

## DETECTION OF POWDERY MILDEW IN GRAPES USING A DNA ASSAY AND NEAR INFRARED REFLECTANCE SPECTROSCOPY, AND ASSESSMENT OF CHARDONNAY WINE QUALITY

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Wines made from powdery mildew-affected grapes have reduced quality and negative sensory attributes, although data for the effects of quantifiable amounts of powdery mildew on wine quality for cultivars commonly grown in Australia are scarce (Stummer et al. 2005).

Estimating disease severity by visual assessment is difficult in large consignments of fruit, particularly after machine-harvesting. Accurate measurement of disease severity is required to assess and grade fruit at the weighbridge, as such information would enable wineries to make more informed decisions about the utilization of fruit and must. The objectives of this work were to assess the effects of known amounts of powdery mildew on wine quality and to develop DNA-based tools and near infrared reflectance (NIR) spectroscopy for detection and quantification of powdery mildew in grape samples at the winery.

Chardonnay bunches were inspected in the vineyard and sorted into disease severity categories based on a bunch assessment key designed by R. Emmett and T. Wicks (pers. com.) over five vintages (2001-2005). Infection categories were later confirmed by microscopic observation. Four batches of grapes representing each of four disease severity categories (0, 1-5%, 10-30%, 31-100% of the bunch with sporulating powdery mildew) were crushed and vinified in 2001 and 2002 (1). Grapes were harvested at commercial maturity on one day in 2001 and over 2 weeks as they reached a standard sugar ripeness in 2002. Juices and wines were subjected to standard chemical analysis. Sensory analysis comprised duo/trio tests to identify differences and descriptive analysis to describe differences in aroma and palate attributes (Stummer et al. 2005).

Southern blots and slot blots were hybridised with the *E. necator*-specific clone, pEnA1, obtained from a plasmid library (Stummer et al. 2000). *E. necator* DNA was quantified by slot blot hybridization using DNA extracted from grapes, must, juice, clarified juice and wine. The amount of DNA per sample was estimated by comparing the hybridization signal with known amounts of *E. necator* DNA.

Chardonnay grapes with various degrees of powdery mildew were homogenized and scanned in reflectance mode with a FOSS NIRSystems 6500. The homogenates were also analysed for total soluble solids (TSS, °Brix), pH and *E. necator* DNA content.

Grapes with powdery mildew generally matured earlier than healthy grapes and were smaller and lighter. Titrable acidity, total phenolic content, hydroxycinnamates, flavonoids and brown pigments in juice increased with increasing infection. Similar trends were observed for the resulting wines.

Wines made in 2002 were similar in alcohol concentration, which facilitated the perception of sensory differences among treatments. Wines made from grapes with powdery mildew were perceived as having pronounced viscosity/oiliness compared to the control, and this was correlated with the phenolic composition of the wines. Wines made from diseased grapes, especially the 31-100% infected category, exhibited pronounced fungal, earthy and cooked tomato aroma attributes compared to those made from uninfected grapes.

Clone pEnA1 hybridised to *E. necator* DNA but not to DNA extracted from grapevine or from a diverse range of microbes associated with grapevines. In addition, the probe was sensitive enough to detect 50 pg of DNA, which equates to less than 1% of the bunch infected or fewer than 100 conidia.

*E. necator* DNA was detected in grapes, juice and must but not in clarified juice and wine. There was a strong positive relationship between the amount of *E. necator* DNA detected in must and juice samples and the infection category assigned to the corresponding bunches following visual and microscopic assessment (2004: must;  $r = 0.85$  and juice;  $r = 0.94$ ).

To determine the relationship between the amount of *E. necator* DNA in juice and disease severity, a linear regression model was assessed was made for the 18 grape samples of varying disease severity obtained in vintage 2005. The equation of  $y = 1.552(x) + 3.289$  and  $r^2 = 0.955$ ,  $P < 0.001$  was obtained for these juice samples, where  $y =$  disease severity (%) and  $x = E. necator$  DNA content (ng/100 ng total DNA).

Strong spectral correlations with disease severity were observed over the 400-2500 nm wavelength range, including spectral changes not related to differences in pH and TSS. Principal component analysis (PCA) of spectral data showed distinct clustering of samples based on disease severity. The PCA scores were used to prepare a discriminant analysis algorithm to classify disease severity category and although a small number of infected samples were predicted in adjacent categories, all non-infected samples were classified correctly and no infected samples were classified as non-infected.

## Conclusions

The *E. necator*-specific DNA clone detected the pathogen in grapes, must and juice. Compared with visual assessment, the *E. necator*-specific DNA probe provides a reliable and objective means of detecting and quantifying powdery mildew in grapes, juice and must. The detection threshold is approximately 100 conidia.

NIR could discriminate the least infection category from uninfected samples, but these samples were from a small number of trial plots. To ensure a robust calibration, further material representing more diversity must be scanned.

Even small amounts of powdery mildew, as little as 1-5% of the bunch infected, resulted in increased oily, viscous mouth-feel characters.

The detection tools described here offer the grape industry a means of obtaining objective, quantitative data on disease severity in grapes, must and juice samples at the winery, particularly when disease is slight and visual assessment is difficult. Such information can then be used to inform decisions about the use of grapes and must to achieve desired outcomes.

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## Literature

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