What is *Botrytis cinerea*?

*Botrytis cinerea* is a weather-driven fungus which causes the grapevine diseases botrytis bunch rot and grey mould, as well as the ‘noble rot’ used for sweet wines. High humidity or prolonged rain in conjunction with cool or mild temperatures results in persistent moisture on berry surfaces and promotes infection and disease development. Previously infected sites and sheltered vineyard areas such as hollows are at greatest risk of developing the disease.

What are the implications for winemakers?

Managing *Botrytis* in the vineyard can be a challenge because many factors contribute to infection and disease development. For winemakers there are two main reasons to be concerned about *Botrytis*-infected fruit. First, *Botrytis* produces the enzyme laccase, which in the presence of oxygen can cause oxidative spoilage. Second, *Botrytis*-infected fruit can result in a mouldy character in wine. Preventing laccase from causing damage requires techniques that minimise or eliminate exposure to oxygen.

*Botrytis*-infected red grapes will require different treatments to white grapes. The following processing strategies will help reduce the issues that can be caused by *Botrytis*-infected fruit.

**Strategies to use for both red and white grapes**

- Minimise the quantity of mould-affected fruit at harvesting. For crops picked by hand, fruit can be selectively harvested and infected fruit avoided. Pickers must be able to recognise *Botrytis* bunch rot and
know which bunches should be avoided. If the vineyard is to be machine-harvested, hand pickers can be used to remove the worst affected fruit prior to machine harvesting.

- Add more sulfur dioxide (SO₂) than usual (in the range of 60-100 mg/L depending on severity) due to the increased risk of oxidation from laccase (although SO₂ does not inactivate laccase) and because there is likely to be a higher than usual population of other unwanted microorganisms if *Botrytis* is present.

- Measure and adjust yeast assimilable nitrogen (YAN), as *Botrytis* and other microbiological growth on grapes can deplete natural levels.

- It is recommended that a test for laccase activity is conducted as early as possible, and if detected, heat treatment (pasteurising) should be considered to deactivate the laccase enzyme before fermentation (see qualitative and quantitative tests for laccase activity below).

- Laccase requires oxygen to cause oxidative damage, therefore *winemaking techniques which reduce or eliminate must, juice and wine contact with oxygen should be considered paramount*.

**Strategies for processing white grapes**

- Whole bunch press with CO₂ cover. Assess press fractions for mouldy taint carefully.

- Add pectic enzyme at the higher end of the recommended range and use flotation to clarify juice if available, otherwise cold settle at low temperature to achieve rapid settling. Extra additions of pectic enzyme might also be required if settling.

  - Rack and discard the heavy lees.
  
  - Trial bentonite additions to remove mouldy characters and settle for 24 hours. Recommended starting rate is 0.5 – 1 g/L bentonite.

  - Rack off bentonite lees.

**Strategies for processing red grapes**

- Minimise the time between crushing and yeast inoculation and avoid cold soaking.

- Initiate fermentation as early as possible, allowing yeast to quickly consume all the available oxygen.

- Add a greater than usual yeast inoculum (~120%) to assist with binding free SO₂ and shortening pre-fermentation lag time.

- Laccase is an enzyme and as such is a protein, which to some extent can be ‘fined’ out using tannin. Consider adding 200-500 mg/L of an oenological tannin at crushing to bind the laccase (note that tannin addition can change wine style).

- Consider pressing earlier than usual, while a large amount of CO₂ is still being produced. While there is active fermentation yeast will scavenge oxygen introduced during pressing, and secondly, the CO₂ cover will also provide a degree of protection from the ingress of oxygen.

- At the end of fermentation, much of the laccase will have been bound to grape solids and will therefore be in the lees. Where practical, separate heavy fermentation lees at pressing, and press to stainless steel (not oak), and avoid ullage.
• Rack off gross lees after 24 hours, being careful not to introduce any oxygen, and test for laccase activity in the wine.

• If laccase activity is still detected, consider adding some tannin as a fining agent. Allow it to settle, rack again, and again test the wine for laccase activity.

• If laccase activity is still detected, heat treatment of the wine might be necessary.

• Consider the use of a suitable enzyme to assist with clarification and filtration. Red wines made from Botrytis-affected grapes are often difficult to clarify and filter due to the presence of long chain polysaccharides.

Qualitative test for laccase activity
A simple bench test can be used to obtain a qualitative result for laccase activity. Sulfur dioxide is added to the sample in question to give a total SO$_2$ concentration of about 60 mg/L. The sample is then poured into two wine glasses (approximately 50 mL of sample in each glass) and each glass is covered with a watch glass or petri dish lid. One sample is placed in a refrigerator, while the other sample is left 'on the bench'. The samples are examined after 24 hours and compared for any change in colour or quality. If there is laccase activity, the sample left on the bench should be browner than the sample left in the fridge and may have an oily film on the surface of the wine.

Quantitative test for laccase activity
Quantitative determination of laccase activity can be achieved using a number of commercially available test kits. Affinity Labs performs simultaneous quantitative and qualitative analysis for laccase activity.

If laccase activity is detected, heat treatment (pasteurising) should be considered to deactivate the laccase enzyme before conducting the fermentation. It is recommended that the juice be pasteurised at a minimum temperature of 65°C for 40 seconds and wine at a minimum temperature of 65°C for 20 seconds.
Figure 1. Deactivating the laccase enzyme is a time and temperature relationship, where the higher the temperature, the shorter the amount of time required for deactivation to occur. Graph prepared from data provided by Dr Roger Boulton (pers. comm.)

If heat treatment is not available, initiate fermentation. Addition of 0.1 – 0.2 g/L of bentonite during fermentation might be beneficial.

As SO₂ can inactivate thiamine, the addition of thiamine to the must should be considered.

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Further reading


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