TECHNICAL BOOKLET

MANAGING BOTRYTISED HARVESTS

Warm and wet vintages, hail or poor aeration in the vineyard are all factors that increase the risk of *Botrytis cinerea* developing as "grey rot". When this fungus develops, the enological consequences can be significant. The winemaker must therefore evaluate the level of grape contamination in order to adapt their vinification process.

In this practical booklet, Lamothe-Abiet describes the key steps that should be monitored and the ways to effectively treat grapes contaminated with *Botrytis*.

***Botrytis cinerea* is a phytopathogenic fungus that takes advantage of the smallest opening to develop. It progresses rapidly with various damaging effects:**

- **Reduced yield**
- **Nitrogen** and **thiamine deficiency** causing problems for fermentations
- **Production of aromatic compounds** causing mouldy, earthy odours
- **Strong laccase activity,** accelerating oxidative mechanisms.
- **Fragile grapes,** sensitive to secondary contaminations (*Penicillium*, *Aspergillus*, etc)

PRODUCTION OF SPECIFIC COMPOUNDS: THE MECHANISMS OF *BOTRYTIS CINEREA* ATTACK

***Botrytis cinerea* secretes numerous compounds that can decrease the wine's quality and which make winemaking more complicated.** These compounds are mainly enzymes, as well as polysaccharides, which act on different aspects:

- **Pectinases** are responsible for hydrolysing the pectin chains of the grapes' pellicules. This facilitates juice extraction, removing the need for adding exogenic pectinases. However, where there is an early contamination, the grapes are fragile and secondary contaminations are induced due to pests.
- **Laccase** is a polyphenol-oxidase specific to *Botrytis*. It is particularly resistant (SO_2, low pH, high temperatures) and powerful. It is not removed during clarification (as it is totally soluble), and can quickly oxidise musts and phenolic compounds.
- **Glycosidases** are responsible for loss of aromas, through hydrolysis of terpene glycosides which are oxidised by *Botrytis* into less aromatic compounds.
- **β-D-glucanes** are polysaccharides with a high molecular weight. They make clarification and filtration of musts or wines particularly difficult. They are not degraded during pre-fermentation maceration or the alcoholic fermentation.

Grapes can be considered to be contaminated by *Botrytis cinerea* if the glucan test is positive or the number of laccase units is above 2 U/mL.

Find more information on this test on our website in "Protocols" section

DETECT CONTAMINATED GRAPES AND TAKE APPROPRIATE ACTIONS

1. Means of detection

OBJECTIVE	METHOD
Evaluate the presence of rot	Gluconic acid measurement (specific *Botrytis* marker)
Quantify laccase activity	Colorimetric test with syringaldazine: Botrytest (available at Lamothe-Abiet), test in presence of air and oxygen consumption test (polarographic method)
Evaluate presence of glucans	Simple or modified glucan test (practical document available). Grapes are judged to be contaminated by *Botrytis cinerea* if the glucan test is positive or if the number of laccase units is > 2U/mL.
Measuring young wine's filterability	CFLA measurement (Lamothe-Abiet filterability criteria)

2. Technical remedies to *Botrytis* contamination

PROBLEM	SOLUTION
Risks associated with *Botrytis* contamination : Polyphenol oxidation (laccase) and aromatic losses (glycosidase, esterase)	Do not allow laccase to come into contact with oxygen until it is removed Early stabilisation of colour and aromas
Botrytis' pectolytic enzymes naturally increase extraction. There is therefore a risk of over-extraction and spoilage	Decrease the dosage of extraction enzymes on grapes (Do not use at all when more than 10% *Botrytis*)
Risk of "mouldy" tastes and odours	Specific treatment (ex: decontaminating carbon)
Risk of nitrogen and thiamine deficiency	Specific nitrogen nutrition, including thiamine
Botrytis glucans complicate clarification and filtration. The microbiological risk is increased as a consequence	Use specific β-glucanases for clarification
Risk of reduction	Aerate once laccase activity has disappeared (to be verified)

SOME TIPS

- *Rigorously sort, in the vineyard or on processing in the winery*
- *Adjust sulfite addition depending on health of grapes*
- *Low extraction (short maceration, reduced mechanical actions, buffer tank…)*
- *Remove the first hectolitres of juice (in receival hopper) and treat them separately*
- *Inert tanks using CO_2*
- *Exclude air until laccase has totally disappeared*
- *Check that laccase activity several times to verify that it has disappeared beforing oxygen addition*

BOTRYTISED WHITE GRAPES

1. Removing laccase activity

Botrytis' laccase activity causes oxidation of must's phenolic and aromatic compounds. Removing this activity as early as possible helps to conserve the must's organoleptic quality.

Before acting, it is important to adapt the SO_2 doses to the sanitary condition. Juices coming from the bottom of the hopper must be separated and treated separately, as they concentrate a strong laccase activity.

Harvest with medium contamination: direct pressing

Objectives: **1. Quickly inhibit laccase activity, remove its substrates (phenolic compounds)**
2. Limite the extraction of undesirable compounds, treat them if needed

L.A SOLUTIONS

Tannin addition: Tanin gallique à l'alcool **- 5 à 15 g/hL**

- Inhibits the laccase activity
- Antioxidant and anti-oxidasic role, allowing for decreased SO_2 addition
- Easy to use and immediate effect
- Precipitates unstable proteins

Highly contaminated harvest: hyperoxygenation

Objectives: **1. Use the laccase activity to oxidise the phenolic compounds and exhaust the substrates**
2. Facilitate the removal of the quinones that have formed through fining/racking

L.A SOLUTIONS

In the case of hyperoxygenation, sulfite **should not be added** to the harvest so that the laccase activity is optimal. Hyperoxygenation **also consumes all of the glutathione**.

After hyperoxygenation :

a) Add **sulfite** to the must (2 to 4 g/hL)

b) **Fine the must in order to remove the quinones that have formed:**
Products of the Greenfine® range **- 20 to 100 g/hL depending on the state of the must**

- Fining based on plant proteins
- Eliminating the oxidised or easily oxidisable compounds
- Preserves aromas
- Readjusts colour.

c) **Increase the antioxidant potential** using formulations of inactivated yeasts rich in reducing compounds, or using tannins
Aroma Protect ® **- 20 to 30 g/hL**

- Yeast extract naturally rich in glutathione

2. Clean an clarify musts

Even if it has been blocked by the prior actions, the laccase activity leaves undesirable compounds in the must. It is therefore important to properly clean the must for an optimal alcoholic fermentation.

Objectives: **1. Facilitate the rapid clarification of musts to obtain clean juice**
2. Remove oxidised polyphenols with fining

L.A SOLUTIONS

a) Optimise clarification using specific enzyme addition

It is important to **remove *Botrytis*' glucans early on**, in order to optimise must clarification. These polysaccharides are structured as a mesh which holds particles in suspension and complicates clarification. The use of **specific enzymes promotes a rapid action**.

- Vinotaste® Pro **- 10 g/hL :** rapid clarification of musts
- Vinoclear® Classic **- 1 to 2 mL/hL :** clarification enzyme, helps to compact solids
- Vinozym® Ultra FCE **- 1 to 2 mL/hL :** liquid enzyme formulation, for maceration and clarification of white and rosé wines

b) Must fining is effective on phenolic compounds

Once the glucans are removed, must fining can be carried out to **eliminate the oxidised or oxidisable phenolic compounds** which have a negative influence on the wine's **aromatic potential**.

- Produits de la gamme GreenFine® **- 20 to 100 g/hL :** pea protein formulations
- Polymix® **- 30 to 100 g/hL :** PVPP, potassium caseinate
- Polymix® Natur' **- 30 to 100 g/hL :** PVPP, calcium bentonite, inactivated yeasts
- Géospriv (poudre & granulé) **- 30 to 100 g/hL :** active carbon from plant origin for removal of mouldy-earthy characteristics

BOTRYTISED RED HARVEST

1. Manage the extraction

The laccase activity caused by *Botrytis* contamination results in extreme oxidation of the phenolic and aromatic compounds in the must. In order to preserve the must's organoleptic quality, it is necessary to remove this activity as early as possible.

As for white wines, the juices at the bottom of the hopper should be separated and treated separately. Aerated pump-overs should be avoided until the laccase activity has been totally eliminated.

Thermovinification (>70ºC)

Objectives: **1. Denature the laccase: laccase is an unstable protein and can be denatured with heat (>70ºC)**
2. Remove or decrease the vegetal characteristics from pyrazines (IBMP is volatile)

NB: thermovinification does not denature Botrytis' glucans

L.A SOLUTIONS

a) Thermovinification: heat the grapes up to a temperature > 70ºC
Warning: the increase in temperature must be fast enough to avoid increasing the laccase activity (which is optimal between 40-50ºC)!

b) Enzymes and clarification

- Vinoclear® Classico **- 1 to 2 mL/hL :** facilitates clarification of thermovinified musts

NB: the enzyme should be added after heating once the must has cooled to below 55ºC; this will give optimal enzyme activity.

Traditional vinification

Objectives: **1. Work quickly, without aeration until laccase activity is removed**
2. Conserve the must's antioxidant potential

L.A SOLUTIONS

a) Tannin addition: use of **Pro Tanin R - 40 to 80 g/hL**

- Inhibits laccase activity
- Antioxidant and antioxidasic role, limit sulfite addition
- Easy to use and works immediately
- Precipitates unstable proteins

b) Enzyme addition: limit use of extraction enzymes since laccase will have already strongly weakened the grapes' cell walls.

2. Optimising the alcoholic fermentation

Botrytis contamination leads to nitrogen deficiencies and naturally facilitates the extraction of pellicular compounds, due to the activity of released pectinases by the fungus. The winemaker must compensate this nitrogen deficiency and moderate extraction during the alcoholic fermentation.

Objectives: **1. Correct the nitrogen deficiencies caused by *Botrytis***
2. Optimise the fermentations to reduce time on skins so as not to over-extract

L.A SOLUTIONS

Laccase activity is stimulated by the presence of oxygen. Therefore aerated pump-overs must be avoided. However, oxygen is required for the synthesis of membrane sterols, which improve the fluidity and functionality of the yeasts' cell walls. Without these, the yeasts' metabolism is incomplete and there is an accumulation of untransformed compounds (squalenes, lanosterols) in the cell walls.

It is therefore important to use a **yeast preparatory** product to add exogenous sterols.

a) Activator: The use of a preparatory product adds exogenous sterols, which allows oxygen additions to be limited during the alcoholic fermentation. This will optimise the cellular metabolism and improve the yeasts' viability.

- **ŒenoStim® - 30 g/hL :** Addition of survival and growth factors

b) Yeast addition: add yeast (20g/hL) as soon as grapes are in tank. Limit time on skins and aerations (until the laccase activity is totally eliminated), as well as mechanical extraction (pumpovers, push downs etc). Use yeast strains which are quick to start and finish the fermentation.

- **Excellence® XR, DS, SP, LAL13 - 20 g/hL**

c) Nutrition: *Botrytis Cinerea* consumes nitrogen during its development and induces a deficiency in assimilable nitrogen. It is therefore necessary to correct the must by adding a specific nutrition, rich in **nitrogen** and **thiamine**.

- **VitaFerment® or VitaFerment® PH - dosage according to level of deficiency**

3. Colour and aroma stabilisation

Certain compounds produced by the fungus and secondary spoilage microorganisms lead to the appearance of unpleasant aromas and tastes. These compounds must be quickly removed to conserve the wine's quality. Furthermore, these compounds can reduce the wine's quality if the grapes are over-extracted. It is therefore necessary to ensure colour stabilisation by limiting extraction as much as possible.

Objectives: **1. Remove mouldy-earthy aromas and taste (GMT) linked to secondary contaminations (*Aspergillus*, etc.)**
2. Stabilise the wines' colour as early as possible (after alcoholic fermentation) since the amount of extracted tannins is too low to ensure anthocyanins stabilisation.

L.A SOLUTIONS

a) Must fining: it is recommended to use a decontaminating activated carbon highly effective against mouldy-earthy tastes, with a low impact on colour.

- **Géospriv - 20 to 40 g/hL :** limit contact to to 24h to avoid the risk of re-release of compounds - max. dosage: 100 g/hL

Regulations (EU) 606/2009 specify the usage of decontaminating carbon. Usage should be done before the end of the alcoholic fermentation. On fermenting red must, the tank can be drained (without oxygen), the must treated then filtered and reintegrated into the tank. Thus, the carbon is removed. A record must be kept when using activated carbon.

b) Fining finished wine: it is possible to fine the wine later, with a product that is effective against GMT.

- **Polymix® - 40 g/hL :** potassium caseinate, PVPP

c) Treatment during AF to stabilise colour:

- **Softan® Vinification - 20 à 40 g/hL :** Vinification tannin, bound to polysaccharides, for colour stabilisation through the formation of stable complexes with anthocyanins.
- **Natur'Soft® - 30 g/hL :** Preparation of yeast autolysates for colour stabilisation, decreasing green notes and adding roundness.

d) Treatment after fermentation to stabilise colour:

- **Tan'Excellence® - 5 to 30 g/hL :** Formulation based on grape proanthocyanidic tannins and stave quality oak ellagic tannins. Recommended for fast stabilisation of colour and maturation of top quality red wines.

4. Improve wine clarification and filterability

Objectives: **1. Remove the glucans formed by Botrytis, which inhibit clarification and filterability**

L.A SOLUTIONS

a) On free run wine: Remove glucans formed by *Botrytis* at the end of the alcoholic fermentation

b) On press wines: Higher amounts of laccase and clogging colloids. It is necessary to add enzymes directly on the pressed wine
NB: only β1-3 ; β1-6 glucanases are effective againstBotrytis glucans

- **Vinotaste Pro - 10 g/hL**

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TS V2/2020