Wine/Enology

Grape Chemistry
Group at Va Tech

FRUIT ROT IN VIRGINIA

DR. BRUCE ZOECKLEIN, PROFESSOR EMERITUS
VIRGINIA TECH

Soren Kierkegaard reminded us that life must be lived forward but can only be understood looking backward. In 1985, the year I arrived in Virginia, the mere rumor of an approaching hurricane would send most growers to the field to begin harvest. Those few that dared to let fruit hang must of had the caviler motto-if you are not living life on the edge you are taking up too much room. Generally, we have learned that delaying harvest until optimum maturity pays off, even when the rot potential due to rain is great. Such choices are not easy or clear cut and may represent the dichotomy of being approximately right vs. precisely wrong. We have evolved since 1985, but wet harvests and fruit rots remain. Those that believe that they cannot face yet another year of annual events, such as our summer rains, perhaps should not be growing grapes in Virginia.

Our goal must be to enhance our understanding of the constraints to producing fine wines in in our region. I believe that luck is the residue of design. Having a viticulture and winemaking HACCP plan (hazard analysis critical control point) can aid in minimizing the negative influences of fruit rot both in the field and in the winery (see Enology Notes at www.vtwines.info)..

An understanding of the nature of fungal degradation and fungal metabolites is essential to crafting fine wines in a warm and humid growing environment such as ours. Grapes are susceptible to a number of fungal complexes, including bitter rot, black rot, sour rot, ripe rot and *Botrytis cinerea*. Temperature, moisture, insects, and the presence of fruit wounds have a strong influence on kaleidoscope of potential fruit rots and what organisms dominate. In addition to yield losses, fungi can negatively impact a wide range of grape, and therefore, wine parameters including color, aroma and flavor properties.

Botrytis infection followed by warm, sunny, windy weather can cause berries to dehydration, increasing the sugar concentration. This is the so-called *pourriture noble,* or noble rot. In our environment secondary infection by other microbes usually follows. Under wet conditions, molds such as *Penicillium, Mucor*, *and Aspergillus* spp., as well as other fungi and yeast, may overgrow *Botrytis*; referred to in France as vulgar rot (*pourriture vulgaire*).

Wounds and breakdown of grape skins provides substrates for the growth of yeasts and bacteria, and may produce a condition called *pourriture acide,* or what we know as sour rot. Sour rot is generally a complex of microorganisms from various species: *Alternanaria, Aspergillus, Cladosporium, Diplodia, Penicillium, Rhizopus* yeast, *Acetobacter* and other bacteria. The pungent odor of sour rot comes from acetic acid produced by the *Acetobacter* bacteria. Sour rot is strongly influenced by the presence of Drosophila fruit flies and berry wounds.

Ripe rot is a bunch rot caused by species of the fungus *Colletotrichum*. This malady occurs predominantly in regions such as Virginia that have warm, wet conditions during the later stages of fruit maturation. Ripe rot causes shriveling and fruit collapse. As the disease progresses, reddish brown circles develop on the fruit. These circular lesions grow in size, eventually covering the entire berry. Fungal bodies contain small pink colored spores. In addition to the typical visual disease symptoms, ripe rot degrades fruit color and produces various volatile and non-volatile compounds that can significantly taint the resultant wines.

Effects of Fruit Rot on Fruit and Wine Chemistry

It is safe to say that for most Virginia winemakers fruit rot in 2018 cause a certain limited amount of stress, much in the same vain that the Pacific Ocean contains a certain limited amount of water. Early on we evaluated the effect of several fruit rots on the chemistry of Virginia grown grapes. As can be noted in Table 1, fruit rots can have significant influence on must composition. A universal question solicited - how much rot does it take to detrimentally impact a wine quality? The answer is complicated due to the variations in rots and the incidence (percentage of clusters with visual rot) severity (percentage of rot per cluster) and the production of metabolites. The determination of potential impact of rot is best made based on the analysis of metabolites.

Table 1. Comparison Between Virginia Riesling Musts

Parameter	**"Clean" Grapes**	***Botrytis cinerea***	**Sour Rot**
Brix	18.5	21	≥ 16.0
Titratable Acidity (g/L)	8.0	6.5	5.0
Tartaric + Malic acid (g/L)	7.2	5.2	4.4
pH	3.3	3.5	> 3.4
Gluconic acid (g/L)	0.5	1-5	≥ .5
Acetic acid (g/L)	0	1.1	≥ 1.5
Glycerol (g/L)	Trace	1-10	Trace
Ethanol (%, v/v)	0	0-trace	≥ 0.2%
Laccase (µg/mL)	Trace	0.1-8	trace to 0.5
Glucan (mg/L)	0	247	65

Source: Zoecklein et al., 2000).

Some Important Rot Metabolites

Laccase and other oxidizing enzymes

Rots can produce substantial array of enzymes such as laccase, esterase, lipase, and those that break down pectin, the cellular glue, such as cutinase, polygalacturonase, glucanase, cellulase, phospholipidase. Each of these can oxidize must and wine components including aroma/flavor and phenolic compounds. My lab demonstrated that the yeast species and strains associated with sour rot possessed the ability to breakdown grape aroma compounds (McMahon *et al*.,1999). The enzyme laccase produced by *Botrytis cinerea* presents a particular hindrance for winemakers because, unlike most enzymes, is resistant to sulfur dioxide, cannot easily be removed and is active in the presence of alcohol, including in bottled wines

Fungal enzymes can cause the oxidation of a large variety of phenolic compounds including anthocyanins and tannins, releasing other phenolic compounds. Ripe rot, for example, produces wines that are particularly bitter as a result of grape skin breakdown and release of phenolic compounds. Additionally, oxidizing enzymes produced by fruit rots destroy a wines reductive strength. Reductive strength is essentially a measure of a wines ability to age and age well. Reductive strength and longevity are an important wine quality feature (See Enology Notes #169).

Glycerol

Glycerol is a type of alcohol produced by molds. Owing to its relatively-high specific gravity (density), it may contribute to the overall perception of wine body or fullness when fruit glycerol levels are high. Most of the glycerol produced by molds will remain inside the defective berry despite berry dehydration and heat, due to the fact that glycerol is not particularly volatile. Glycerol itself possesses no significant problem for the winemaker, but can be used as a barometer of fruit rot. Musts from healthy berries usually contain less than 1 g/L of glycerol, while musts from infected berries contain 5-30 g/L glycerol, depending on the nature of the rot.

Gluconic Acid

Rot infected fruit can contain a relatively-high (25 g/L) gluconic acid level as a result of glucose metabolism. Since this acid is not utilized by yeast or bacteria, it may be used as an indicator of fruit deterioration. Gluconic acid levels in “clean” fruit, and in wines made from clean fruit, are near 0.5 g/L, whereas in wines produced from fruit infected with *B. cinerea*, levels range from 1 to 5 g/L.

The ratio of glycerol to gluconic acid indicates the “quality” of the rot. Higher ratios indicated the growth of true noble rot, whereas lower ratios generally suggest sour rot or ripe rot complexes.

Acetic Acid

Acetic acid or vinegar is a normal byproduct of yeast and bacteria. When acetic acid bacteria and yeast are combined with fungal growth, high levels of this volatile acidity can be produced. Rot complexes may show significant variations in acetic acid content in the fruit. Acetic acid is volatile at normal vineyard temperatures and can be detected by scent during a vineyard stroll.

In some cases, fruit enters the winery showing limited visual rot, only to have excessive acetic acid produced during fermentation due to the large concentrations bacteria. Several species of *Lactobacillus* present are very efficient in converting grape sugars to acetic acid, thus raising the spoilage characteristics of the wine excessively, even prior to the completion of alcoholic fermentation! We have seen that occur this season.

Ethyl Acetate

The volatile character or “acetic nose” is not exclusively the result of acetic acid production. Esters, most specifically ethyl acetate, contribute significantly to this defect. Ethyl acetate formation by yeast can create the characteristic “finger nail polish remover”-like odor.

Galacturonic Acid

Molds can cause an increase in the galacturonic acid content as a result of enzymatic breakdown of cell wall components. This acid may be transformed by enzymatic oxidation to form an insoluble salt, calcium mucate. Wineries with calcium levels greater than 40 mg/L in their water run the risk of having rot compromised fruit produce wines with this insoluble salt. We have seen this in Virginia.

Clarification Difficulties and Instability

Fruit rots can form protective colloids in juices and wines, inhibiting clarification. Pectins (complex sugars that hold plant tissues together) are hydrolyzed or broken down by mold-produced enzymes, with the formation of soluble pectins and glucans (glucose polymers). In wine, alcohol causes pectin and glucan chains to aggregate, thus inhibiting clarification and filtration. Winemakers dealing with rot-compromised fruit frequently choose to add pectinolytic enzymes and glucanase enzymes to help abate clarification problems.

Aroma/Flavor

Wine aroma compounds can be lost as a result of the oxidizing effect of fruit rots. Zoecklein *et al*., 2000, demonstrated that sour rot reduced the concentration of Riesling grape aroma compounds (terpene alcohols) by 37% and increase the concentration of oxidized aroma compounds (terpene oxides) by 75.9%.

Equally important is the fact that most fruit molds can produce off aromas and flavors. Infected fruit can contribute sensory characteristics such as earthy, vegetal/herbal-like, mushroom and moldy, etc. Off-flavors compounds include a wide range such as geosmin (the so-called the mushroom alcohol) and the ketone 1-octen-3-ol, detectable at extremely low concentrations.

Practical Considerations

Even though the season is over it may be wise to review the steps utilized to help minimize the effects of fruit rots. After all, no matter how late it is it is never as late as it will be later on!

- Have a viticulture and winemaking HACCP plan
- Crop level: Avoid over-cropping, which could delay maturity.
- Carefully review your spray programs in the context of your HACCP plan. Some success has been reported by using sulfur dioxide spray (in pH adjusted water) to help limit sour rot. It should be noted that although this material has GRASS status it is not an approved vineyard material. Additionally, some have used ozone treated water as a vineyard spray.
- Control Drosophila fruit flies and wounds. Hall et al (2018) reported that insecticides targeting fruit flies significantly reduces sour rot severity.

- Fruit culling: Cull as much visible fruit rot out as possible in the field.

- Sort fruit at the winery: A very small concentration of rot can have a large impact. It is not the incidence of rot, but the level of various rot metabolites that determines the impact on wine quality. The best rule of thumb: no rot is acceptable. The current generation of optical sorters allows separation based color, among other things which can help eliminate rotted berries.

- Rinse fruit: Wineries may consider rinsing the fruit with water (containing a wetting agent) if the fruit delivered to the winery is high in rot. That may aid in lowering some of the rot metabolites. This practice can slightly lower the Brix level as a result of dilution.
- Muté production/cryoextraction: Mutés (juice held, or mutéd, from fermentation) can add life and freshness back into the base wine. A small quantity of muté produced from non-degraded fruit can help recover aroma, while masking some of the spoilage and oxidized notes which may have resulted from rots.
- Pressing: Whole cluster press whites by discarding the initial juice. Press very lightly and take press fractions. Short vat red grapes.
- pH adjustment: Adjust the juice pH – the lower, the better. Expect about 2.0 g/L TA will drop out during fermentation or shortly following completion. High pH values may lead to both biological and oxidative problems.
- Sulfur dioxide: Keep the initial sulfur dioxide level low during pressing. You want the low molecular weight tannins to polymerize or bind together. Then raise the sulfur dioxide, depending on the fruit condition and pH.
- Ascorbic acid: The addition of ascorbic acid to the juice or wine and limit the extent of oxidation cause by rot-derived enzymes.
- Test YAN: Test the YAN (yeast assimilable nitrogen) content and make adjustments accordingly. Rots deplete YAN and micronutrients. As such, the addition of a complex nutrient formulation is wise. Most fruit rots use grape ammonia nitrogen, reducing the levels available for wine yeast metabolism. Additionally, thiamine (vitamin B_1), and pyridoxine

(vitamin B_6) are depleted. This is a primary reason why wines produced from rot-infected grapes generally require supplementation with nitrogen and vitamins to help avoid protracted fermentations, sticking, and possible sulfur-like off odor formation. Low thiamine levels can also be the result of excessive addition of sulfur dioxide to the must, binding and inactivating this important yeast growth promoter.

- Measure the NTUs for whites: Ferment fairly-clean juice. Measure the NTUs (nephelos turbidity units) and adjust to about 100 to 150.If the juice is not clarifying add enzymes or more enological tannin.
- Cold settle: Adequate cold settling with the use of pectinolytic enzymes will help lower the level of rot metabolites.
- Tannin addition: Enological tannin addition help clarify the juice and bind with some of the rot-produced enzymes. Tannins can act as oxygen buffers and may bind with enough protein to lower the bentonite requirement needed for wine protein stabilization. This is an important consideration for rather delicate varieties such as Pinot gris and Sauvignon blanc.
- Pectinolytic enzymes: The addition of pectic enzymes aids in clarification, which is particularly important if juice is produced from compromised fruit.
- Yeast Inoculation: Inoculate with a high volume of a vigorous, not too nitrogen -dependent yeast. Use more than the standard 24 g/hL or 2 lb/1000 gallons. Make sure the starter is properly prepared, and understand that oxygen is a yeast nutrient.
- Co-ferment: If you are planning on an MLF co-fermentation, make sure you check with your suppliers regarding yeast and MLF strain compatibility. If you do not desire an MLF (including from indigenous spoilage lactic acid bacteria) consider the use of lysozyme.

- Fermentation temperature: Begin the fermentation at a slightly warmer temperature to help lower the concentration of undesirable aroma characters, and to assure a rapid yeast fermentation.

- Mid-fermentation racking: Rack mid fermentation. This helps to remove wine from the primary lees.

- Rack immediately post-fermentation.

- Consider short vatting reds, avoid cold soak and extended post-fermentation maceration. Use short vatting, and possibly délestage, to help remove fermenting wine from lees

- Fining agents. Addition of fining agents such as PVPP to the juice or young wine will help to remove oxidized phenols. Other agent can be used to lower the concentration of some rot-derived volatile metabolites. These agents include deodorizing carbons, caseinate, bentonite, etc.

References

Hall, M., G. Loeb and W. Wilcox. (2018). Fruit flies play a role in sour rot complex. Wines and Vines, January.

McMahon, H., B. Zoecklein, K. Fugelsang and Y. Jasinski. (1999). Quantification of glycosidase activities in selected yeasts and lactic acid bacteria. J. Industrial Microbio and Biochem. 23: 198-2003.

Sadoughi, N. (2016). Effect of Ripe Rot of Grapes (*Colletotrichum* spp.) on the Chemical Composition and Off-Flavour Compounds in Grapes and Wine. Thesis National Wine and Grape Industry Centre. School of Agricultural and Wine Sciences Charles Sturt University

Zoecklein, B., J.W. Williams, and S.E. Duncan. (2000). Effect of sour rot on the composition of White Riesling (Vitis viniferia L.) grapes. Small Fruit Review. Vol 1. 63-

77.