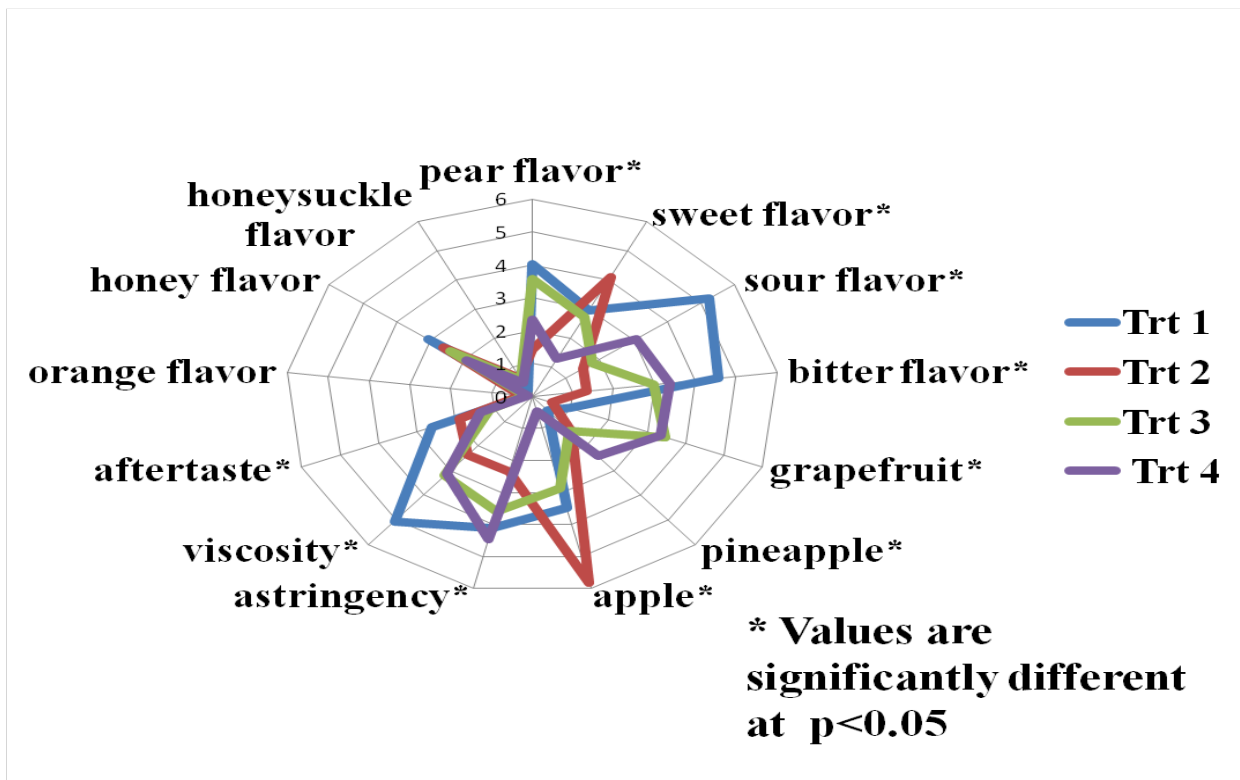
These results suggest that fruit composition of Petit Manseng was impacted by soil nitrogen, foliar nitrogen, and foliar nitrogen plus sulfur applications.

In this study, nitrogen application impacted fruit nitrogen components and had qualitative and quantitative impact on aroma and flavor perceptions. It is hoped that in the future fertilization practices could be optimized to alter the aroma and flavor profile of Petit Manseng.

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3. Winery Planning and Design, Edition 16, Available.

This publication, in CD format, is the result of a number of short courses and seminars, covering various aspects of winery planning, in several wine regions around the country. While not regionally specific, the information provided is from a number of authoritative sources, covering such diverse topics as sustainable design, winery equipment, and winery economics. *Winery Planning and Design, Edition 16*, is available through the industry trade journal Practical Winery and Vineyard (phone 415-444 6695, email: tlv100@sonic.net, or don@practicalwinery.com). The entire index and additional information is available at www.vtwines.info.