SUSCEPTIBILITY OF GRAPES TO *BOTRYTIS CINEREA* IN RELATION TO BERRY NITROGEN AND SUGAR CONCENTRATION

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ABSTRACT

The percentage of detached grape berries inoculated in the laboratory that became infected by *Botrytis cinerea* during ripening was correlated with increasing sugar concentration and increasing yeast assimilable nitrogen (YAN) concentration. These factors could not be separated to provide a causal relationship between YAN and % berries with disease due to the close relationship that existed between sugar concentration, YAN, vine development and time. Wounding increased the percentage of berries with disease, particularly when the berries were immature. These findings do not support the hypothesis that the inherent susceptibility of grape berries to botrytis infection increases with increasing YAN. Field observations by growers that nitrogen fertiliser increases bunch rot may be due to nitrogen causing increased canopy density, which in turn causes a microclimate more conducive to the development of botrytis bunch rot.

Keywords: grape, botrytis bunch rot, berry nitrogen, sugar accumulation, wounding.

INTRODUCTION

Poor nitrogen fertility in the vineyard has a detrimental effect not only on shoot growth, leaf function and fruit development, but also on fermentation during winemaking (Spayd et al. 1995). Conversely, too much nitrogen can contribute to excessive vigour, reduced fruit set (Bell & Henschke 2005) and, reportedly, increased disease incidence (Christensen et al. 1994; Keller et al. 2001). Some of these responses may result directly from changes in berry nutrition; other responses may occur indirectly, for example because of increased shoot growth and canopy shading. Botrytis bunch rot in grapes, caused by *Botrytis cinerea,* is present in all vineyards to some extent, but only results in damaging bunch rot epidemics when physiological triggers cause berry tissues to become susceptible. Berry sugar concentration and physical damage are known to be key infection triggers (Hill et al. 1981).

Grapes grown on some New Zealand soils are typically low in yeast assimilable nitrogen (YAN) and anecdotally, berries with low YAN seem to have a lower incidence of botrytis bunch rot. At lower berry YAN, fermentation of grapes can be impaired, so lowering YAN *per se* is not a practical suggestion to manage botrytis. However, an understanding of how nitrogen levels influence susceptibility to disease could help in the overall management of botrytis bunch rot.

This study used a field experiment to provide fruit of different sugar and YAN concentrations for use in a laboratory experiment to investigate the direct influence of these factors on the susceptibility of berries to *B. cinerea* infection. The experiment was conducted on artificially inoculated berries in the laboratory so that indirect effects, such as canopy density could be eliminated. Susceptibility was measured as the percentage of inoculated berries that become diseased after laboratory incubation.

METHODS

A Sauvignon blanc vineyard in the Marlborough region that showed leaf yellowing symptomatic of low nitrogen fertility was selected for the field experiment. Four soil-applied nitrogen treatments were used, with eight replicates in a double Latin square design. Each experimental plot was a single bay or panel of four vines, and the middle vines in each bay were sampled.

The rates and timing of nitrogen applications were determined from experimental work conducted on Sauvignon blanc by Goldspink & Frayne (1996). Urea was used as the source of nitrogen and was broadcast in the dripper zone. Sauvignon blanc berries from two of the field treatments (no added nitrogen and 200 g urea added per vine at fruit set) were used in the susceptibility experiment. Samples of five bunches per plot were collected four times (Table 1) between veraison (berry softening) and harvest.

At each sample date, berries of similar sugar concentration were selected by grading them according to specific gravity using sucrose solutions. The berries were then surface sterilised to remove contaminating fungal spores. Susceptibility of berries to *B. cinerea* was determined by artificially inoculating both wounded and non-wounded berries with 1-5 x 10^4 spores/ml in the laboratory. A non-inoculated/non-wounded control was also used, giving a factorial design with two levels each of wounding and inoculation. A sterile needle was used to artificially wound berries prior to inoculation. Three incubation trays, each with 10 berries, were used for each wounding x inoculation treatment. The percentage of berries per tray showing *B. cinerea* sporulation after 6 days of incubation at 20°C was the measure of susceptibility to infection and subsequent disease development.

Nitrogen content of berries was assessed using standard wine YAN kits (Unitech Scientific, UNITAB^{TM} Reagent, PAN$_{bl}$-500 and AMM-500). Sugar content was assessed using standard wine industry analytical techniques.

RESULTS

The control and wounded-only treatments had a significantly lower incidence of diseased berries after incubation than the corresponding inoculated treatments, indicating that disease arising from background *B. cinerea* in the vineyard could be ignored in relation to the susceptibility testing method (Table 1).

TABLE 1: Mean percentage (%) of grape berries with *Botrytis cinerea* sporulation from four sample dates and four inoculation/wounding treatments after incubation for 6 days at 20°C.

Sample date	Non-inoculated/ non-wounded	Wounded	Inoculated	Inoculated/ wounded
16-Mar-05	0.4	0.2	18.8	57.1
30-Mar-05	2.7	7.3	77.5	92.3
7-Apr-05	6.5	12.1	64.8	92.3
12-Apr-05	10.7	7.1	81.6	95.1
Mean	4.1 a[1]	6.5 a	54.3 b	81.1 c

[1] Means with the same letter within the row are not significantly different (P>0.05)

Data from all sample dates were pooled and the *B. cinerea* disease incidence after incubation (mean percentage) was regressed separately on sugar concentration (°Brix) and YAN (Fig. 1). Percentage of berries with disease of both non-wounded and wounded inoculated berries increased significantly with increasing sugar content (Figs 1a & 1c). There was also a significant increase in % diseased berries with increasing YAN, although the relationship was much weaker than for sugar content (Figs 1b & 1d). Wounding increased the % berries with disease and it tended to cause a relatively greater increase for berries with a lower sugar concentration, as shown by the lower regression slopes for the wounded berries compared with the non-wounded berries (Figure 1a compared with 1c, and 1b compared with 1d). YAN and sugar concentration were highly correlated and both increased over successive sample dates. When % berries with disease in both non-wounded and wounded inoculated berries was regressed with both sugar concentration and YAN as X-variates, the regression coefficient for sugar was significant (P<0.001) but that for YAN was not (P>0.1).

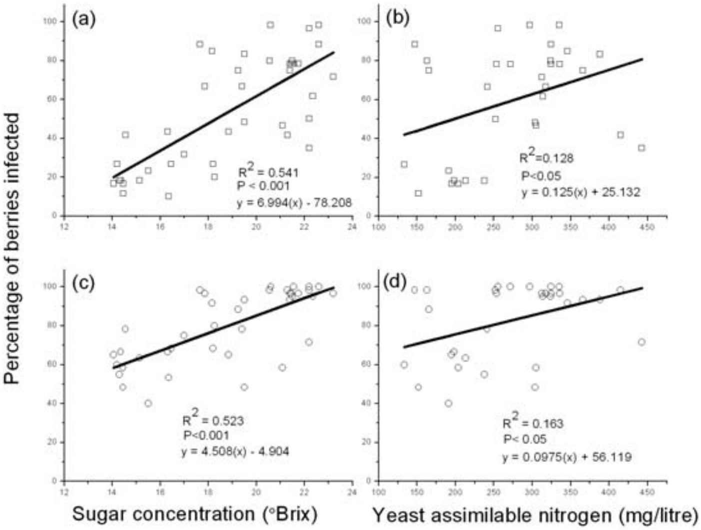

FIGURE 1: Mean percentage (%) of berries with *B. cinerea* sporulation after incubation, in relation to sugar concentration (a and c) and yeast assimilable nitrogen (b and d) for inoculated/non-wounded (a and b) and inoculated/wounded (c and d) treatments.

Further analyses investigated the effects of field-applied nitrogen (with or without urea at fruit set) and date of sampling on % berries with disease for non-wounded and wounded inoculated berries, using YAN and sugar concentration as covariates. A descriptive summary of these is given, as follows. There were significant effects for both date (P<0.001) and nitrogen (P<0.05) and there were significant date x nitrogen interactions (P<0.01).Thus, added urea gave a mean of 63% berries with disease compared with 46% without urea for the inoculated/unwounded treatment (P<0.001). For the inoculated/wounded treatment, the mean susceptibility for added urea gave 86% berries with disease and compared with 76% without urea (P=0.013). Neither Brix nor

YAN were significant as covariates (P>0.1), indicating that these factors did not help to explain more of the variation in % berries with disease than that which was explained by sample date and field-applied nitrogen. When these analyses were repeated with an arcine transformation of the data, there was no improvement in the normality of the residuals, nor in the homogeneity of the variance.

DISCUSSION

Yeast assimilable nitrogen (YAN) appeared to influence the susceptibility (the ability of a berry to become infected and express disease when spores are present) of grape berries to *B. cinerea,* as evidenced by the significant relationship between % berries with disease and YAN, and the greater % berries with disease from field plots with added nitrogen. However, the results need to be interpreted with caution because of the high correlation between YAN, sugar concentration and time. The increase in susceptibility may reflect changes in some other factor not measured in the study, such as decreasing concentration of anti-fungal phenolic compounds in the berry.

For a given sample date, sugar concentration tended to be lower in berries that had greater YAN. This was probably because sugars accumulated more slowly in berries with high YAN and so for a given sample date high YAN berries were less mature with respect to sugar accumulation. A further study, in which the rate of increase in susceptibility during ripening could be related to the rate of increase in sugar content for different levels of YAN, may allow the relationship between berry nitrogen, sugar concentration and berry susceptibility to be better defined.

Apart from the many physiological changes that take place within the grape berry during ripening, physical structures that reduce the susceptibility of immature grape berries to infection also change (Hill et al. 1981; Commenil et al. 1997; Gabler et al. 2003). In the present study, wounding was found to increase the susceptibility of berries, and the more immature the berries, the greater the relative increase in susceptibility with wounding. Thus, bird or leafroller damage during early ripening could have a large effect in predisposing the crop to a botrytis epidemic.

The anecdotal difference in botrytis bunch rot observed in the field on vines may be an indirect response to low vine nitrogen levels, rather than a decreased susceptibility of berries because of low YAN levels. Increased canopy size and delayed harvest due to increased nitrogen fertilisation have been proposed as indirect mechanisms for increased incidence of botrytis bunch rot, although incidence was not determined (Spayd et al. 1994). Changes in canopy microclimate have been reported to change botrytis bunch rot incidence and severity in grapes (English et al. 1989), which would support changes in canopy size as a possible mechanism for increased disease incidence. Another study defined bunch rot as four or more berries in a bunch showing decay (Christensen et al. 1994), and when 112 kg nitrogen/ha was applied at budbreak, decay was significantly higher than in the control. The high nitrogen treatment at budbreak also resulted in significantly higher petiole nitrate and total nitrogen at bloom and veraison, and lower soluble solids for French Columbard grapes (Christensen et al. 1994). No measurements of vine vigour or canopy density were reported.

The need for adequate berry nitrogen for fermentation may require the addition of nitrogen fertiliser to vines. This may increase the risk of botrytis bunch rot, but probably through an increase in leaf canopy density and prolonged ripening period, both of which increase the risk of a favourable microclimate allowing botrytis bunch rot development, rather than through a direct increase in the inherent susceptibility of the berries to botrytis. However, if appropriate use of fungicides and cultural methods (e.g. leaf plucking) in the vineyard are adopted, this should not mean that the requirement for application of nitrogen fertiliser will result in increased losses due to botrytis bunch rot.

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