

## DISEASE ASSESSMENT AND EPIDEMIC MONITORING METHODOLOGY FOR BUNCH ROT (*BOTRYTIS CINEREA*) IN GRAPEVINES

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### ABSTRACT

Epidemic descriptors that quantified the location in time and rate of disease increase of botrytis bunch rot were developed using percentage disease severity data. Data were obtained from fungicide evaluation trials in three regions of New Zealand and from Tasmania in Australia. Mean percentage severity versus time was logit transformed and linear regression was used to predict the date at which 5% severity was reached, the daily severity increase rate at 5% severity, the severity on the date of harvest and the daily severity increase rate at harvest. These descriptors will be of general use for the critical comparison of bunch rot epidemics between sites and between seasons. They will be used in quantitative analyses to identify factors that drive botrytis bunch rot epidemics in order to develop botrytis prediction models.

**Keywords:** grapevine, botrytis bunch rot, disease assessment, disease progress, disease prediction.

### INTRODUCTION

Botrytis bunch rot, caused by *Botrytis cinerea*, affects grape yield and wine quality in wine growing regions with cool or cold climates (Smart & Dry 1980) and a high probability of rainfall close to harvest. Botrytis damage is often measured at harvest time as the percentage of berries within bunches that are affected. Although this allows the winery to quantify the level of botrytis in a given season and allows researchers to compare the efficacy of various treatments within individual disease control trials, it is of limited use for comparing epidemics between vineyards or regions, or even between seasons within the same vineyard. This is because harvest severity does not describe the dynamics of botrytis disease progress. A given disease severity could arise from a sudden late epidemic or one that developed gradually during the season. Such differences could arise from quite different sets of crop management or environmental conditions. The ability to describe the dynamics of epidemics is a prerequisite for quantitative analysis of factors that drive botrytis bunch rot.

Botrytis disease datasets from vineyards in three regions of New Zealand and from Tasmania in Australia were used to develop a simple method for collecting disease

progress data and analysing epidemic dynamics using readily available spreadsheet software. These methods are intended for use in disease prediction research and wherever else comparisons need to be made between botrytis epidemics.

### MATERIALS AND METHODS

The botrytis disease data used were taken from four vineyard field trials containing various fungicidal spray programmes against botrytis and untreated controls. Each trial used a randomized complete block design with five replicates of each treatment. Data from two treatments from each site were used, the non-fungicide control and one treatment with fungicides (Table 1). The Hawke's Bay and Auckland sites were experimental vineyards and the Marlborough and Tasmania sites were commercial vineyards.

Disease was assessed non-destructively on several dates in each trial (Table 1), using 20-50 bunches for each treatment in each replicate. Bunches were either tagged or mapped in the vineyard row so that the same bunches could be assessed each time. At harvest in Tasmania, bunches were collected, then incubated in humid chambers at room temperature before further assessment.

**TABLE 1: Vineyard and fungicide treatment details for the botrytis bunch rot datasets. Analyses included data from non-fungicide treated plots at each site.**

Vineyard location and season	Variety	Fungicide application dates and growth stages <sup>1</sup>	Fungicide products <sup>2</sup>	Disease assessment dates
Hastings, Hawke's Bay 2000/2001	Sauvignon blanc	11, 17 Dec 2000 (F1, F2) 24 Jan 2001 (PBC) 18 Feb 2001 (V)	Sw × 4	26 Feb, 13, 27 Mar 2001
Pukekohe, Auckland 2001/2002	Sauvignon blanc	10 Jan 2002 (PBC)	Sw	19 Feb, 4, 15, 21, 27 Mar, 3 Apr 2002
Rapaura, Marlborough 2002/2003	Sauvignon blanc	11, 30 Dec 2002 (F1, F2) 16 Jan 2003 (PBC) 25 Feb 2003 (V) 24 Mar 2003 (PH)	Sh, EM, E, Sc, Sc	27, 31 Mar, 8 Apr 2003
Campania, Coal River Valley Tasmania 2005/2006	Riesling	24 Nov (PF) 2, 16 Dec 2006 (F1, F2) 12 Jan 2006 (PBC) 16 Feb 2006 (V) 15 Mar 2006 (PH)	B, B, Sc, Sw, R, R	14, 21, 31 Mar, 7 Apr 2006

<sup>1</sup>Growth stages for fungicide applications: PF= pre-flowering, F1= first flowering spray, F2= second flowering spray, PBC= pre-bunch closure, V= veraison and PH= pre-harvest.

<sup>2</sup>Fungicide products: B=Bravo® 420 ml/100 litres (720 g/litre chlorothalonil); E=Euparen DF® 200 g/100 litres (500 g/kg dichlofluanid); EM=Euparen Multi® 200 g/100 litres (500 g/kg tolyfluanid); R=Rovral AquaFlo® 100 ml/100 litres (500 g/litre iprodione); Sc=Scala® 200 ml/100 litres (400 g/litre pyrimethanil); Sh=Shirlan® 100 ml/100 litres (500 g/litre fluazinam); Sw=Switch® 80-100 g/100 litres (375 g/kg cyprodinil+250 g/kg fludioxinil).

Disease severity was estimated visually as either the percentage of berries within the bunch with visible symptoms, or the percentage of projected surface area of the bunch with symptoms, when viewed from one side. Mean percentage disease severity at each date was calculated as:

$$\text{Mean \% severity} = (\Sigma \% \text{ severity for each bunch} / \text{no. bunches assessed}) \times 100 \quad (1)$$

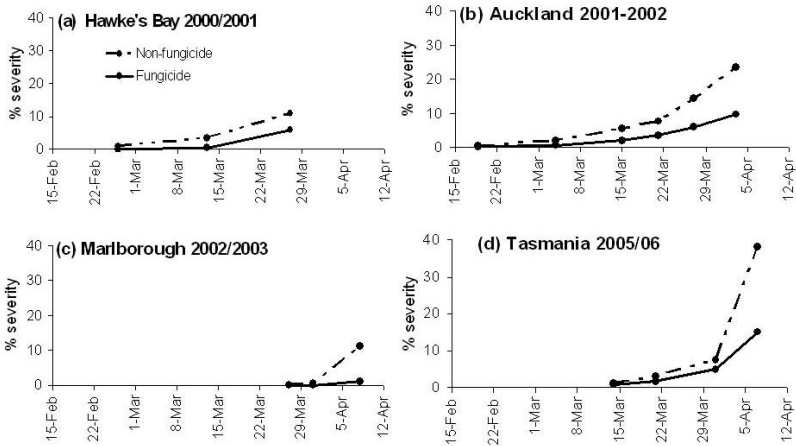
This definition of disease severity is consistent with that generally used in plant disease epidemiology (Nutter et al. 1991). At Auckland and Tasmania separate severity records were made for berries showing *B. cinerea* sporulation and those showing brown discoloration without sporulation.

## RESULTS

The disease severity data were first examined for their characteristics in order to identify the appropriate statistical analysis to test for treatment effects. Both raw percentage severity and an arcsine transformation of percentage severity were unsuitable for analysis of variance because means and variances were highly correlated and residuals had a highly skewed distribution. Logit transformation of the individual disease scores gave the best correction of these tendencies.

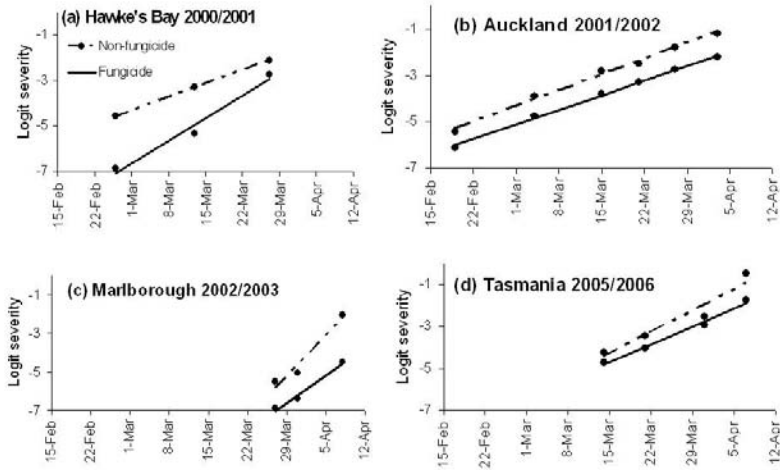
Disease progress for raw mean percentage disease severity showed exponential increase, but with different timings and different rates of increase for the various epidemics (Fig. 1). The disease progress data were linearised (Fig. 2) by transforming to logit disease severity (van der Plank 1963), using the following equation:

$$\text{Logit severity} = \ln((\% \text{ severity} + 0.1) / (100.1 - \% \text{ severity})) \quad (2)$$



**FIGURE 1: Bunch rot disease progress in non-fungicide and fungicide treatments at four sites. Percentage severity included symptoms of *B. cinerea* sporulation plus associated discoloured berries.**

Linear regression of logit transformed data produced regression equations with high  $R^2$  values, even though two of the data sets had non-significant regression coefficients because of the small number of observations (Hawke's Bay fungicide,  $P=0.104$  and Marlborough non-fungicide,  $P=0.132$ ; Table 2).



**FIGURE 2:** Botrytis disease progress as logit transformed percentage severity, with fitted linear regression lines. Regression parameters are given in Table 2. Note that 50% severity = 0 logit severity and 0.1% severity = -6.909 logit severity.

**TABLE 2:** Regression parameters from regression of logit severity of botrytis bunch rot on days from 1 January 1900<sup>1</sup>, for non-fungicide and fungicide sprayed plots at four vineyards.

Site/season	Treatment	n <sup>2</sup>	Slope	Intercept	P-value	R <sup>2</sup> (adj.)
Hawke's Bay 2000/2001	Non-fung.	3	0.085229	-3153.6	0.011	0.999
	Fungicide	3	0.143011	-5291.1	0.104	0.947
Auckland 2001/2002	Non-fung.	6	0.097154	-3629.7	0.000	0.993
	Fungicide	6	0.090386	-3378.0	0.000	0.996
Marlborough 2002/2003	Non-fung.	3	0.300868	-11350.7	0.132	0.915
	Fungicide	3	0.204475	-7717.1	0.077	0.971
Tasmania 2005/2006	Non-fung.	4	0.147284	-5717.6	0.035	0.896
	Fungicide	4	0.123366	-4790.2	0.007	0.978

<sup>1</sup>Microsoft® Excel date convention.

<sup>2</sup>n=number of time points when assessments were made.

The linear regression parameters in Table 2 were used to calculate epidemic descriptors that defined the dynamics of each epidemic (Table 3). These included the timing of each epidemic, as the date that 5% severity occurred, and the rate of disease increase at 5% severity, using the following back-transformation:

$$\text{Back-transformed \% severity} = (100.2 / (1 + e^{-(\text{logit severity})})) - 0.1 \tag{3}$$

The fitted harvest severity and calculated rate of severity increase at harvest were similarly determined.

**TABLE 3: Botrytis bunch rot epidemic descriptors from the regression parameters in Table 2. Values shown are the date of 5% severity, the increase rate (%/day) at 5% severity, the severity at harvest (%) and the severity increase rate (%/day) at harvest.**

Site/season	Treatment	Actual harvest date	Date for 5% severity <sup>1</sup>	Increase rate at 5% severity <sup>2</sup>	Severity at harvest <sup>3</sup>	Increase rate at harvest <sup>4</sup>
Hawke's Bay 2000/2001	Non-fung.	11-Apr-01	17-Mar-01	0.43	30.26	1.83
	Fungicide	11-Apr-01	27-Mar-01	0.74	30.80	3.14
Auckland 2001/2002	Non-fung.	14-Apr-02	15-Mar-02	0.49	48.90	2.43
	Fungicide	14-Apr-02	25-Mar-02	0.46	24.19	1.70
Marlborough 2002/2003	Non-fung.	8-Apr-03	5-Apr-03	1.67	9.88	3.05
	Fungicide	8-Apr-03	16-Apr-03	1.09	0.93	0.23
Tasmania 2005/2006	Non-fung.	29-Mar-06	24-Mar-06	0.76	9.65	1.38
	Fungicide	29-Mar-06	29-Mar-06	0.63	4.71	0.60

<sup>1</sup>Date for 5% severity (T) = (-2.926 – Intercept) / Slope (from Table 2).

<sup>2</sup>Difference in back-transformed severity between T+1 and T using equation (3).

<sup>3</sup>Slope × harvest date + intercept (from Table 2).

<sup>4</sup>Difference in back-transformed severity between (harvest date+1) and (harvest date) using equation (3).

The Auckland non-fungicide epidemic was the most severe with the earliest date for 5% severity and the greatest severity at harvest (Table 3). However, the Marlborough non-fungicide epidemic had a greater severity increase rate at harvest than the Auckland non-fungicide epidemic. The least severe epidemic was in the Marlborough fungicide treatment, where the calculated date of 5% severity actually occurred after harvest. The epidemic in the Hawke's Bay fungicide treatment had a greater increase rate than that in the non-fungicide treatment (Fig. 2). Thus, although the severity measured in the field was less in the fungicide treatment it was predicted to be similar to that in the non-fungicide treatment by harvest on 11 April 2001 (Table 3).

At Auckland and Tasmania, separate percentage severity records were available for sporulating botrytis and for brown discoloration of berries. There were substantial differences between these two variables and apparent inconsistencies between sites. Final severity values for total bunch rot compared to sporulating *B. cinerea* were 23.4 compared to 15.6 for Auckland non-fungicide and 9.7 compared to 2.5 for Auckland fungicide. For Tasmania, severity values for total bunch rot compared to sporulating *B. cinerea* were 38.1 compared to 11.3 for non-fungicide, and 15.1 compared to 2.6 for fungicide. The relative differences between Auckland and Tasmania may have arisen from differences in interpretation of visible symptoms.

## DISCUSSION

The methods for describing the dynamics of grape botrytis epidemics used in this study are consistent with methods used elsewhere in plant disease epidemiology (Xu 2006). The time scale chosen for the X-variate in the regression of logit-transformed severity on time was, for convenience, the date convention used in Microsoft® Excel, where day 1=1 January 1900. The time scale became arbitrary when the regression equations were used to calculate the epidemic descriptors shown in Table 3.

The calculated date at which 5% severity occurred was used to locate the epidemics in time. Dates for other severity values suited to different purposes could similarly be calculated from the regression equations, e.g. 3% severity is often the threshold at which a price penalty is imposed by the winery for botrytis-infected grapes. Thus the date at which 3% severity would be reached could be predicted using these methods from a series of disease assessments made in the vineyard during the early stages of an epidemic. Both the location and rate descriptors used in this study would be suitable for use as dependent variables in multivariate analyses to identify factors that drive botrytis epidemics.

In using this disease assessment method it will be important to standardise the way in which bunch rot symptoms are recorded by different researchers. Standard area diagrams for percentage severity and standard photographic images of specific symptoms need to be used. Symptoms of total bunch rot (berries with *B. cinerea* sporulation plus discoloured berries not showing sporulation) can be recorded if all discolouration can be attributed to botrytis. If other bunch rot organisms are present it will be necessary to record separate symptom categories. Recording the same variables at every assessment is important in order to analyse disease progress and a minimum of three, but preferably four to six, assessment dates are required.

The use of severity range categories could reduce recording time in the field. Categories with equal percentage intervals should be used and the percentage severity data should be reconstructed, using the mid-point of each category, before analysis.

The number of bunches assessed per experimental unit in this study varied from 20-50, determined mostly by the time available to make assessments. It is more important to assess the same bunches each time and to use the same symptom categories than to always sample a fixed number of bunches. However, better precision does result from a larger sample size, especially in the early stages of epidemic development.

The epidemic analysis methods described here will allow critical comparison of bunch rot epidemics between sites and between seasons. The epidemic descriptors will be used in quantitative analyses to identify factors that drive botrytis epidemics and these will then be used to develop botrytis prediction models. The use of these methods will be of particular benefit for collaborative studies comparing botrytis epidemics in different wine production regions.

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