

Grape Fungal Infection and Wine Quality

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I) Effects of diffuse powdery mildew on bunch rot, berry microflora, and wine quality.

Production of grapes (principally cultivars of the European species *Vitis vinifera*) for high-quality wines requires a high degree of suppression of powdery mildew (*Uncinula necator*). Severe infection of either fruit or foliage has well-documented and deleterious effects upon crop and wine quality. We found that berries nearly immune to infection by *U. necator* due to the development of ontogenic resistance may still support diffuse and inconspicuous mildew colonies. Fruit with diffuse mildew colonies appear to be healthy and free of powdery mildew in late-season visual vineyard assessments. Nonetheless, the presence of these colonies was associated with: (i) elevated populations of spoilage microorganisms on berries, (ii) increased evolution of volatile ethyl acetate, acetic acid, and ethanol from berries, (iii) increased infestation of fruit clusters by insects attracted to the aforementioned volatiles, (iv) increased rotting of clusters by *Botrytis cinerea*, (v) increased frequency of perceived defects in wines prepared from fruit supporting diffuse powdery mildew colonies, and (vi) higher incidence of perceived unripe, green flavors in the resulting wines. Prevention of diffuse infection required extending fungicidal protection until fruit were fully resistant to berry infection, approximately four weeks after bloom is completed. Despite the fact that this may not produce a perceived improvement in disease control due to the insidious nature of diffuse powdery mildew, the prevention of diffuse colonization will eliminate secondary deleterious effects upon crop and wine quality.

Wine Quality. Wine produced using Pinot Noir grapes in which 20-30% of the berries supported diffuse powdery mildew colonies were described as flat and bitter, with plastic, dusty and moldy flavors. Sensory analysis of similarly-prepared Chardonnay wines showed that those made from mildew-free grapes were preferred to those made with grapes with diffuse powdery mildew. Grapes with less infection (1-2%) were also described as earthy and with plastic, paper, and rotten apple flavor. Wines

prepared from grapes supporting substantial diffuse powdery mildew infection were generally perceived as inferior due to their lack of cleanness and body, compared to wines prepared from mildew-free grapes.

Berry microflora. Berry surface microflora was studied using various agar media appropriate for fungal and bacterial growth: YM agar to grow yeast and mould, YM agar added with cycloheximide (YMC) to select yeasts such as *Brettanomyces/Dekkera bruxellensis* and *Hanseniaspora spp*, Lysine agar (LYS) to select non-*Saccharomyces* yeasts, and MLB to enhance bacteria growth. Results indicated a quantitative (30- to 100-fold) difference in microbial populations on berries supporting diffuse powdery mildew versus mildew-free berries. Additionally, high populations of non-*Saccharomyces* yeast and lactic acid bacteria were recovered from berries with diffuse powdery mildew. Colonies that developed on YMC were identified based upon their microscopic characteristics as *Hanseniaspora*. Sixty colonies (20 for each of the three replicated samples) randomly isolated on YM agar and LYS agar were further analyzed. A portion of the 26S rDNA gene was amplified by PCR, purified, sequenced, and compared to registered sequences from the NCBI Blast database to discriminate the yeast species involved. *Hanseniaspora* yeast predominated on berries with diffuse powdery mildew (70%) along with *Metschnikowia* or *Candida*. Although *Hanseniaspora* will not persist during alcoholic fermentation, and will be quantitatively passed by *Saccharomyces*, its activity may be detrimental to wine quality because of its capacity to produce acetic acid and various ethers.

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II) A study on *Botrytis cinerea* noble rot in Italian Passiti Wine

Passiti wines are special wines produced in Italy characterized by a post harvest drying step of the grapes. The quality of these grapes is beneficially influenced by development of *Botrytis cinerea* as noble rot, like in the more famous Sauternes and Hungarian Tokay wines. Few studies can be found in the scientific literature related to Passiti production and quality. Among the various Passiti produced in Italy, Caluso Passito is the appellation of the Passito wine produced in Piedmont. The appellation Caluso Passito DOC belongs to wines produced in the Canavese area of Turin Province, with Erbaluce grape, at least 15 degree of alcohol, obtained from grape harvested in September and let dry in special rooms until March. From the production of the grape must to the end of the aging process 4 years pass. Average production is 15,000 liters per year (about 4,000 gallons).

A three year study on Caluso Passito was carried out at the University of Turin (DIVAPRA department, Agricultural Faculty) with the main aim to highlight the peculiar characteristics of this product in order to promote its production and consumption. In this project, funded by the Piedmont Region, winemaking practices, microbiology and flavor aspects were analyzed.

The grapes are stored in boxes which allow very good airflow or individual clusters are hung on special structure in well aerated rooms with large opened windows protected by wire screens. During drying one of the more interesting microbiological events is encouraged to occur: the growth of *Botrytis cinerea* as noble rot. While *Botrytis* grows, the grapes must be protected from development of saprophytes like *Penicillium*, *Aspergillus*, *Mucor* mold that are just consuming sugars and producing substances that are inhibiting alcoholic fermentation and enzyme that most likely will influence color stability and wine aroma.

In order to encourage the noble rot occurrence and avoid detrimental fungi, great care must be taken during the drying step. Unfortunately this step is still left to empiric practices and the actual factors affecting the fungus biology and development are still unknown. The frequency of opening and closing windows is the only control exerted so far: too dry an environment will lead to too dry raisins, with low must yield. Too high humidity will promote sour rot that will cause grape loss and decreased quality of grape must (high volatile acidity).

We monitored the temperature and humidity over the entire after harvest grape drying period but could not observe a correlation with laccase activity of gluconic acid (noble rot markers). Probably these two parameters alone are not enough to provide useful suggestion in order to enhance the beneficial *Botrytis* establishment. Grape composition, skin thickness and grape health may also play an important role. It has been found in literature (mostly reviewed in Doneche, 1993) that grape berries must be undamaged, and short periods of humidity (3-4 days) alternated with long dry periods (10 days) will help in the development of *Botrytis* as the noble rot infection. This seems to start only later in the drying step,

in January. Up to 50% of the berries will be infected by the fungus hyphae. Their penetration can occur through microscopic ruptures originated by drying of the tissues. The fungus will invade the epidermal layer giving to the berry a bruised color (blue-brown and opaque). The skin becomes more permeable and the water evaporation increases. A profound chemical transformation occurs: although sugars are consumed as well as acids (especially tartaric acid), water evaporation leads to an increase in the sugar concentration (30-40%) but to a moderate acidity. The glycerol content in the grape must is 5-7% and poly alcohols and gluconic acid (1-2.5 g/L) are increased. The presence of oxidase enzymes, colloids (glucans), characteristic aromatic compounds, and their precursors also characterize the juice. The content of yeast available nitrogen availability is lowered, with obvious consequences on the alcoholic fermentation. Antimicrobial compounds identified as heteropolysaccharides termed "Botrytine" cause the increase of production of glycerol and acetic acid (Ribéreau-Gayon *et al.*, 1979).

In this study an attempt to improve the winemaking process was carried out.

The limited production by itself does not allow to invest in proper machinery for crushing, and destemming is very often done by hand. Grapes are crushed and usually macerated for 6 to 24 hours in order to increase the juice yield. The effect of the pectolytic enzyme Rapidase CB in 24 hours of maceration has been evaluated. As expected, treatment with Rapidase CB resulted in musts with higher content in poly-phenols (in particular polymeric tannins and pro-antocyanidins). However, the treated musts did not reveal a darker color, compared to the untreated one. The longer maceration time increased the juice yield by 6% (Zeppa *et al.*, 2001). In order to protect the juice from unpredictable development of wild micro flora (wild yeast and acetic acid bacteria) it is recommended to add a yeast starter culture to the grapes already at the beginning of the long skin maceration. Browning reaction during skin contact time (maceration) will lead to earlier color stability. The use of this enzyme did not improve the process but further studies with different enzymes preparation may lead to different results. Long maceration time did not cause the appearance organoleptic defects, and no increase in costs.

Sensory analysis of several Passiti (different wineries and different vintage) also was part of this study and revealed a high variability among year of production and among wineries indicating rather diverse production practices. However wine analysis by GCO revealed that the longer the wine is aged the lower is the amount of fruity (raspberry, wild strawberry) and floral aromas detected, and the higher is the amount of compounds described like, caramel and hazelnut. Aging also lead to a lower amount of grassy and hay like aromas. Citrus and rose aromas tend to be stronger in young wines. Predominance of these compounds in most but not in all young wines may also be related to the winemaking practice and to the grape quality.

A comparison among Caluso Passito, Cinque Terre Sclacchetrà, Passito di Pantelleria (three among the more important Italian passiti) volatile compound highlights the higher contents in ethyl esters and

alcohols (Gerbi *et al.* 2003). Caluso Passito showed the higher concentration. Acetyl esters are also in higher concentration in Caluso Passito. All three wines contain isobutanol, and isopentanol, but Caluso Passito is characterized by the presence, almost exclusive, of 2-ethyl-1-ethanol and furfural. Originated by *Saccharomyces* fermentation and found in all three wines, were the γ -butyrolactone (from glutamic acid) and the 2-phenylethanol. Characteristic of Caluso Passito and Sciacchetra is benzaldehyde (bitter almond).

Varietal terpene and norisoprenoid aroma compounds were characterizing the three wines: linalool is characteristic of Passito di Pantelleria (from Muscat grape). If found in Caluso Passito and in Cinque Terre Sciacchetra it must come from small amount of aromatic variety accidentally used or to the Bosco, Albarola e Vermentino (typical grapes of Sciacchetra) the aromatic composition of which is still unknown. The prevalent terpenes in Caluso Passito are 1,4-cineol and 4-terpineol, meanwhile in Cinque Terre Sciacchetra 1,4-cineolo, limonene, alpha-terpineol, and 4-terpineol are found in higher quantity.

The norisoprenoid β -damascenone was always found in the wines, especially in Cinque Terre Sciacchetra. Vitispirans (*cis* and *trans* isoform) were not detected in Passito di Pantelleria, but in Caluso Passito and Sciacchetra, probably because of the long maceration, characteristic of their winemaking process. They may also be originate during aging in bottle.

Data analysis by Principal Component Analysis clearly distinguished Passito di Pantelleria from a second group where Caluso Passito and Cinque Terre Sciacchetra were not clearly discriminated.

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