PROTOCOL P

Compromised Fruit – *Botrytis* and Rot

White & Rosé wines

Botrytis cinerea is the most common form of "rot" in vineyards. *Botrytis* infection can cause many quality-degrading issues in winemaking. Using the tools and tips in this article will reduce the negative impact of rot infection on the finished wine.

QUALITY RISKS

- Off-aromas and flavors from the rot infection (geosmine, octenone).
- Browning from laccase enzyme activity (juice oxidation).
- Secondary infection of fruit when rot breaks berry skins.
- Settling and filtration complications due to glucans produced by *Botrytis*.
- Mycotoxin and biogenic amines in wine.
- Increased acetic acid and gluconic acid in must.
- Reduced levels of amino acid and YAN levels in juice.
- Reduced organic acids, leading to unbalanced wine acidity.
- Higher protein levels in juice leads to challenging protein stabilization in white & rose wines.

HOW TO EVALUATE THE LEVEL OF ROT INFECTION

- Visual inspection of fruit – estimate percent of rot.
- Laccase – reported in units/mL.
- Gluconic acid – reported in g/L.

Before the day of harvest, a vineyard sample can be taken and analyzed in a laboratory to determine the level of rot infection. Professional wine laboratories offer tests for laccase activity, reported in units/mL, and gluconic acid analysis, reported in g/L. **LAFFORT® USA** sells Vintessential enzymatic kits for measuring gluconic acid at your winery with either a spectrophotometer or Chemwell Discrete Analyzer.

For a short review of the effect of different mold species and wine chemistry, read Bruce Zoecklein's "Wine Enology Notes #161", May 12th 2012. Virginia Tech, Wine / Enology Grape Chemistry Group (www.apps.fst.vt.edu/extension/enology/EN/161.html).

	Low rot contamination		Moderate rot contamination	High rot contamination		
Visual inspection - rot (%)	< 1	1 - 5	6 - 10	11 - 25	26 - 50	51 - 100
Laccase activity (U/mL)	0.39	0.78	2.25	6.56	8.12	15.86
Gluconic acid level (g/L)	1 - 2		2	> 3		

**Note: gluconic acid measurement is recommended for must, not on vineyard samples.

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The protocol below is outlined in chronological order, starting with the day of harvest. Each phase of winemaking is covered from grape harvesting, fermentation, and cellar aging.

PHASE 1 – VINEYARD HARVEST AND TRANSPORTATION TO WINERY

Estimate the level of rot.

Do a visual assessment or run laccase or gluconic acid analysis to estimate the level of laccase in the fruit. The rot and subsequent laccase level determine the dosage for the tools in this protocol.

Hand harvest and sort out as much mold-affected fruit as possible.

Machine-harvested fruit will have more juice in harvest bins, extracting mold character and increasing oxidation. General tips for working with machine harvested rot infected fruit:

- Add **SUPRAROM®** for antioxidant protection during transport from vineyard to winery. **SUPRAROM®** combines gallic acid tannin (25%), KMBS (50%), and ascorbic acid (25%). A 100 ppm addition of **SUPRAROM®** provides 28 mg / L of SO_2.
- Adding a fining agent to the harvest gondolas is recommended before pressing to begin the phenolic profile correction. **VEGECOLL®**, pure vegetable protein fining agen, is recommended for application on grapes at 50 ppm. When composting is desired, using a vegetable-based fining agent will ensure no polymer (PVPP) particles in the grape pomace.
- Remember that the juice from the harvest gondolas will have a higher microbial load, laccase concentration, and negative mold compounds.

A non-*saccharomyces* yeast preparation for **BIO**Protection can be used to control unwanted indigenous microflora on botrytized grapes. **ZYMAFLORE® ÉGIDE**^{TDMP} (*T. delbrueckii* and *M. pulcherrima*) will out-compete negative microorganisms, reduce VA levels, and scavenge oxygen to protect must from oxidation. **ZYMAFLORE® ÉGIDE**^{TDMP} can be used directly on fruit in picking bins or grape-receiving hopper. **ZYMAFLORE® ÉGIDE**^{TDMP} can also be applied to harvesting equipment (harvest machine, grape transport bins, cellar equipment).

ZYMAFLORE® ÉGIDE^{TDMP} additions can be used in combination with low additions of Potassium Metabisulfite (KMBS), keeping the additions below 30 ppm of KMBS. **SUPRAROM®** is an excellent alternative to KMBS, and can be used in combination with **ZYMAFLORE® ÉGIDE**^{TDMP} at a full dose of 100 ppm. **ZYMAFLORE® ÉGIDE**^{TDMP} **should NOT be in direct contact with KMBS or SUPRAROM®.**

Chitosan is ineffective for antimicrobial control at the grape or juice stage. Chitan-glucan has a fining effect, not an anti-microbial action.

PHASE 2 – FRUIT PROCESSING

2.1. General TIPS.

- Use a non-*saccharomyces* yeast early in fruit processing if not already added to picking bins. **ZYMAFLORE® ÉGIDE**^{TDMP} **can be dry-pitched on the fruit** at 30 - 50 ppm and will quickly begin to populate the must and out-compete the negative microflora.
- Reduce time on skins and maceration. Avoiding a destemmer and must pump will help reduce fruit maceration and further extraction of mold characters. Hand harvesting and **whole cluster pressing is optimal**.

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- **Cool fruit temperature** will slow down laccase enzyme activity and reduce harmful oxidation. Harvesting at night and/or cooling fruit before processing is recommended. Dry ice can assist in cooling fruit and protecting fruit from oxygen. Keep tank temperature set points below 45° F.
- **Protect must and juice from air/oxygen** exposure with inert gas or dry ice cover. Limiting oxygen exposure will reduce browning and aroma loss.

2.2. Pressing

- **Pressing can be difficult with moldy fruit,** and lower juice yields can be expected. If you are working with machine-harvested fruit or destemmed fruit, adding rice hulls to the press during press filling is recommended to help create juice channels and improve juice release.
- **Keep all initial juice collected during press filling separate.** The first juice (10 to 20 gallons / ton) collected is higher in botrytis metabolites and negative microflora. This juice can be added to the hard press fraction and treated as high rot-infected juice; refer to the high dosage range of products in this protocol.
- Protect juice in the press-pan from oxygen exposure with an **inert gas cover or dry ice.**
- Adding a vegetable protein fining agent can jump-start the **phenolic fining in the press. VEGECOLL®**, pure potato protein, is recommended for application on grapes. When composting is desired, using a vegetable protein fining agent will ensure no polymer (PVPP) particles in the grape pomace.

2.3. Treatments for juice settling

- **The treatments in this section can be added to the juice in the press-pan or to the juice in the collection tank.**
  - → SO_2 addition – Consult the table for SO_2 dosage recommendations. Keep in mind SO_2 additions that have already been made to the fruit in the vineyard. The chart recommendations are based on the total amount of SO_2 used from the vineyard through juice settling. **Note: Do not exceed 40 ppm SO_2 when using **ZYMAFLORE® ÉGIDE^{TDMP}** for BIOProtection.
  - → Tannin addition – Gallic acid tannin effectively binds to laccase, reducing its activity, and acts as an antioxidant. Add **TANIN GALALCOOL®** or **GALALCOOL® SP** to the press-pan or tank during the press cycle.
  - → Enzyme addition – Molds excrete high levels of pectins and glucans. It is essential to use a robust pectinase enzyme at higher dose rates for juice clarification. **LAFAZYM® 600 XL^{ICE}** maintains high activity at cold temperatures and over a wide pH range.
- **The treatments in this section are best added to juice in the collection tank.**
  - → Fining addition – Multiple fining treatments might be necessary with mold-affected fruit. At the juice clarification stage, the contact time is short and rapid settling is desired. A combination fining agent is recommended for best results: **POLYLACT®, POLYMUST® ROSE,** or **OENOFINE® PINK**.
  - → Yeast Hull addition – **OENOCELL®** (yeast hulls) have an absorptive capacity, binding toxins produced by mold and negative microflora. Adding a low dose can help detoxify the juice before fermentation.
  - → Bentonite addition – Mold-affected fruit has higher protein content and can require a high amount of bentonite for protein stability. Early treatment at juice settling can remove the unstable proteins and reduce the amount of bentonite needed during cellar aging. Try adding 2 – 4 lbs / 1000 gallons at least 6 hours **AFTER** your enzyme addition. Bentonite inactivates pectinase enzymes.

NOTE - Laccase and bentonite are negatively charged at typical juice pH. Meaning there is no attraction or binding; therefore, bentonite will NOT remove laccase. Only at a pH of 2.9 and lower will bentonite bind laccase effectively.

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Settle juice for 24 to 48 hours. Rack as clean as possible, desired NTU 75- 100. Protect juice during transfer with an inert gas cover.

Chart 1 - Quick reference for product dosage (Phase 2)

	Level of rot contamination		Low	Moderate	High
Timing	Product	Action		Dosage rates	
Transport from vineyard to winery	ZYMAFLORE® ÉGIDE™ - reduces SO_2 addition	BIOProtection	30 ppm	40 ppm	50 ppm
	SUPRAROM®	Antimicrobial & antioxidant	100 ppm	150 ppm	250 ppm
Fining on grapes in press	VEGECOLL®	Phenolic correction	50 ppm	75 ppm	100 ppm
Juice addition in press-pan or tank	SO_2 Addition	Antimicrobial & antioxidant	50 ppm	80 ppm	100 ppm
	TANIN GALALCOOL®	Antioxidant	50 - 70 ppm	80 - 100 ppm	150 - 200 ppm
	LAFAZYM® 600XL™	Clarification	1 to 2 mL / hL	2 mL / hL	2 to 3 mL / hL
Fining agent at juice settling (choose one)	POLYLACT®	Phenolic correction	200 - 300 ppm	400 ppm	500 - 700 ppm
	POLYMUST® ROSE	Phenolic correction	200 - 300 ppm	400 ppm	500 - 700 ppm
	OENOFINE® PINK	Phenolic & color correction	300 ppm	400 ppm	500 ppm
Addition 6 hours after enzyme addition	OENOCELL®	Detoxify juice	75 ppm	150 ppm	200 ppm
	MICROCOL® ALPHA (bentonite)	Protein stability	2 lbs / 1000 gal	3 lbs / 1000 gal	4 lbs / 1000 gal

**Note: The fining treatment dosages given in the chart are based on doing one fining treatment on the must and a second fining treatment during fermentation. Reference Chart 2 for fining dosages during fermentation.

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PHASE 3 – FERMENTATION

3.1. Yeast & nutrition.

- Desired NTU between 75 – 100 ppm. If the juice is over-clarified, use **TURBICEL®** (cellulose powder) or **OENOCELL®** (yeast hulls) to increase turbidity. Do not add back juice lees. Lees will be high in mold compounds.
- Use a strong aromatic fermenting yeast strain with a short lag phase. Increase the dose rate to 300 ppm or 2.5 lbs / 1000 gal to help the yeast inoculation implant successfully in a challenging juice environment. Recommended yeast strains: **ZYMAFLORE® CX9, ZYMAFLORE® X5, ZYMAFLORE® X16** or **ZYMAFLORE® XAROM** (any Laffort 'X-Series' yeast strain).
- Rehydrate the yeast with **SUPERSTART® BLANC** at 300 ppm or 2.5 lbs / 1000 gal to ensure a strong fermentation finish.
- Mold-affected fruit is often depleted of YAN and other micronutrients important for healthy yeast fermentation. Measure YAN and supplement with **NUTRISTART® ORG** and **THIAZOTE® PH** as needed.

3.2. General fermentation TIPS.

- Fermentation at warmer temperatures will minimize yeast stress and aid in a fast, clean fermentation. For moderate to high rot levels, ideal fermentation temperatures are between 60 – 70˚F.
- Add a β-glucanase enzyme, such as EXTRALYSE, to break down glucans produced by botryis and other microorganisms. Treatment with EXTRALYSE at 60 - 100 ppm in the last 1/3rd of alcoholic fermentation will help improve the wine's settling and filterability in the future.
- It is recommended to conduct a secondary fining treatment during fermentation to remove the residual oxidized and oxidizable phenolic compounds. Use a different fining product to what was used in the must. The recommended doses in the charts are determined for applying a double fining treatment on the must and during fermentation. If only one fining is performed, either at juice or during fermentation, the doses noted can be increased.
- Utilizing mannoprotein products during fermentation will help build up the wine texture, which juice cleanup techniques may have decreased. **FRESHAROM®** can be added early in fermentation at 100 – 300 ppm for improved mouthfeel and aroma protection with glutathione.
- Add NOBILE® alternative oak for masking "off" flavors from rot infection.
  - → Aromatic White & Rose wines
    - Granular or Chips - **NOBILE® FRESH** (French or American oak) will boost fruit flavors and mask mold character. Dosage 1 – 2 g / L.
    - Staves or Blocks – **NOBILE® 18-XBASE** will build texture, boost fresh fruit flavors, and mask mold character. Dosage 1 – 2 staves / hL .
  - → Chardonnay wines
    - Granular or Chips - **NOBILE® SWEET VANILLA** enhances midpalate weight, giving notes of toasted marshmallows and stone fruit. Dosage: 1 – 2 g / L.
    - Staves or Blocks – **NOBILE® ELITE** builds structure and volume, respectful of fruit. Dosage: 1 – 2 staves / hL.
- If malolactic fermentation is desired, sequential inoculation with a robust strain such as **LACTOENOS® B7 DIRECT** or **LACTOENOS® BERRY DIRECT** is recommended.

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Chart 2 – Quick reference for product dosages (Phase 3)

	Level of rot contamination		Low	Moderate	High
Timing	Product	Action		Dosage rates	
Yeast inoculation	ZYMAFLORE® Yeast	Fermentation	1.5 lbs / 1000 gal	2 lbs / 1000 gal	2.5 lbs / 1000 gal
	SUPERSTART® BLANC	Yeast-rehydratation	1.5 lbs / 1000 gal	2 lbs / 1000 gal	2.5 lbs / 1000 gal
Start of fermentation	NOBILE® OAK	Mask off flavor	See text for options		
Fermentation nutrition	NUTRISTART® ORG	Organic Nitrogen	2 lbs / 1000 gal	3 lbs / 1000 gal	4 lbs / 1000 gal
	THIAZOTE® PH	DAP & Thiamine	2 lbs / 1000 gal	3 lbs / 1000 gal	4 lbs / 1000 gal
Fining in fermentation (choose one)	POLYLACT®	Phenolic correction	50 ppm	100 ppm	300 ppm
	POLYMUST® ROSE	Phenolic correction	75 ppm	125 ppm	200 ppm
	OENOFINE® PINK	Phenolic & color correction	75 ppm	100 ppm	200 ppm
1/3 fermentation	EXTRALYSE®	Beta-glucanase enzyme	60 ppm	80 ppm	100 ppm
	FRESHAROM®	Antioxidant & mouthfeel	100 ppm	200 ppm	300 ppm

**Note: The fining treatment dosages given in Chart 2 are based on doing one fining treatment on the must and a second fining treatment during fermentation. If only applying one fining treatment during fermentation, reference Chart 1 for recommended dosages.

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PHASE 4 – MATURATION

4.1. General wine cellaring TIPS.

Post-fermentation analysis - In the case of rot levels above 30%, analyzing the wine for residual laccase activity is recommended. If laccase is present, additional **TANIN GALALCOOL® SP** can be utilized. With **GALALCOOL® SP**, the 'SP' stands for 'soft palate', which can be added to wines without adding astringency.

Monitor VA and SO_2 levels often. It is difficult to maintain levels of free SO_2 in wine made from fruit with high laccase activity. Additional microbial protection tools are:

- **OENOBRETT®, a chitosan product**, can be added after primary and secondary fermentation is complete. **OENOBRETT®** is an effective antimicrobial that will continue protecting the wine for up to three months. The addition range is from 50 – 100 ppm.
- When malo-lactic fermentation is NOT desired, the use of fumaric acid can prevent or arrest ML fermentation. Yeast can metabolize fumaric acid via the Krebs pathway, so **FUMARIC*trl*®** should only be used post alcoholic fermentation for malolactic control. A **FUMARIC*TRL*®** addition at 300 ppm will prevent ML fermentation, while a 600 ppm **FUMARIC*TRL*®** addition can stop an active ML fermentation.

Keep wine away from oxygen exposure. Gas any headspace and keep cooperage topped with tight bungs.

Wines produced from fruit with moderate to high levels of rot have a low oxygen consumption capacity, meaning lower antioxidant power. During cellar aging, minimize the number of wine rackings and transfers. Unfortunately, the wines will not have the aging capacity either. It is recommended to bottle early and get the wines to market.

4.2. Oxidation prevention, masking off-flavors, and rebuilding mouthfeel.

- Add **POWERLEES® LIFE**, a product rich in protective compounds (glutathione), to the wine after racking. **POWERLEES® LIFE** will protect the wine from oxidation during aging. Dosage: 100 – 300 ppm.
- **TAN'FRESH®**, a tannin formulated specifically for white and rose wines, can help refresh wine, restoring fruit flavors. Bench trial additions at 10 - 30 ppm.
- Oak alternatives are effective at masking off characters and building mouthfeel.

→ Aromatic White & Rose wines

- Chips - **NOBILE® BASE** will boost fruit flavors and build volume. Dosage: 1 -2 g/hL.
- Staves or Blocks – **NOBILE® 18-XBASE** will build texture, boost fresh fruit flavors, and mask mold character. Dosage: 1 – 2 staves/hL.

→ Chardonnay wines

- Chips - **NOBILE® DARK ALMOND** enhances midpalate weight, giving toasted brioche and hazelnut barrel-aged character. Dosage: 1 – 2 g/L.
- Staves or Blocks – **NOBILE® 18-XBASE** builds volume and respects fruit. Subtle oak influence, suitable for use post-fermentation. Dosage: 1 – 2 staves/hL.

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- If mold aromas and flavors persist, treatment with **GEOSORB®** might be necessary. **GEOSORB®** is a deodorizing carbon for binding compounds associated with rot and mildew. Bench trials are recommended, dosage range of 150 – 400 ppm.
- Continue to work with mannoprotein products to build mouthfeel during aging. **AUTOLEES®** and **MANNOSWEET®** can bring midpalate weight and a perception of sweetness.
- If **EXTRALYSE®** was not used during fermentation, adding at least one month before bottling is recommended. **EXTRALYSE®** is a beta-glucanase enzyme that will break down the glucans produced from mold and improve settling and wine filterability. Dosage: 60 ppm.
- If malolactic fermentation is desired, sequential inoculation with a robust strain such as **LACTOENOS® B7 DIRECT** or **LACTOENOS® BERRY DIRECT** is recommended.

Chart 3 – Quick reference for product dosages (Phase 4)

	Level of rot contamination		Low	Moderate	High
Timing	Product	Action	Dosage rates		
After fermentation-cellar aging	TANIN GALALCOOL® SP	Laccase removal	30 ppm	50 ppm	75 ppm
	OENOBRETT®	Microbial stability	NA	50 ppm	100 ppm
	FUMARIC ACID	Microbial stability	NA	300 ppm	600 ppm
	POWERLEES® LIFE	Antioxidant & mouthfeel	100 ppm	200 ppm	300 ppm
	TAN'FRESH®	Aroma correction	10 ppm	30 ppm	30 - 40 ppm
	NOBILE® OAK	Mask off flavor	See text for options.		
	GEOSORB®	Aroma correction	NA	200 ppm	400 ppm
Preparation for bottling	MANNOSWEET® or AUTOLEES®	Mouthfeel	50 ppm	100 ppm	200 ppm
	EXTRALYSE®	Beta-glucanase enzyme	60 ppm	80 ppm	100 ppm

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