Compromised Fruit: *Botrytis Bunch Rot* and *Powdery Mildew*

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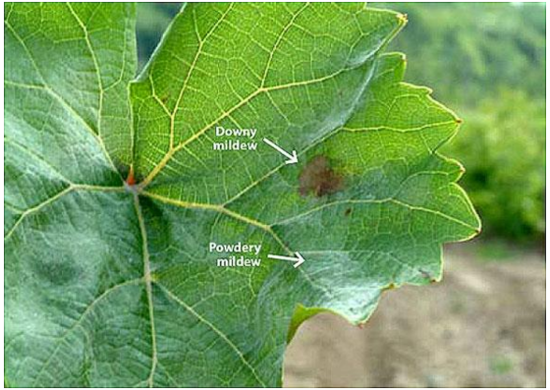
Webinar Formalities

- This Webinar is being recorded and will be published on the Enartis website
- Please refrain from using the chat box during the presentation, there will be 15 minutes for questions at the end of the presentation
- If you are having technical difficulties please use chat box 2, Whitney will be there to help

Overview

- Monitoring tools for Powdery mildew and Botrytis Bunch rot (JT Jaeger)
- Acceptance Levels
- Control Points
- Wine Matrix Alterations
- Grapevine Diseases
  - Powdery Mildew
    - *Erysiphe necator & Oidium tuckeri*
  - Noble Rot or Grey Rot
    - *Botrytis cinerea*



INTRODUCTION TO BOTRYTIS & POWDERY MILDEW

- Introduction to the diseases
- When are we most at risk for these diseases?
- What weather conditions are critical for infection?
- How do we manage these diseases in the vineyard?
- What do we do viticulturally to combat these diseases that might compromise wine quality or interfere with the winemaking process?

BOTRYTIS (*Botrytis cinerea*)- Common Botrytis Rot, grey mold, or noble rot

BOTRYTIS (Botrytis cinerea)

- Found in all major grape producing regions
- Dominant fungus in harvested wine grapes
- Can impart a ‘moldy’ character in wine
- Can reduce yield up to 50 to 60% in severe cases
- Significantly weakens the vines ability to fend off further disease

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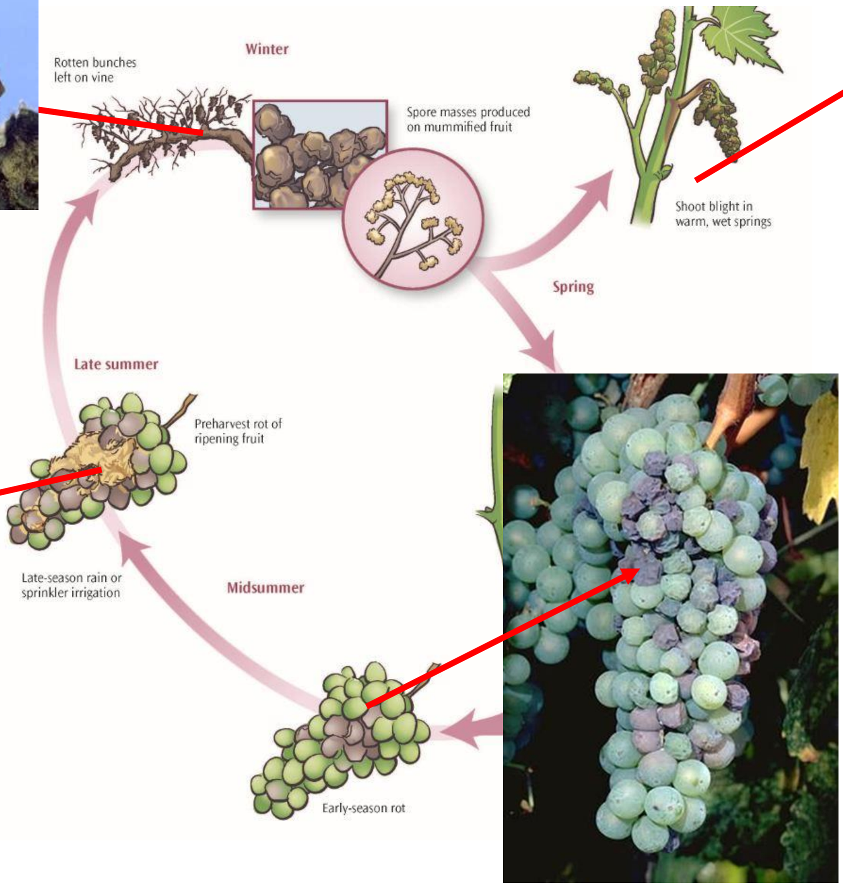
Winter
Rotten bunches
left on vine
Spore masses produced
on mummified fruit
Shoot blight in
warm, wet springs
Spring
Late summer
Preharvest rot of
ripening fruit
Late-season rain or
sprinkler irrigation
Midsummer
Early-season rot


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BOTRYTIS (Botrytis cinerea)

SYMPTOMS OF INFECTION

- Can occur on vegetation (necrosis)
- Berries turn brown (white grapes) or reddish color (red grapes) and shrivel
- Fungus visible on surface in extreme cases
- Single berry infection or whole cluster

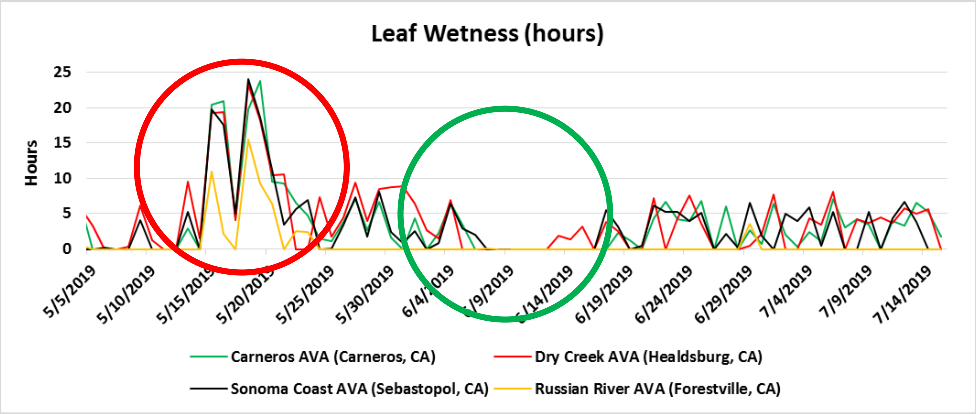
BOTRYTIS (Botrytis cinerea)

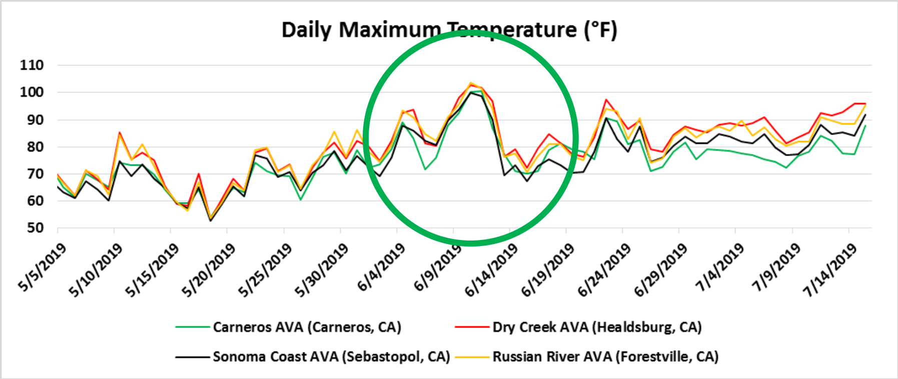
DISEASE RISK

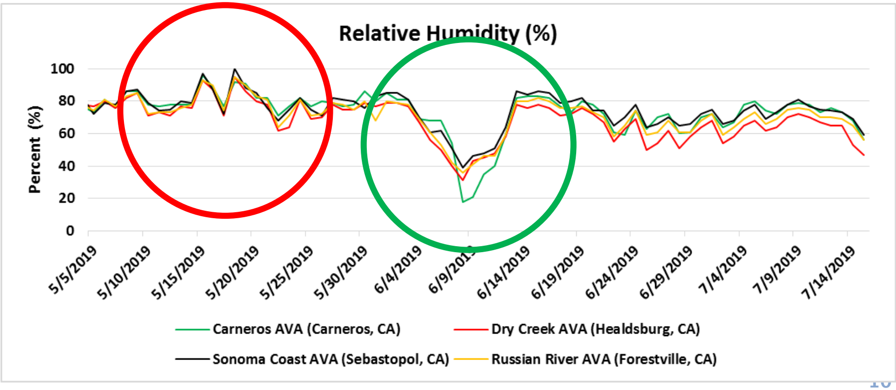
- **High humidity or prolonged rain** (>95% RH) in conjunction with **mild to moderate temperatures** (temps between 50 to 85F)
- Rot can develop when vineyards recieve more than one day of rain on mature grapes
- Optimum temperature is 65 to 72ºF for growth
- Cannot grow above 90 ºF

BOTRYTIS (Botrytis cinerea)

CURRENT CONDITIONS AND SEASON OUTLOOK







Data source: *Western Weather Group*, sonoma.westernweathergroup.com; Data interpretation: *Broome et al. 1995*

BOTRYTIS (Botrytis cinerea)

HIGH RISK VINEYARDS

- Previously infected vineyards
- Sheltered areas such as hollows
- Vineyards with vigorous and dense canopies
- Varietals known for having compact clusters and a thin skin

Most susceptible varietals include Chardonnay, Chenin Blanc, Grey Riesling, Pinot Noir, Sauvignon Blanc, White Riesling, and Zinfandel.

Note: For more information see http://ipm.ucanr.edu/PMG/selectnewpest.grapes.html

BOTRYTIS (Botrytis cinerea)

DISEASE MANAGEMENT

- Canopy management can be more effective then fungicides
- Hand-harvested: fruit can be selectively harvested and infected fruit discarded
- Machine-harvested: hand pickers can be used to remove the worst affected fruit prior to machine harvesting
- Fungicide applications

Note: See the UC IPM grape pest management guidelines for further information (www.ipm.ucdavis.edu)

BOTRYTIS (Botrytis cinerea)

FINAL THOUGHTS

- Botrytis can cause direct crop loss
- Produces an enzyme called laccase that reduces flavor, color, and storage stability of wine
- Makes way for further wine taints
- Early harvest decisions → Under ripe fruit
- Increased farming costs associate with harvest

POWDERY MILDEW (Erysiphe necator and Odium tuckeri)

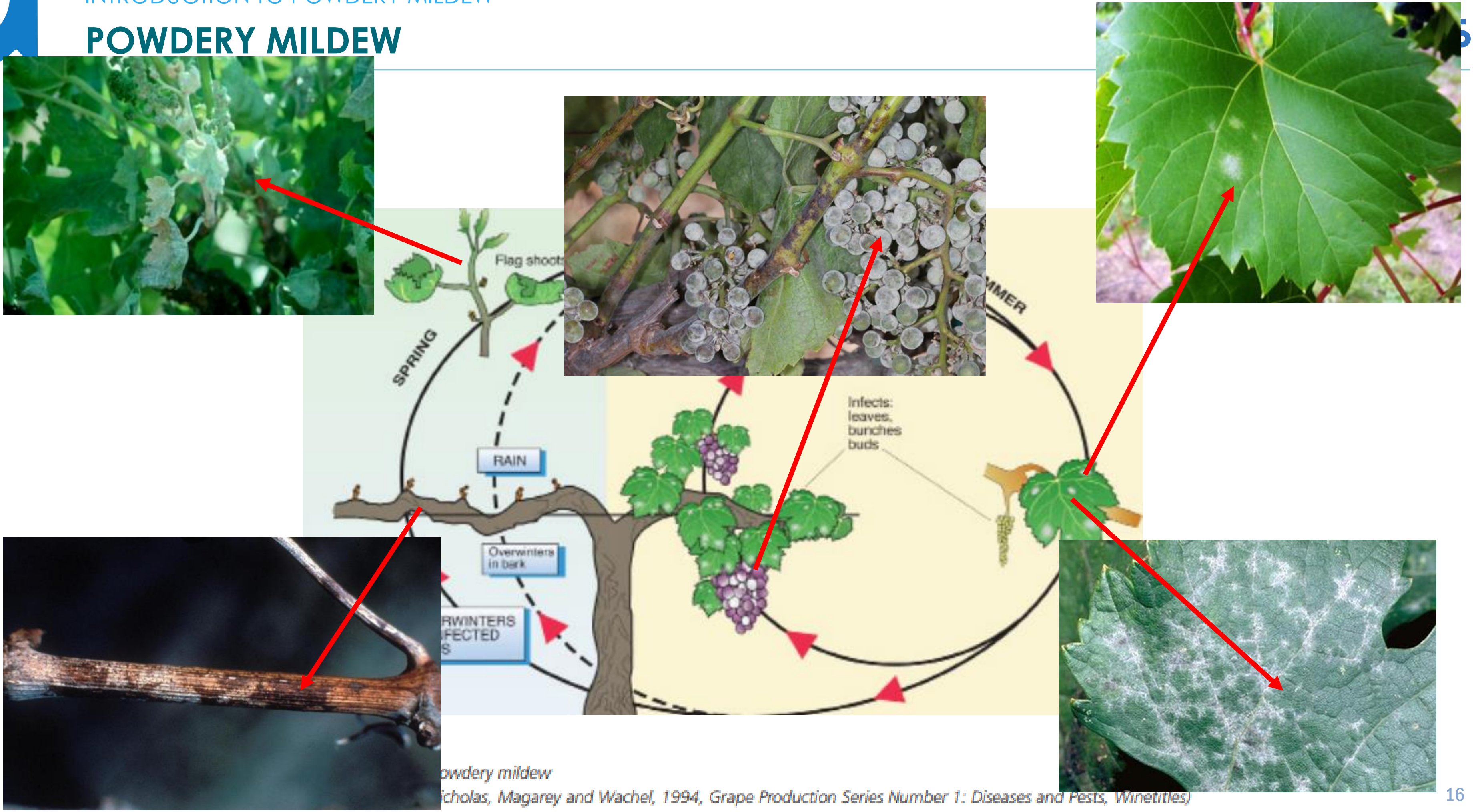
POWDERY MILDEW

WHY SHOULD WE CARE?

- Most widespread and destructive disease in grapevines
- Very good at slowing/stopping the ripening of grapes
- Mushroom-like aroma in the vineyard which can lead to wine associated faults (green, mushroom)
- Damage to fruit leads to secondary infections (i.e. Botrytis)
- Common tool to kill the fungus is elemental sulfur

Note: Not to be confused with Downy Mildew

POWDERY MILDEW



owdery mildew

icholas, Magarey and Wachel, 1994, Grape Production Series Number 1: Diseases and Pests, Winetitles)

POWDERY MILDEW

SYMPTOMS OF INFECTION

- Chlorotic spots (yellowish spots) on leaves
- Infection takes on white, dusty (powdery) appearance on leaves and fruit
- Brown or black scaring on fruit and shoots/canes

POWDERY MILDEW

DISEASE RISK

- **Does not need free moisture to germinate after initial germination** (i.e. rain or foggy weather)
- Ideal temperatures for growth are between 70° and 85°F
- High temps >95°F does not kill the fungus
- One spore can multiply to 50 million spores in just 3 weeks

POWDERY MILDEW INDEX (PMI)

SPRAY INTERVALS BY FUNGICIDE GROUPS BASED ON DISEASE PRESSURE USING THE UC DAVIS POWDERY MILDEW RISK INDEX MODEL

			Suggested spray schedule			
Index	**Disease pressure**	**Pathogen status**	**Biologicals[1] and SARs[2]**	**Sulfur**	**Demethylation-inhibitors (DMI)[3]**	**Strobilurins and Quinolines[4]**
0-30	low	present	7- to 14-day interval	14- to 21-day interval	21-day interval or label interval	21-day interval or label interval
40-50	moderate	reproduces every 15 days	7-day interval	10- to 17-day interval	21-day interval	21-day interval
60 or above	high	reproduces every 5 days	use not recommended	7-day interval	10- to 14-day interval	14-day interval

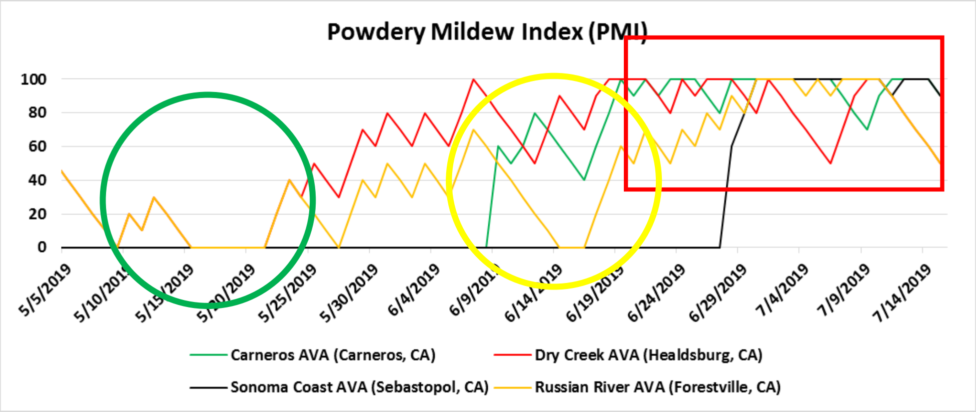
1 *Bacillus pumilis* (Sonata) and *Bacillus subtilis* (Serenade Max)
2 SAR = Systemic acquired resistance products
3 Tebuconazole (Elite), triflumizole (Viticure), and myclobutanil (Rally)
4 Trifloxystrobin (Flint), kresoxim-methyl (Sovran), and pyraclostrobin/boscalid (Pristine)

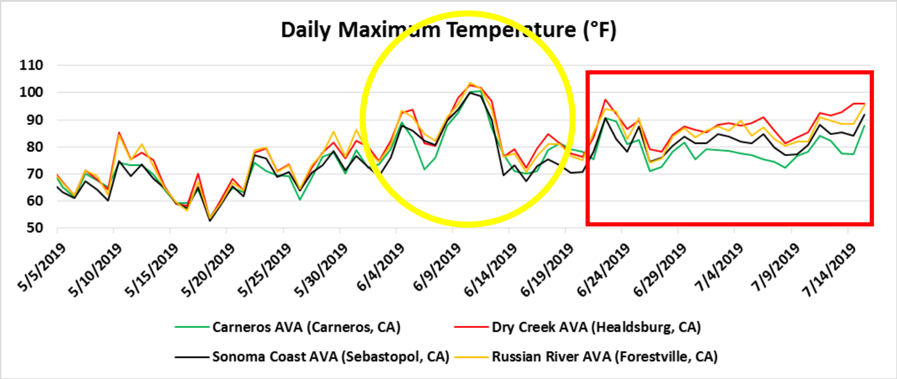
- This chart is used by vineyard personnel to know how often to spray various products to combat Powdery Mildew

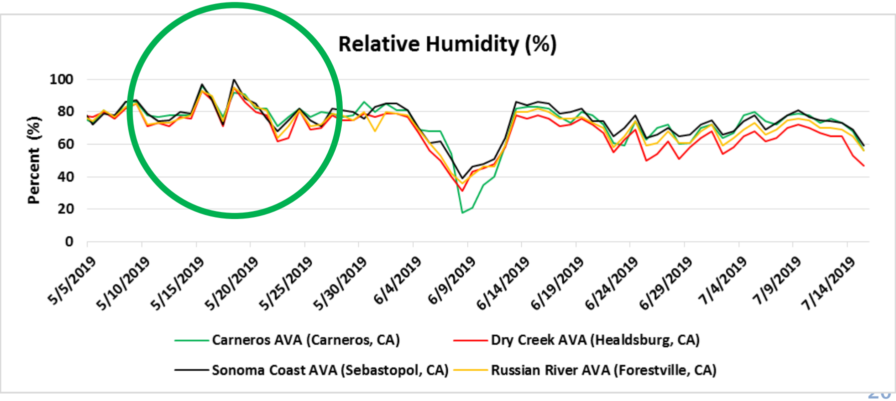
- Risk Index for Sonoma county: http://forecasts.westernwx.com/sonoma/SonomaPMhistory.htm
- Risk Index for other CA counties: http://ipm.ucanr.edu/calludt.cgi/GRAPEPMVIEW1

POWDERY MILDEW

CURRENT CONDITIONS AND SEASON OUTLOOK







Data source: *Western Weather Group*, sonoma.westernweathergroup.com; Data interpretation: *UC IPM* www.ipm.ucdavis.edu

POWDERY MILDEW

HIGH RISK VINEYARDS

- Previously infected vineyards
- Areas down wind of infected vineyards
- Shaded or dense parts of vine canopies
- Sheltered vineyard sites such as hollows

Note: most susceptible varietals include Barbera, Cabernet Franc, Cabernet Sauvignon, Chardonnay, Chenin Blanc, Merlot, Pinot Blanc, Pinot Noir, Sauvignon Blanc, and White Riesling

Note: For more information see http://ipm.ucanr.edu/PMG/selectnewpest.grapes.html

DISEASE MANAGEMENT

- Early season:
  - Cover crop management
  - Application of lime sulfur or stylet oil before budbreak
- Mid-season to pre-harvest:
  - Fungicide applications
  - Sulfur most common
- Year round: canopy management

Note: See the UC IPM grape pest management guidelines for further information (www.ipm.ucdavis.edu)

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FINAL THOUGHTS

- A severe infection of powdery mildew can decrease crop production for several years
- Diseased berries can cause off flavors in wine
- Splitting of infected berries increases susceptibility to bunch rots (i.e. Botrytis)
- Frequent and season long usage of sulfur can cause significant amounts of residues on clusters at harvest

CITATIONS

- Broom et al. 1995. Development of an infection model for Botrytis bunch rot of grapes based on wetness duration and temperature. Phytopathology 85:97-102
- Gadoury 1995: UC IPM Pest Management Guidelines 2002, www.ipm.ucdavis.edu/PMG/selectnewpest.grapes.html
- Weather data exported from Western Weather Group, sonoma.westernweathergroup.com

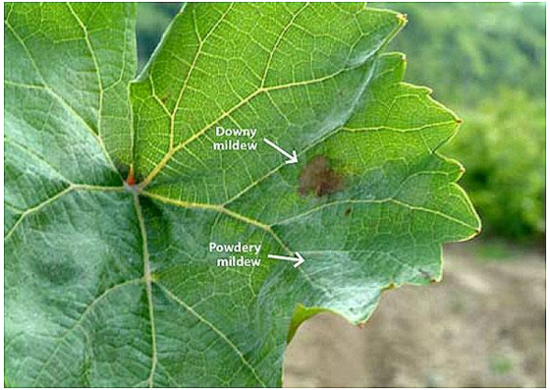
- Please see the UC IPM grape pest management guidelines (www.ipm.ucdavis.edu) for continually updates information of fungicides, including both organic and inorganic materials.

PLEASE SAVE QUESTIONS UNTIL THE END OF THE PRESENTATION

Compromised Fruit: *Botrytis Bunch Rot and Powdery Mildew*

Overview

- Monitoring tools for Powdery mildew and Botrytis Bunch rot (JT Jaeger)
- **Acceptance Levels**
- **Control Points**
- **Wine Matrix Alterations**



Acceptance Levels

How much is to much?

Assessment Methods: *Defining Incidence and Severity*

- **Visual assessment**
  - % Bunch area with symptoms and average severity in vineyard (crop infection)
  - Bunch rot assessment trainer (BRAT)
- **Image analysis**
- **Quantitative PCR**
- **Spectroscopic techniques**
  - Near-infrared (NIR)
  - Mid-infrared (mid-ir) spectroscopy

BRAT Bunch Rot Assessment Trainer

Vinquiry Lab analysis

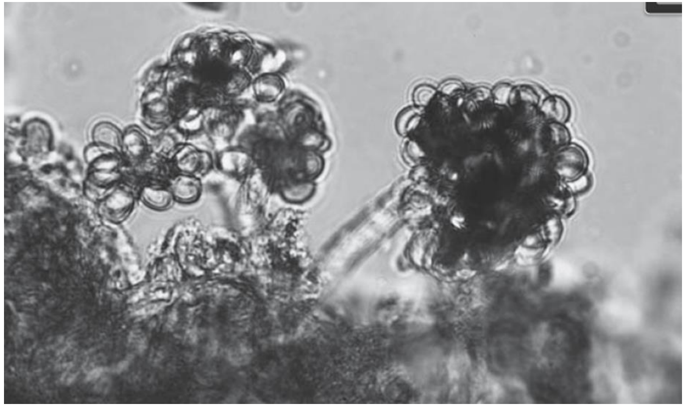
- Volatile acidity (acetic acid)
- Glycerol → >15 g/L
- Filterability Panel
  - Glucans→ Filterability

- Botrytis Panel
  - Gluconic acid→ >1 g/L
  - PCR
    - Low Risk 100 spores/mL
    - Medium risk 100-500 spores/mL
    - High risk >500 spores/mL

VINQUIRY LABORATORIES

Grapevine Diseases Alterations to Must

Grey Mold, Noble Rot
Botrytis cinerea



Sour rot and Botrytis infections are the most common causes of heavy grape berry crop losses

Noble Rot-Grey Rot *Botrytis cinerea*

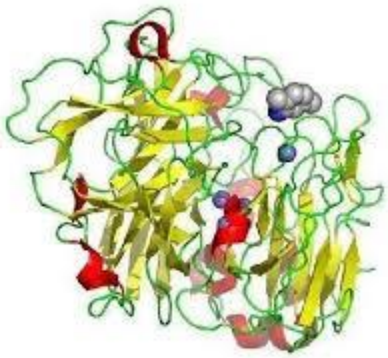
- **Enzymes**→ laccase
  - Oxidation→ color and aroma
- **Extracellular glucans (β 1-3, β 1-6) contribution**
- **Biogenic amines, mycotoxins**
- **Depletion of nitrogen and amino acids** → Low free amino nitrogen
- **Mold and earth off flavors**→ Sotolone, *l-octen-3-ol (mushroom)*
- **Oxidizes and depletes organic acid**
- **Secondary Infections**

- **Depletion of Free SO_2**

Wine Matrix Alterations

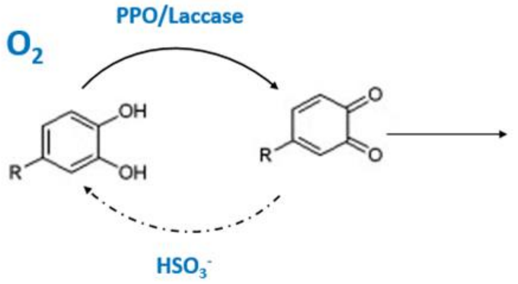
- **Secondary metabolites**
  - Glucans
  - Biogenic amines
  - Sensory modification

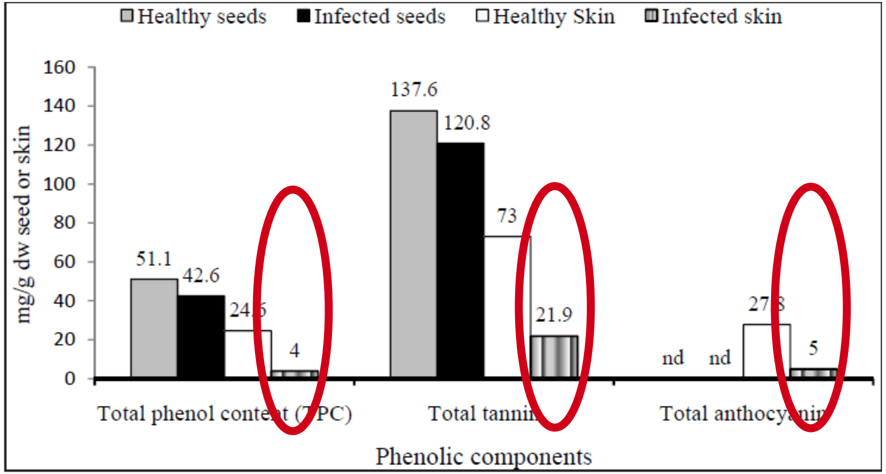
- **Enzyme activity Laccase**
  - **Resistant to SO_2**
  - Ethanol tolerant
  - Heat sensitive →Thermovinification, Flash
  - Copper dependent→ chelators
  - Bentonite fining can help reduce activity



Oxidative enzymes- Polyphenol oxidases

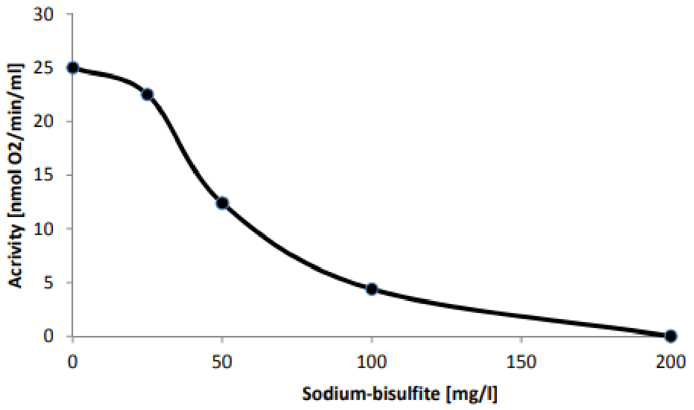
- Laccase → *Botrytis cinerea*



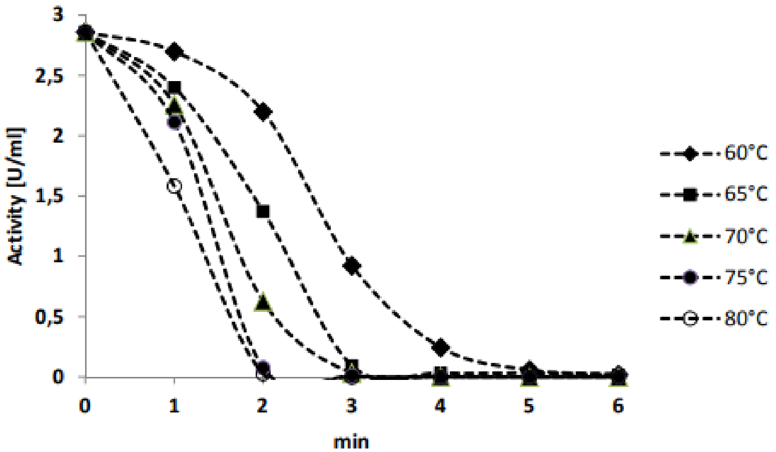


Concentration of phenolic components in seeds and skins of healthy and botrytized grapes
(Ky et al, 2012)

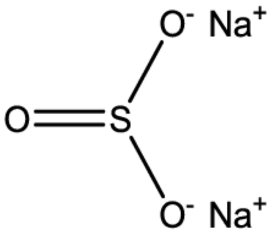
Inhibition of Laccase in Must



Inhibition of laccase activity by sulfite

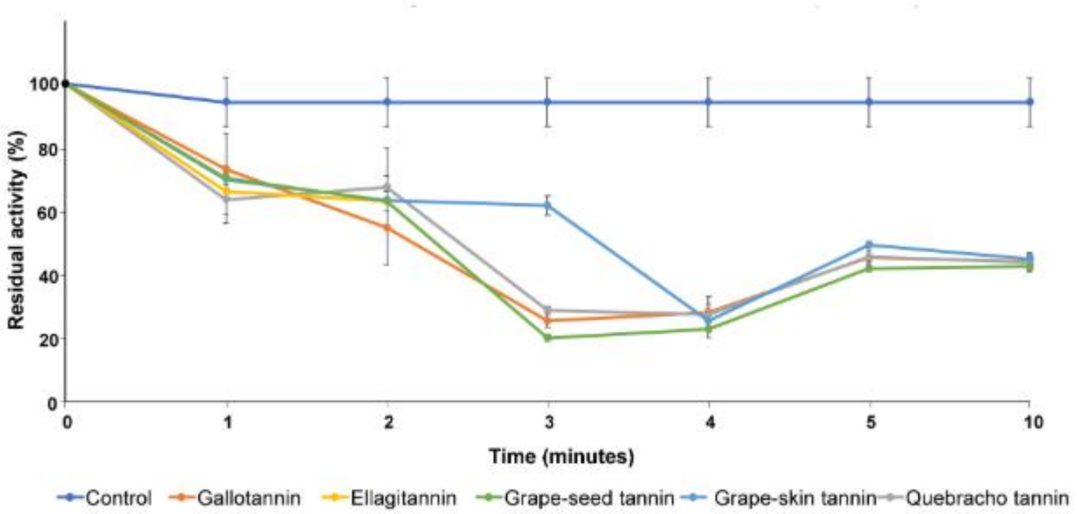


Inhibition of laccase activity by must heat treatment



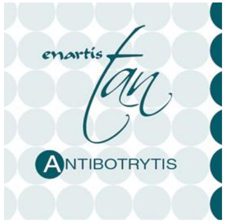
(Claus et al, 2014; Zivkovic et al, 2011)

Inhibition Kinetics of Enological tannins on laccase activity



(Vignault et al, 2019)

- Dosage 40 g/hL
- Maximal inhibitory effect after 3 minutes
- All enological tannins were effective in inhibiting laccase



PRODUCT	DOSAGE	REDUCTION OF OXIDASIC ENZYME ACTIVITY
SO_2	50 ppm	25%
	75 ppm	62%
ENARTIS TAN ANTIBOTRYTIS	20 g/hL	60%

Grapevine Diseases Alterations to Must: *Botrytis cinerea*

- **Microbial inhibition and competition**
  - botryicine
- **Nutrient depletion**
  - thiamine (vitamin B1), and pyridoxine (vitamin B6)
- **Acid metabolism**
  - 70 to 90% of the tartaric acid
  - 50 to 70% of the malic acid
  - Increase in pH and decrease in TA

Impact of Botrytis cinerea on must and wine

Increasing	Decreasing
Glucose, Fructose, Galactose, Arabinose	Glucose/Fructose
Polyols (Glycerol, Mannitol, Sorbitol, Inositol, Erythrol)	
Gluconic acid, Malic acid, Acetic acid, Citric acid, Galactaric acid, Galacturonic acid , 2-Ketoglutaric acid, Pyruvic acid	Tartaric acid
Polysaccharides (β-Glucan), Viscosity	Filtrability
Laccase, Glucosidases, Esterases, Pectinases	Polyphenol content Colour (Anthocyanins)
Proteases	Nitrogen content (Amino acids, Proteins); Foaming properties of sparkling wine
Ethanol, Methanol Botrycins Osmolarity	Vitamins (Thiamin)
Microbial diversity Osmotolerant and fructophilic yeasts Acetic- and Lactic acid bacteria	*Oenococcus oeni*
SO_2 demand	

(Claus et al, 2014)

Secondary Infections

- Skin rupture of the berry by physical or biological factors opens the door for opportunistic yeast, mold and bacteria

- Insects are main vector for transporting these yeast and bacteria
  - *Drosophila melanogaster*

- Sour Rot
  - *Gluconacetobacter saccharivorans*
  - Acetic acid bacteria





Control Points: *Botrytis cinerea*

- Vineyard managment
- Selective sorting harvest
- Reception and fruit sorting → Oxidation and antimicrobial control!
  - Ascorbic Acid
  - Hydrolyzable tannins
  - Chitosan
  - SO_2
- Press fraction separation
- Minimize maceration for reds
- Increase settling velocity for whites

Control Points: *Botrytis cinerea*

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Fermentation Problems

- Higher sugar due post-infection dehydration → increased osmotic stress
- Botryicine → leads to yeast inhibition coupled with high acetic acid and glycerol
- 7-61% loss of amino acids (organic nitrogen)
- Supplement! + 100-200 mg/L

NUTRIFERM AROM PLUS

NUTRIFERM AROM

NUTRIFERM ENERGY

Filterability

- Conversion of grape sugars into polymers of glucose→ Glucans
- Large molecular weight→ 80 kD
- Commercial glucanases→ Effective, add to wine at first racking
- Pre-fermentation additions → require higher dosage and extended time

Powdery Mildew:
Erysiphe necator & Oidium tuckeri

Powdery Mildew Erysiphe necator & Oidium tuckeri

- 9% contamination incidence will decrease wine quality
- Elevated mycotoxins and biogenic amines
- Moldy off-flavors, green aromas and loss of varietal characters
- Nutrient and vitamin depletion
- High protein instability
- High pH and potassium levels
- Higher phenolic content
- Wines lacking mid-palate, volume and balance

Managing Metals

- **High disease pressure**
  - Late fungicide applications of $CuSO_4$ and sulfur
- **Claril HM**
  - Copolymers of vinylimidazole and vinylpyrrolidone PVI/PVP
  - Chitosan
- **Stabyl MET**
  - PVI/PVP
  - Silicon dioxide
- **Pro FT**
  - PVI/PVP
  - Mannoproteins
  - S-containing antioxidants and peptides

Remediation

all is not lost…

Please refer to supporting documents for detailed full protocols for Powdery Mildew and *Botrytis cinerea* compromised fruit.

Powdery Mildew Remediation: Red and White Wine

- Hand harvest and sorting
- Antioxidants→ prevent browning
- Microbial stabilization
- Limit skin contact for reds
- Fast settling and clarification for whites
- For reds utilize tannins to promote stable color condensation and co-pigmentation
- Select a resistant, aromatic and dominant yeast strain with low nitrogen needs
  - Yeast nutrition supplementation
- Balance wine mouthfeel with mannoproteins and fermentation tannins

Picture of juice settling 2 hours after 4 g/hL of Enartis Zym RS(P) addition. Enartis Zym RS(P) improves clarification and increases speed of settling.

Botrytis Winemaking strategies: White wine

- Whole cluster press → separate first 10+ gallons/ton mold metabolites
- Evaluate browning of separate press fractions
- Sulfite 0.8 ppm molecular and immediately cold clarify with bentonite
- Control pH <3.5
- Allow polymerization and precipitation of oxidized phenols at low temperatures prior to SO_2 and fining
    - Gelatin, cellulose and silica

Botrytis Winemaking strategies: Red Wine

- Tricky…
- Short vatting
- Acidulate to <3.5 pH
- Microbial control → SO_2 and Chitosan
- Heat treatment→ Thermovinification or heat exchange
- Claril SP and phenolic fining→ get rid of quinones and browning products if there is visual browning!
- Problems with solids and post-fermentation clarification→ Enzymes

Thanks!
Questions?

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Citations

- Claus, H., Sabel, A., & König, H. (2014). Wine phenols and laccase: an ambivalent relationship. *Wine: Phenolic composition, classification and health benefits*, 155-185.
- Ky, I., Lorrain, B., Jourdes, M., Pasquier, G., Fermaud, M., Geny, L., Rey, P., Doneche, B. and Teissedre, P.L. 2012. Assessment of grey mould (Botrytis cinerea) impact on phenolic and sensory quality of Bordeaux grapes, musts and wines for two consecutive vintages. Australian Society of Viticulture and Oenology, 18: 215-226.
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- Zivkovic, K., Kˆnig, H., Claus, H., (2011). Wirkung von Bentonit auf die Laccase-Aktivit in Most und Wein. Deutsche Lebensmittel-Rundschau 107, 575-582.

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