GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Practical Management Strategy for Bunch Rot of Grapes

Project DAN 92/1

Final Report

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Preface

The work contained in this report was carried out during the normal and supplementary funding periods of the Grape and Wine Research and Development Corporation project **DAN 92/1** entitled **"Practical Management Strategy for Bunch Rot of Grapes"**. The report also includes results obtained from research performed beyond the funding period up to the current time.

Botrytis cinerea on grapevines is an insidious pathogen characterised by ubiquity. Consequently, our knowledge on the epidemiology of the *Botrytis* rot of grapes is incomplete, thus making the disease difficult to manage. *Botrytis* management is further complicated by the continuing action of the extracellular enzymes of the pathogen during wine production.

The work reported here contains the most recent published work in Australia on *Botrytis* rot of grapes and includes a practical management strategy for the disease based on, (1) the measurement of disease risk by monitoring *Botrytis* incidence and by using a model which describes the spatial and temporal aspects of the disease and, (2) the adoption of a fungicide resistance management strategy by monitoring resistance. The report also points out areas needing further investigation.

Acknowledgments : I wish to acknowledge the financial support of the Grape and Wine Research and Development Corporation (GWRDC) which enabled me to carry out the work on *Botrytis* rot of grapes. I am grateful to Mr Peter Hayes, Executive Director, GWRDC, for his encouragement and his foresight about the need to solve the vexing problem of *Botrytis* on grapes. I thank the Cooperative Research Centre for Viticulture for providing partial funding for the *Botrytis* monitoring and fungicide resistance testing service which is an outcome of the GWRDC project.

I am also grateful to many grape growers and research colleagues for stimulating discussions on various aspects of the disease. I thank particularly Maria Castillo-Pando, Stacey Carpenter, Karleen Villagran and Emma Borchert who assisted me in the laboratory and field work at various times during the project.

I thank, above all, the silent but ever present Spirit for watching over my wife Yvonne and daughter Hannah while I was away countless times chasing *Botrytis*.

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Botrytis cinerea is perhaps the commonest and best known fungus and has been the centre of mycological research since the time of de Bary. Few, if any other fungi, have been studied so thoroughly by so many able investigators, or are the subject of so extensive a literature.

W. B. Brierley, 1921

Disease exists in natural host populations, usually in the endemic state; it becomes epidemic in uniform pioneer vegetations. Crops are just like latter and thus favour epidemics. With effective control systems, crop diseases tend to become endemic, yet causing losses that may be intolerable, and still necessitating conscious efforts to keep them at a low level.

J. C. Zadocks & R. D. Schein, 1979

1. Executive summary

Grape berry rot caused by *Botrytis* cinerea Pers. is an economically important disease of grapevines (*Vitis vinifera* L.) worldwide. In Australia the disease causes significant reduction in quantity of grapes and quality of wine produced in most of the cold and warm viticultural regions. The principal objective of this project was to develop a practical management strategy for *Botrytis* rot disease. Prior to 1985 the growers relied heavily on calendar spraying and additional applications when rainfall was predicted. The discovery of flower infection by Nair (1985) saw the beginning of a rational use of fungicides to control *Botrytis* rot. Further research (Nair 1990) leading to the establishment of a *Botrytis* monitoring and fungicide resistance testing service for the grape industry (Nair et.al. 1994) has resulted in timing the application of fungicides with maximum effect on the pathogen. This service has also enabled the growers not only to overcome the reliance on calendar spraying but also to choose fungicides to avoid resistance.

Field trials conducted in several vineyards in New South Wales, Victoria and South Australia to examine the population dynamics of *Botrytis* showed that the levels of this pathogen fluctuated considerably during the various stages in the growth of grapevines. However, by developing a model, it was demonstrated that there was a quantitative relationship between carry over populations of *Botrytis,* flower infection and berry infection. This model was successfully used as a guide to measure disease risk in the vineyards and to time the application of botryticides appropriately. Consequently, there has been a reduction in the number of spray applications of to manage the disease.

Varieties of grapevine that may be considered as inherently predisposed to *Botrytis* rot such as Riesling, Semillon, Chardonnay, Shiraz and Pinot Noir are likely to respond more to the prediction model developed in this project. Varieties that are less susceptible such as Cabernet Sauvignon are unlikely to be

found with much *Botrytis* rot at harvest, despite having high incidence of flower infection.

Previous studies on the timing of application of botryticides to manage *Botrytis* rot were largely based on a knowledge of the epidemiology of the disease (Pearson 1982; Pearson and Rigel 1983; Nair *et.al.* 1987) and fungicide resistance of *Botrytis* strains (Gullino and Garibaldi 1982; Gullino *et al.*1989; Beever *et.al.* 1989, 1991; Akagi *et.al.* 1995). The work carried out in this project (DAN 92/1) is the first instance where application of fungicides to manage *Botrytis* rot of grapes is based on the measurement of disease risk by periodic monitoring of the incidence of the pathogen. For instance, carry over incidence of *Botrytis* >15% indicated the need to apply fungicide at pre-flowering. The type of fungicide to be used during the season depended on the frequency of resistance.

One of the objectives of this project was to develop a fungicide resistance management strategy. A study of the seasonal fluctuations of the populations of benzimidazole resistant and dicarboximide resistant strains of *Botrytis* showed that the dynamics were complex. The frequency of dicarboximide strains declined during the early part of the season. The *Botrytis* population responded to dicarboximide applications with an increase in the strains resistant to the chemical. Conversely, temporary withdrawal of the chemical resulted in a reduction in the resistant strains.

In a few instances, benzimidazole and dicarboximide resistance occurred in vineyards where these chemicals appeared to have not been applied. This may have been due to the ease with which *Botrytis* spores could move within vineyards and come from adjacent fields or due to special characteristics of the resistant strains. However, in most instances, there was a predictable fluctuation in the populations of resistant strains within a vineyard. The project was therefore able to determine the type of fungicide which could be used to avoid

problems of resistance affecting *Botrytis* rot management. In general, the occurrence of benzimidazole and dicarboximide resistance in Australian vineyards is relatively low.

A review of the data on incidence of *Botrytis* and fungicide resistance from three growing seasons (1994/95, 1995/96 and 1996/97) showed that there was a gradual increase in incidence of *Botrytis* rot during this period. In the same period there has been a decline in the frequency of dicarboximide resistance with no significant shift in the frequency of benzimidazole resistance.

The outcomes of the *Botrytis* monitoring and fungicide resistance testing service have been generally effective in managing fungicide resistance and *Botrytis* rot of grapes. However, the data obtained by using the methodology prescribed for the last three growing seasons has indicated that certain areas required modifications so that the service can offer greater predictability in disease management. As a result, two areas that have been modified in the protocol for 1997/98 season are : (1) the number of samples collected each time for testing has been increased from 30 to 100; and, (2) the timing and method of collection of these samples have been changed to involve one sampling only for monitoring *Botrytis* (at dormant stage of the crop) and one stage for monitoring resistance (at harvest - with an optional monitoring at end of flowering). The protocol for 1997/98 incorporating these changes are presented in this report.

The direct outcomes of this project of practical significance to grape growers are the recommendations on :

- the timing of application of botryticides based on monitoring disease risk; and,
- a fungicide resistance management plan based on monitoring resistance.

The relatively low incidences of *Botrytis* and frequencies of benzimidazole and dicarboximide resistance in Australian vineyards bear proof of the success of the strategy developed by this project. There were exceptions where the disease could not be managed by the recommended strategy; in such cases the disease

was mainly driven by weather. A paucity of knowledge on the factors responsible for the explosive nature of the disease between post-veraison and harvest has at times contributed to the failure to manage the disease. Therefore, the inclusion of weather factors in the disease risk model appears to be necessary. This is being currently investigated.

2. Economic importance of *Botrytis* rot of grapes

Botrytis rot caused by *Botrytis cinerea* Pers. is an economically important disease of grapes (*Vitis vinifera* L.) worldwide. In Australia the disease causes significant reduction in the quantity of grapes and quality of wine produced in most of the cool and warm viticultural regions. Many growers report crop losses of up to 70-80% in 1 year out of 10. Others report 5% crop loss 4 years in 10, 25% loss 5 years in 10 and 5-10% loss 1 year in 10.

Botrytis can also be severe where rainfall or prolonged periods of moisture coincide with pre-harvest berry ripening. An example of such occurrence is seen in the summer rainfall in the Hunter Valley, New South Wales, at pre-harvest leading to picking the fruit earlier than is optimum from a perspective of yield and quality. In general, approximately 4 years in 10, grape growers harvest early to avoid increasing crop losses due to *Botrytis* rot when rain occurs prior to harvest. Usually, 1 Baume unit is sacrificed as a result. Estimates of quality reductions and penalties vary from winery to winery but significant losses have been recorded.

Botrytis infection can also result in loss of colour when fruits are picked early to avoid berry rot. In approximately 4 years out of 10, growers loose payments for colour units (approximately $ 40 per colour unit at the current rate) per tonne in some districts (Stoneham 1995).

There are also penalties incurred by wineries in the form of additional costs associated with processing (e.g. pasteurization of musts extracted from *Botrytis* infected fruits).

Some wineries test for laccase activity. The laccase produced by *Botrytis* oxidizes the various phenolic derivatives and causes tanning of red wines. Penalties are, therefore, imposed on growers on the basis of laccase concentration in the harvested grapes.

Crop losses in individual vineyards can reach 60-80% and some vineyards are not harvested due to *Botrytis* rot. In general, crop loss as a result of *Botrytis* rot disease can amount to several millions of dollars in moderate to severe disease years.

3. *Botrytis* rot disease cycle

The disease cycle begins with primary infection of flowers or damaged plant tissue (Nair 1990). The spores which cause primary infection come from different sources such as sclerotia and mycelium in canes from the previous season, debris on the ground etc. The pathogen causes little or no harm to the crop at this stage as it lives in harmony (as a biotroph) with the host plant (grapevines). The different stages of flowering are indicated in Fig. 1. The floral parts infected by *Botrytis* in apparently healthy flowers are shown in Fig. 2. Fig. 3 shows an apparently healthy flower. When such an apparently healthy flower was surface sterilised (in 70% ethanol for 1 minute followed by 1% sodium hypochlorite for 3 minutes and then washed three times with sterilised water) to remove surface colonisation by *Botrytis* and other microorganisms and incubated for 3 days at 20^0 C, *Botrytis* grew readily from within different parts of the flower (Fig. 4). Flower infection rarely reaches epidemic proportions and at such times infection becomes obvious leading to bunch thinning and loss of yield potential.

Fig. 1 - Stages of flowering in grapevines

Fig. 2 - Floral parts infected (shown in red) with *Botrytis* in grapevines

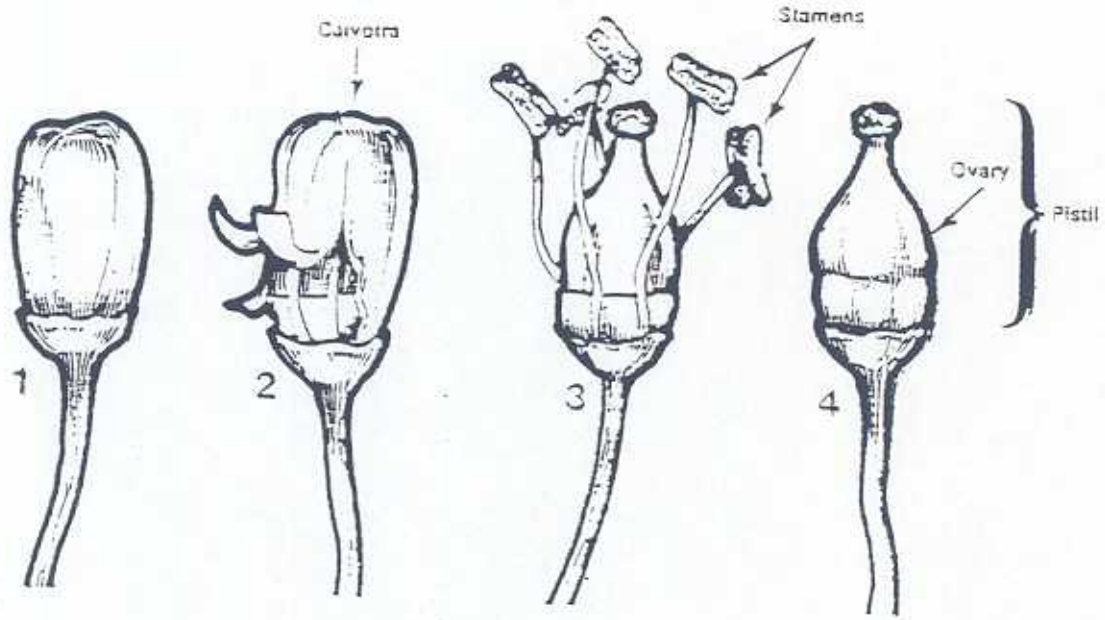

Calyptra
Stamens
Ovary
Pistil
1
2
3
4

Fig. 1

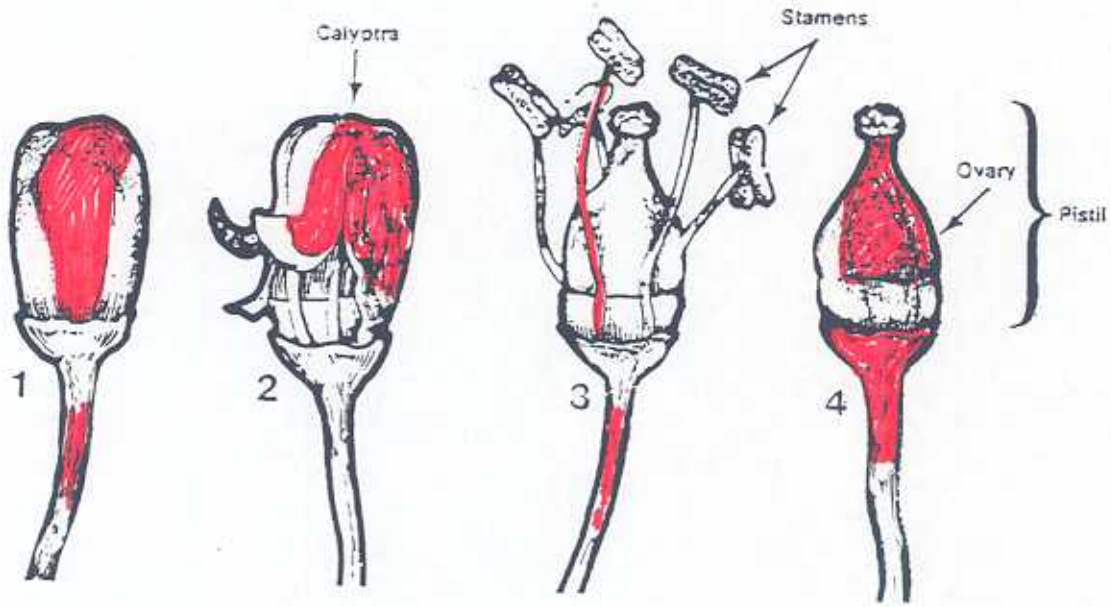

Calyptra
Stamens
Ovary
Pistil
1
2
3
4

Fig. 2

Fig. 3 - An apparently healthy flower of grapevine

Fig. 4 - Floral parts of the apparently healthy flower (Fig. 3) showing infection by *Botrytis* after it was surface sterilised and incubated in the laboratory



Fig. 3



Fig. 4

In such instances, the pathogen behaves as a necrotroph. However, this necrotrophic behaviour occurs mostly on mature berries in Australia (Figs. 5, 6).

The incidence of leaf infection is relatively low in Australia in both high and low disease pressure areas. When leaf infection occurs, it can be identified by a brown sector on the surface which is normally surrounded by a whitish margin. The growth of the necrotic areas on the leaves stops as soon as the leaves dry.

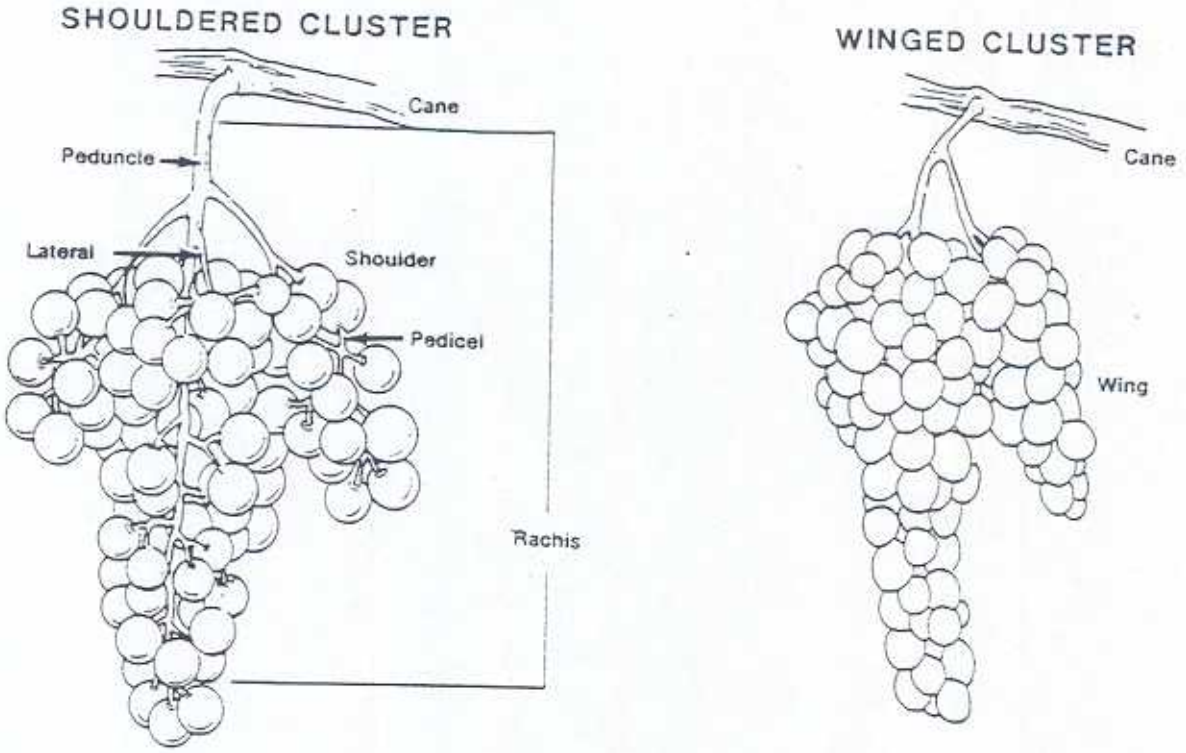
Botrytis has an inactive period in the disease cycle (latent phase) after fruit set and hence, is often inconspicuous to monitoring process between the end of flowering and veraison. This is because the immature berries are relatively more resistant to infection. Grapevines also produce substances such as stilbenes in young berries which inhibit the growth of *Botrytis* (Nair and Hill 1992).

Botrytis resumes its growth after veraison, causing rotting of mature berries. The amount of rotting increases with increasing concentration of sugars in the berries (Nair and Hill 1992). Disease severity also increases if the bunches have been damaged by insects such as the caterpillar of light brown apple moth, or adverse weather such as hail storms (Nair, Emmett and Parker 1988). Secondary infection from maturing berries occurs from spores produced various tissies of grapevine within the canopy or from outside. One such source is the undeveloped flowers trapped inside developing bunches (Fig. a).

After harvest, *Botrytis* again goes into an inactive or dormant phase during winter by producing structures called sclerotia on the canes, or as mycelium. These sclerotia are capable of germinating to produce spores (Fig. b) which causes primary infection of flowers or damaged plant tissue during the following spring, thus initiating the disease cycle in the next season (Nair and Nadtotchei 1987).

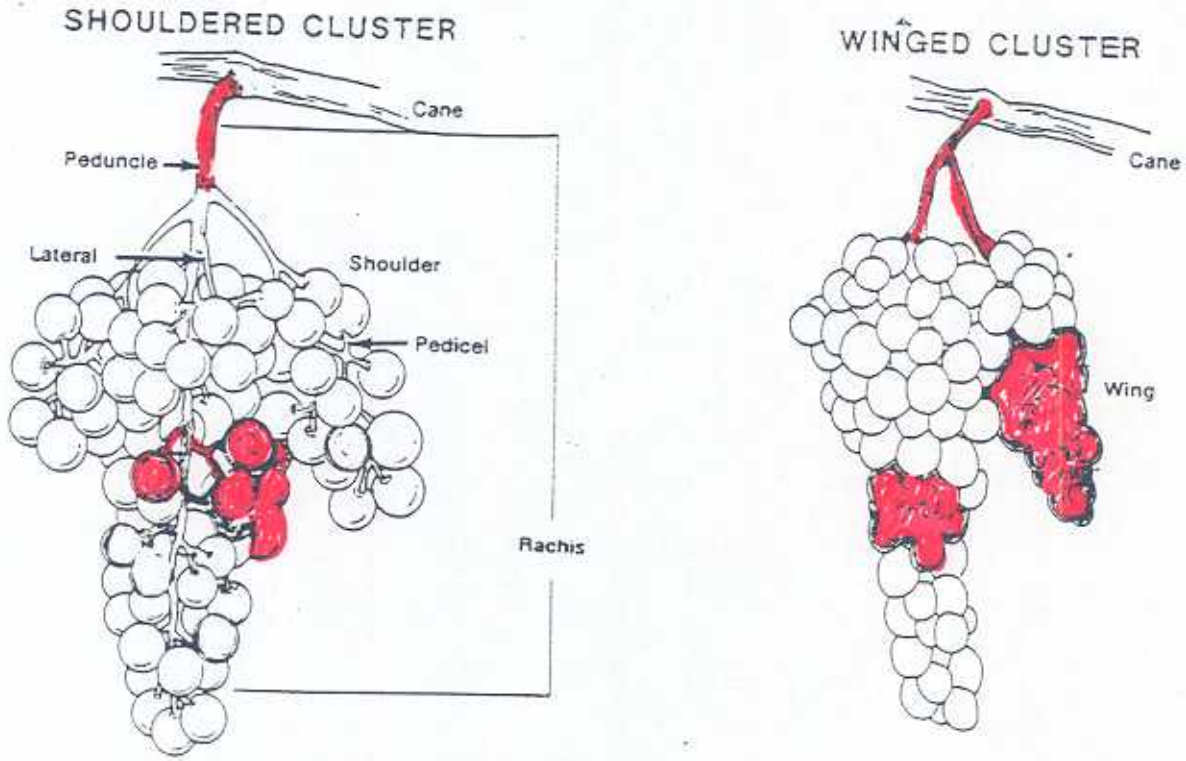
Fig. 5 - Healthy bunches of grapes

Fig. 6 - Bunches of grapes infected with *Botrytis* (note the infected parts of the bunches shown in red)



Typical shapes and structures for grape cluster.

Fig. 5



Typical shapes and structures for grape cluster.

The teleomorph (sexual form) of *Botrytis* is an ascomycete called *Botryotinia fuckeliana* which probably is of limited importance in the disease cycle in vineyards. However, this requires further investigation.

It is now known that the disease is not only based on *de novo* infection of mature berries but also on latent infection established earlier in flowers (McClellan and Hewitt 1973; Nair and Parker 1985; Nair 1990). Latent infection results from infection of flowers by *Botrytis* and occurs during flowering (Nair and Hill 1992). An effect of latent (flower) infection is that in grapevines subject to similar viticultural management and climate, the rate of development of *Botrytis* rot seems to be related to the latent infection of the flowers (Guilbaud-Oulton and Nair 1995). The effect of latent infection on disease development was studied in the current project because of its importance in disease management and fungicide resistance management.

4. Objectives

The main objective of the project was to obtain a clear understanding of the *Botrytis* rot disease cycle and thus, be able to develop a practical management strategy.

This was achieved by :

- understanding the behaviour of *Botrytis* during the growth of grapevine;
- developing a model (based on seasonal dynamics of *Botrytis* populations) to measure disease risk and to respond by timing the application of botryticides to have maximum effect on the pathogen
- preparing a fungicide resistance management plan; and,
- establishing a service for the grape industry to enable the growers to manage *Botrytis* rot.

Fig. a - Undeveloped flowers infected by *Botrytis* trapped inside a grape bunch

Fig. b - One-year-old cane showing germination and production of spores by sclerotia of *Botrytis*



b



a

Additionally, an infection model will be developed on the basis of data obtained in this project (Figs. 7, 8, 9, 10). This will enable us to characterize infection response of grape flowers and berries in relation to wetness and temperature; and, to provide the growers with a second tool in addition to monitoring *Botrytis* populations for measuring disease risk.

5. *Botrytis* rot management - Evolution of a practical strategy

a. Background

The evolution of *Botrytis* rot management during the 1980's and 1990's is given in Fig. 11. Initial attempts (1991/92) at managing *Botrytis* rot relied on three applications of "at-risk" fungicides during flowering. The aim was to reduce flower infection and therefore disease incidence at harvest. A dicarboximide, procymidone, was widely used for this purpose resulting in reduction of the disease. However, when the flowering applications were followed by further applications after veraison, more significant control of the disease was obtained (Grape and Wine Research and Development Corporation Project DAN IV, Final Report, 1991, p. 23)

The next stage (1992/93) in the evolution of *Botrytis* rot management plan was to reduce the number of applications of the "at-risk" fungicides and to include multi-site (contact or protectant) fungicides. The experimental trials consisted of reduction in the number of dicarboximide applications from three to two and the inclusion of fluazinam or chlorothalonil before bunch closure. These treatments resulted in significant reductions in the disease (Grape and Wine Research and Development Corporation Project DAN IV, Final Report, 1991, p. 25). The third stage (1993/94 to the present) in *Botrytis* rot management plan was brought about by new regulations on MRL imposed on Australia by other countries. This

Fig. 7 - Relation between predicted percentage infection of grape flowers by Botrytis cinerea (lines) and infection observed when flowers were incubated wet at 5-30^0 C for 1-24 hours (points). Predictions were based on Nair and Allen (1993)

Fig. 8 - Relation between predicted percentage infection of grape berries by *Botrytis cinerea* (lines) and infection observed when berries were incubated wet at 5-30^0 C for 1-24 hours (points). Estimates of infection were adjusted relative to inoculated controls not exposed to wetness, hence negative values in some instances. Predictions were based on Nair and Allen (1993).

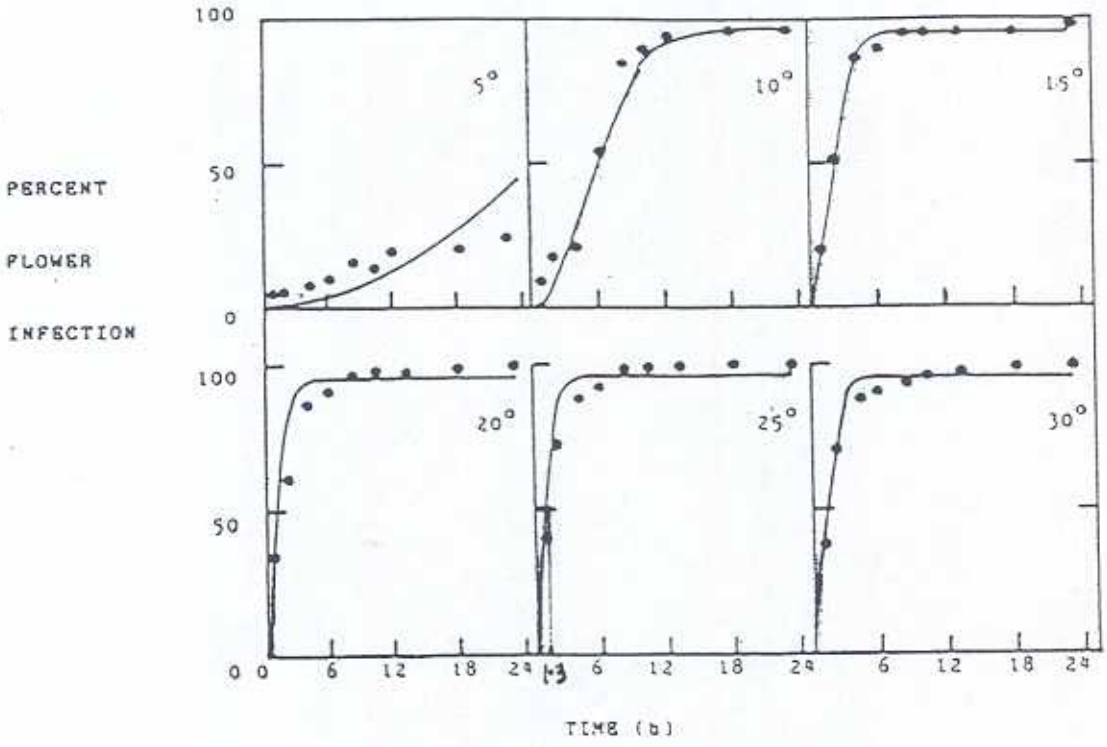

PERCENT
FLOWER
INFECTION
100
50
0
5°
10°
15°
20°
25°
30°
0 6 12 18 24
TIME (h)

Fig. 7

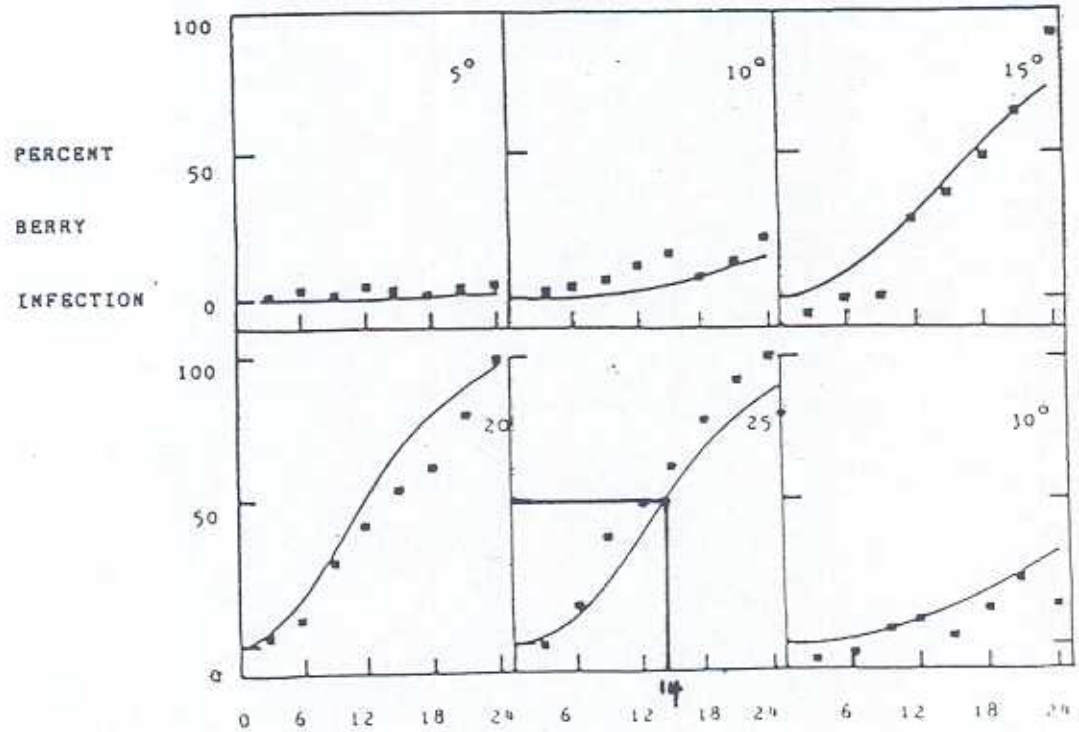

PERCENT
BERRY
INFECTION
100
50
0
5°
10°
15°
20°
25°
30°
0 6 12 18 24

Fig. 8

Fig.9 - Infection of grape flowers by *Botrytis* as a function of duration of wetness and temperature

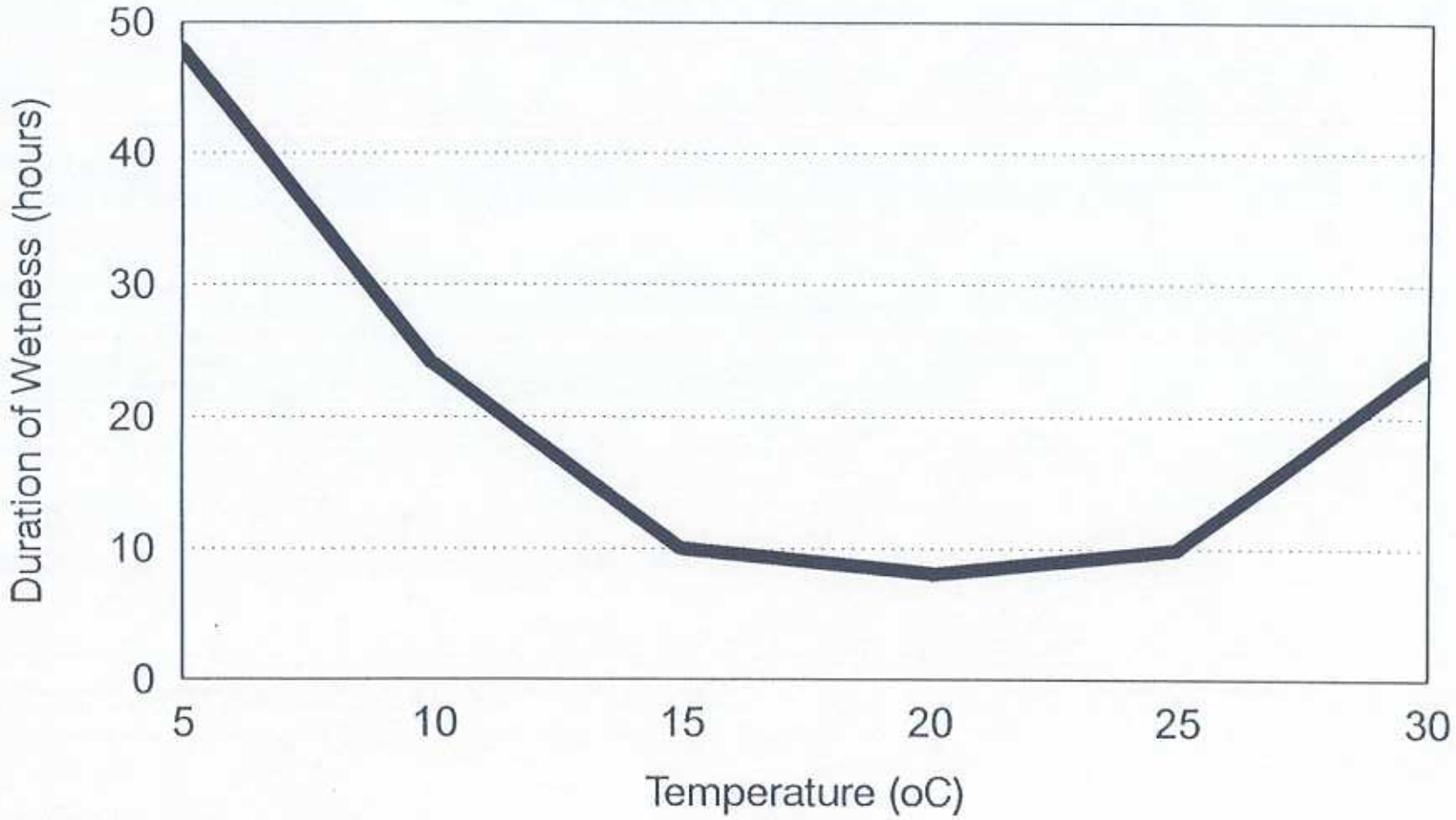

50
40
30
20
10
0
Duration of Wetness (hours)
5
10
15
20
25
30
Temperature (oC)

Fig. 10 - Infection of grape berries by *Botrytis* as a function of duration of wetness and temperature

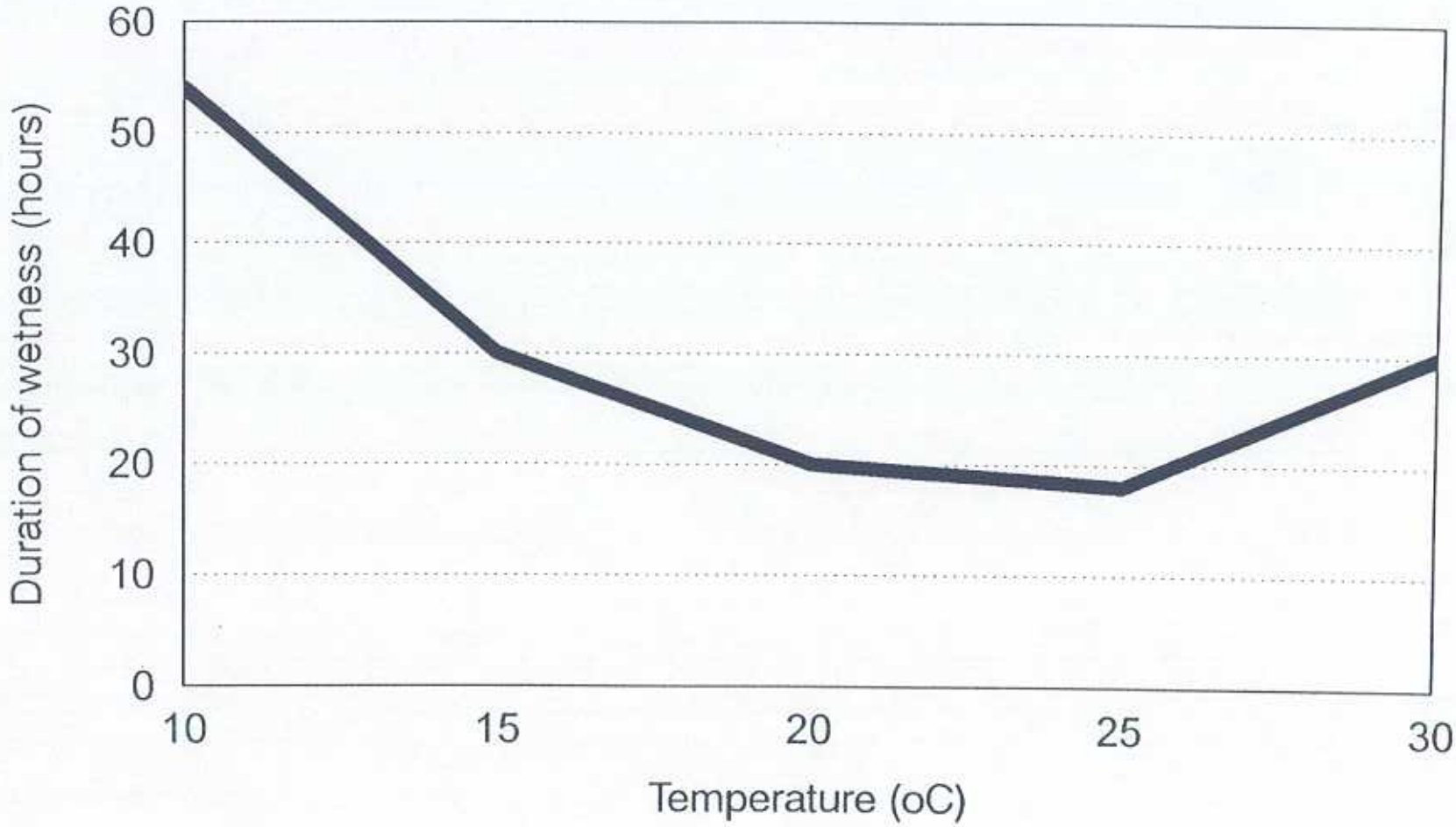

60
50
40
30
20
10
0
Duration of wetness (hours)
10
15
20
25
30
Temperature (oC)

Fig. 11 - Evolution of *Botrytis* rot management strategy during the 1980's and 1990's in Australia

Date	Management strategy	Determining factor
Pre 1980's	Fungicide application mainly after veraison and at pre-harvest	Tradition / Calender spraying
Early 1980's	Fungicide application during flowering and pre-harvest	Detection of flower infection
Mid 1980's	Rationalisation of fungicide application during flowering and pre-harvest	Additional information on disease cycle
Late 1980's	Monitoring infection levels to determine timing of application of fungicides	Quantitative epidemiology
Early 1990's	Monitoring infection and fungicide resistance levels to determine timing of application of fungicides and type of fungicides for use	Measured response to measured risk
Mid to late 1990's	*Botrytis* monitoring and fungicide resistance testing service	National strategy

resulted in limiting the number of applications of "at-risk" fungicides such as benzimidazoles and dicarboximides and using protectants at flowering.

An important change in the way in which *Botrytis* rot is managed at present by the growers is the move away from relying on calendar spraying. This was brought about by the establishment of a monitoring procedure for incidence of *Botrytis* and fungicide resistance (see section 5b of this report).

The spray schedule for *Botrytis* rot management * generally used at present is as follows :

- Chlorothalonil at 10-20 cm shoot length and/or 5% cap fall.
- Benzimidazole at 80% cap fall (if no resistance); if resistance is present, pyrimethanil.
- Dicarboximide at pre-bunch closure (or pyrimethanil if wine is not for export).
- Dicarboximide at pre-harvest.

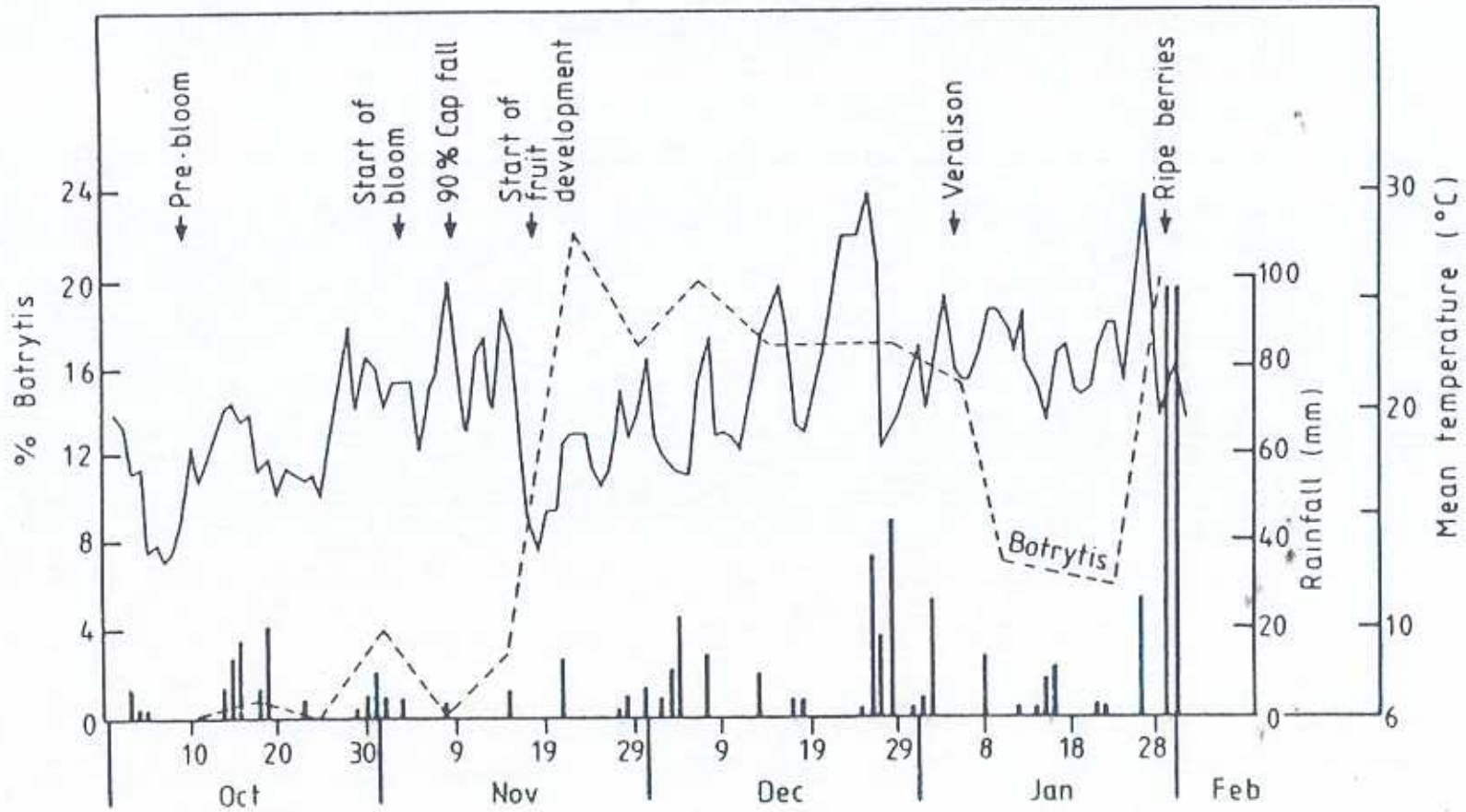
* This management program is based on the monitoring protocol given later in this report.

In all the trials relating to the timing of application of the fungicides, the populations of *Botrytis* was measured during the season. An example of the fluctuation in population of *Botrytis* during grape season is shown in Fig. 12.

b. Measurement of disease risk

(i) Introduction : The publication of Vanderplank's mathematical treatise onplant disease epidemiology in 1963 was followed by the development of a range of infection models by plant pathologists to describe the dynamics of plant disease.. Recent research has resulted in the development of infection models that relate the effects of environmental variables to disease caused

Fig. 12 - Fluctuations in populations of *Botrytis* in grapevine during the season


% Botrytis
Rainfall (mm)
Mean temperature (°C)
Botrytis
Pre-bloom
Start of bloom
90% Cap fall
Start of fruit development
Veraison
Ripe berries
0
4
8
12
16
20
24
0
20
40
60
80
100
6
10
20
30
10
20
30
9
19
29
9
19
29
8
18
28
Oct
Nov
Dec
Jan
Feb

by *Botrytis* spp. on a range of crops (Bulger *et.al.* 1987; Vincelli and Lorbeer 1989; Nair and Allen 1993; Zhang and Sutton 1994; Balasubramaniam and Moore 1994; Broome *et.al.* 1995). However, few models have been used to provide spray recommendations for the control of these diseases (Built and Lafon 1970; Strizyk 1983; Vincelli and Lorbeer 1989; Balasubramaniam and Moore 1994).

The infection models developed by these plant pathologists were mainly deterministic models which assume events occur according to strict rules and do not allow for working of chance. An alternative approach is through stochastic models (similar to the one developed in this project) which are, in general, more realistic (Hau 1988) in their representation of natural variabile. Stochastic models, in which the current disease severity of a given plant is dependent only on its severity at the previous time and not on other history, can be viewed as Markov chains (Feller 1968).

A regression model was developed in this project to measure risk of *Botrytis* rot [in contrast to a deterministic model, which implies that factors significantly influencing an event are known and accounted for, a stochastic model allows the inclusion of unexplained variability in the model - Sall (1980); Smyth (1992)]. The results obtained in the present investigation have demonstrated the ability of the model to describe the spatial and temporal aspects of the disease (Nair *et.al.* 1995).

Since latent infection of flowers is related to berry infection at harvest (McClellan and Hewitt 1973; Nair 1990), a quantitative relationship between these is likely and worthy of quantification. Similarly, the influence of carry over inoculum on flower infection and subsequent berry rot at harvest has not been measured. It is important to quantify these relationships so that they can be used as tools to predict disease risk.

(ii) Methods : *Vineyard profiles* - Fourteen vineyards located in the Hunter Valley, Mudgee and Cowra districts of New South Wales and seven cultivars (Chardonnay, Cabernet Sauvignon, Rhine Riesling, Sauvignon Blanc, Shiraz, Pinot Noir and Traminer) grown in these vineyards were included in the study. The vineyards were located in inland warm regions and the climate varied during the study. Mean annual rainfall ranged from 600-700 mm and the number of rainy days from 80-100. The age of the vines ranged from 20-25 years and were drip irrigated. The canes were spur-pruned and the canopy had, in general, hanging canes. The soil varied from clay loam to sandy loam.

The incidence of *B. cinerea* in two vineyards in the Hunter Valley was studied from 1984 to 1994 and those of the remaining vineyards from 1991 to 1994.

Collection of samples and assessment of Botrytis incidence - Thirty samples of mummified berries and attached stalks left on the vine from previous season and flowers and berries (mature and immature) from the new season each with sixty individuals were collected at random every two to three weeks during the growing season over the period 1984-94. The flowers and berries were surface-sterilized in 2% sodium hypochlorite for 3minutes, rinsed four times in sterile water, packed in clip-seal polyethylene bags containing a piece of wet filter paper (about 4 cm^2) to maintain relative humidity and left overnight at 17^0 C. The samples were then removed and incubated at room temperature at 22^0 C ($\pm 2^0$ C) for seven days. The mummified berries and stalks were incubated at room temperature only for seven days without surface sterilization. The incidence of *Botrytis* was measured by visual observation of sporulating colonies of the fungus and percentage incidence was calculated.

Statistical analysis - A simple linear regression was adopted to relate the proportion of berry infection to the proportions of carry over inoculum and flower infection. Preliminary analyses were carried out by fitting either the actual berry infection (P_B) or logit (P_B) or $\log_{10}[-\log_{10}(1-P_B)]$ on flower infection (P_F), carry over inoculum (P_{CO}), $\log_{10} P_F$, and $\log_{10} P_{CO}$. It was found that the complimentary $\log_{10}(-\log_{10})$ on $\log_{10} P_F$ and P_{CO} was best based on the coefficient of determination values (R^2).

The model used for determining berry infection as it relates to carry over inoculum and flower infection is given below:

$$\text{Log}_{10}[-\log_{10}(1-P_B)] = a + bX$$

where P_B is the proportion of berry infection, X is a covariate such as $\log_{10}(P_F)$ and P_{CO}. As P_{CO} and P_F were highly correlated, a multiple regression was less appropriate to relate the two variables to P_B. For a given value of X, the expected value of P_B and its 95% confidence limits were calculated by re-transforming the linear predicted value and its conventional confidence limits (Draper and Smith 1981).

The same technique was used to relate latent infection to carry over inoculum from the previous season.

(iii) Results and discussion : The incidence of *Botrytis* fluctuated during the entire growth phase of the grapevines irrespective of the location of the vineyard, type of cultivar, weather or cultural factors. The data on the relationship between the incidence of *Botrytis* at critical stages in the growth of grapevine are presented in Table 1.

Cary over infection rate (transformed) explained as much as 62% of variation of flower infection. However, carry over infection only accounted for 21% variation in berry infection. Carry over infection was directly related to both flower and berry infections; increased carry over infection resulted in

Table 1 - Relationship between carry over inoculum, flower infection and incidence of *Botrytis cinerea* in berries at harvest

P_F, flower infection; P_{CO}, carry over inoculum; P_B, berry infection (disease incidence); P_L, latent infection

Values in parentheses are ± s.e.

y-value	*x*-value	*a* (intercept)	*b* (coefficient)	R^2 (%)
log [–log (1–P_F)]	Log (P_{CO})	–0.534 (± 0.290)	0.782 (± 0.123)**	61.74
log [–log (1–P_B)]	P_{CO}	–2.588 (± 0.324)	1.821 (± 0.695)**	20.87
log [–log (1–P_B)]	Log (P_F)	–0.705 (± 0.253)	0.639 (± 0.102)**	59.94
log [–log (1–P_{CO})]	P_L	–1.349 (± 0.355)	2.562 (± 0.610)**	57.58

increased flower and berry infections (Figs. 13a, 13b). Infection rate (transformed) of flowers explained 60% of variation of berry infection (Table 1); increased flower infection resulted in increased berry infection at harvest (Fig. 14). Latent infection resulted in 58% of infection carried over to the following season. As the latent infection increased, there was a corresponding increase in carry over infection (Fig. 15).

This project has shown that the incidence of *Botrytis* at critical stages in the growth of grapevines such as pre-flowering, flowering and harvest are quantitatively related. We have therefore developed a model on the basis of these relationships to enable the grape growers to predict disease risk (infection levels) at specific stages in the growth of the crop. Such predictions are meant to serve as general guidelines for deciding on the timing of application of botryticides.

Previous studies on the timing of application of fungicides to control *Botrytis* rot have been based largely on a knowledge of the epidemiology of the disease and of fungicide resistance. The work reported here is the first instance where application of the fungicides to manage the disease is based on the measurement of disease risk by periodic monitoring of incidence of the pathogen.

c. Fungicide resistance management strategy

(i) Introduction : Fungicide resistance is a reality for "at-risk" chemicals and a threat to many more. This project had three objectives in relation to the development of a fungicide resistance management plan. These were :

- to prevent, delay or manage fungicide resistance in order to maintain the shelf-life of botryticides available in Australia;
- to foster a responsible attitude towards resistance problems among

Fig. 13 - Relationship between carry over infection by *Botrytis* and (a) flower infection and (b) berry infection in grapevines

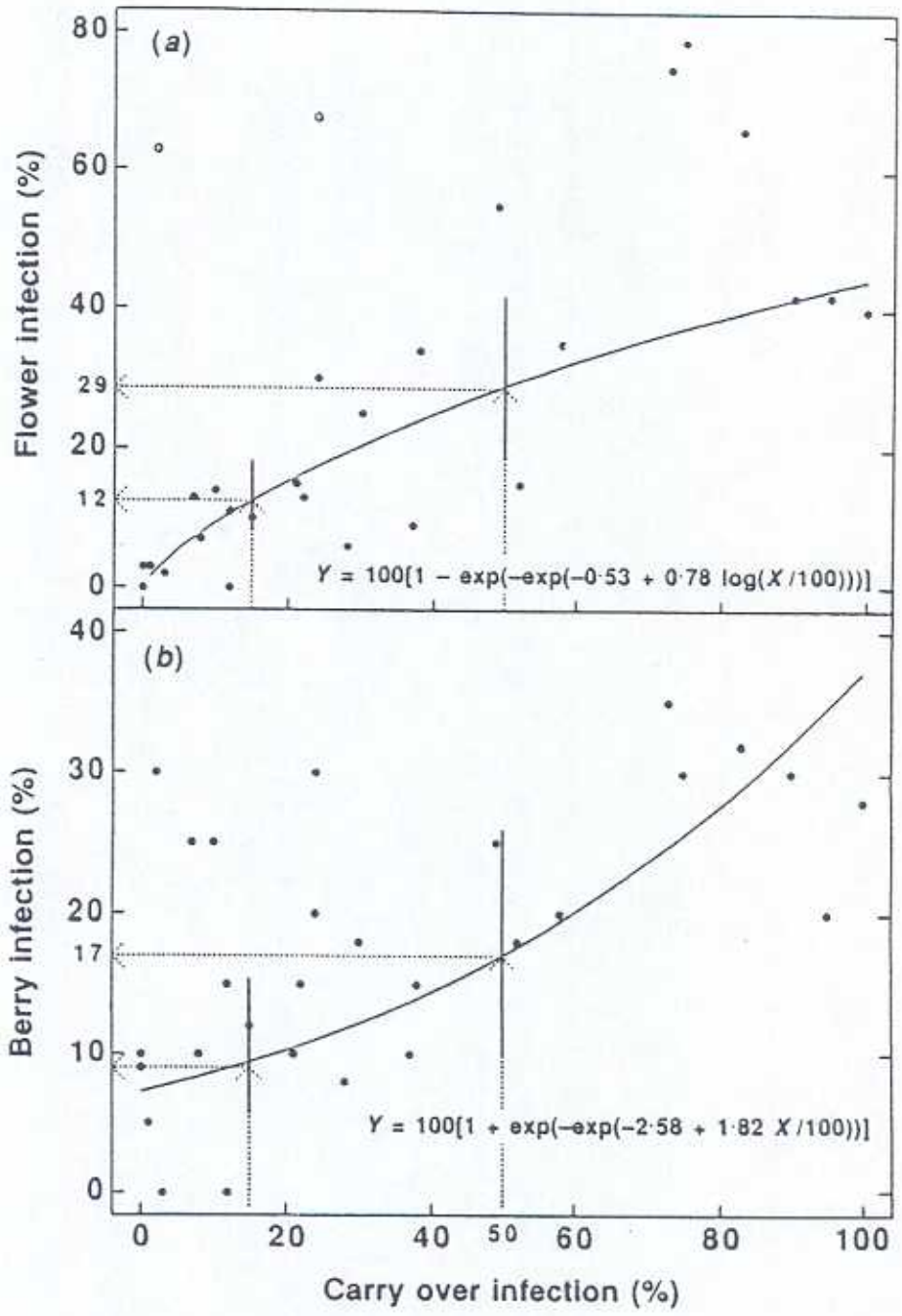

(a)
Flower infection (%)
80
60
40
29
20
12
0
Y = 100[1 − exp(−exp(−0·53 + 0·78 log(X /100)))]
(b)
Berry infection (%)
40
30
20
17
10
0
Y = 100[1 + exp(−exp(−2·58 + 1·82 X /100))]
0
20
40
50
60
80
100
Carry over infection (%)

Fig. 14 - Relationship between flower infection by *Botrytis* and berry infection in grapevines

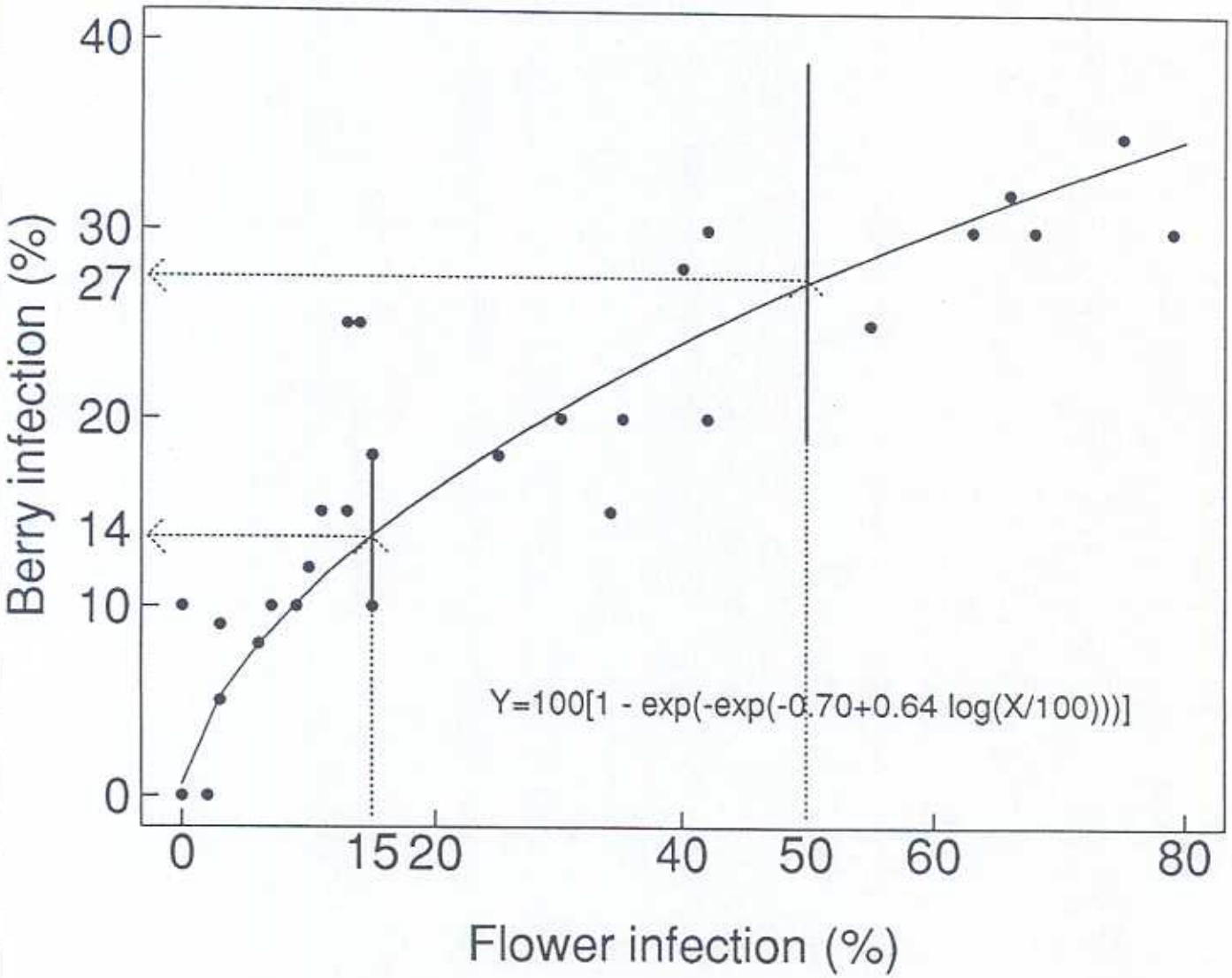
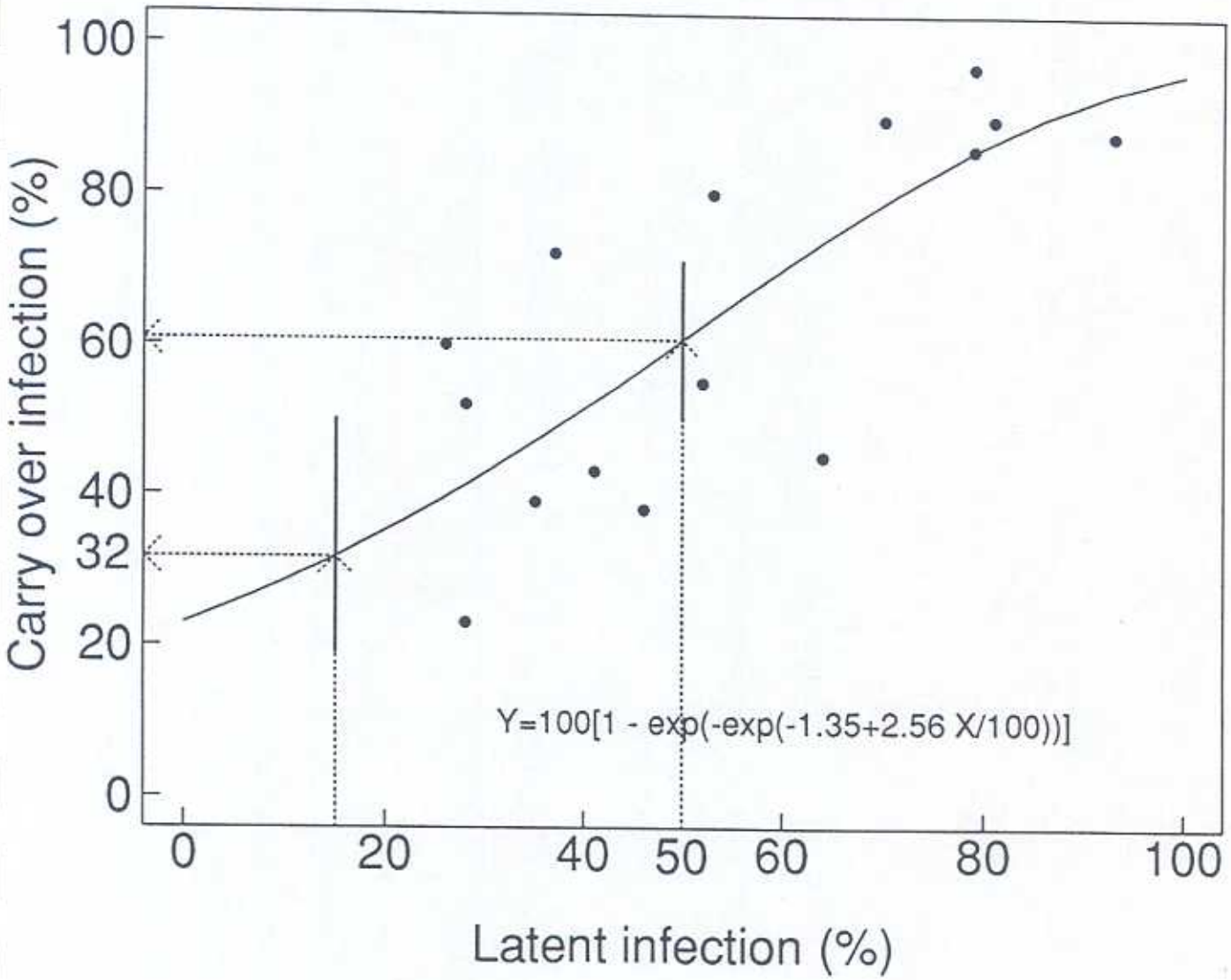

40
30
27
20
14
10
0
Berry infection (%)
Y=100[1 - exp(-exp(-0.70+0.64 log(X/100)))]
0
15
20
40
50
60
80
Flower infection (%)

Fig. 15 - Relationship between latent infection by *Botrytis* and carry over infection in grapevines


100
80
60
40
32
20
0
Carry over infection (%)
Y=100[1 - exp(-exp(-1.35+2.56 X/100))]
0
20
40
50
60
80
100
Latent infection (%)

the grape growers and to encourage them to use botryticides in a rational manner; and,

- to demonstrate the fact that fungicide resistance management is a vital component in an integrated approach to pest and disease management.

These objectives were in keeping with the aims of the Australian Fungicide Resistance Action Committee (AFRAC) and therefore, management of *Botrytis* rot of grapes should be a model for the AFRAC strategy.

Botrytis can develop resistance to modern fungicides, but does not normally develop resistance to multi-site fungicides. Factors of concern regarding fungicide resistance in *Botrytis* are :

* a short pathogen cycle;
* a freely sporulating target pathogen;
* is haploid in the asexual stage and therefore expresses resistance directly - no possibility of masking by a dominant gene;
* is multinucleate with an average of 4-5 nuclei per spore - leading to resistance at different levels;
* the need for frequent, widespread application of fungicides; and,
* the ability to survive out of season.

The fungicides commonly used in Australia for management of *Botrytis* rot are listed in Table 2.

Work carried out on fungicide resistance in this project has enabled the growers to choose the right type of chemical at critical times during the season to manage *Botrytis* rot.

(ii) Methods : The isolates of *Botrytis* were tested for resistance to benzimidazole and dicarboximide *in vitro*. Malt agar medium was amended with 10 ppm of benomyl and 2 and 5 ppm of iprodione individually. Mycelial

Table 2 - Fungicides commonly used in Australia for the management of *Botrytis* rot of grapes

Multisite fungicides

- Chlorothalonil

"At-Risk" fungicides

- Benzimidazoles
  - ◊ Benlate
  - ◊ Carbendazim
- Dicarboximides
  - ◊ Iprodione
  - ◊ Vinclozolin
  - ◊ Procymidone
- Anilinopyrimidines
  - ◊ Pyrimethanil

(Any omission of fungicides is unintentional)

disc (4 mm) from an axenic culture of *Botrytis* isolate was placed on the fungicide amended medium in petri dishes. The growth of the isolate was noted after 24 hours. Absence of growth indicated that the isolate was susceptible. On the other hand, if the isolate grew in the presence of the fungicide it was considered resistant to that fungicide (Fig. 16). The resistance frequency was expressed as a percentage.

Two other methods were used to confirm the results of the mycelial growth test. One was specifically for testing benzimidazole resistance; and, this was a polymerase chain reaction (PCR) method (Fig. 17) where the susceptible and resistant strains were finally identified by gel electrophoresis (Luck et.al. 1995). The other (suitable for both benzimidazole and dicarboximide) consisted of testing spore germination in these fungicides at different concentrations similar to that used in the mycelial growth test.

Mycelial growth was then adopted as the standard test method for all fungicide resistance monitoring.

(iii) Results and discussion : The three different methods used for testing gave the same result (Table 3). All further resistance tests were, therefore, carried out using the mycelial growth test. Occurrence of fungicide resistance in the different viticultural regions is discussed in section 5d of this report. A few general observations on resistance in the vineyards studied could be given here. There has been a drop in the resistance level, especially dicarboximide resistance, when the number of applications of the fungicides was reduced or temporarily removed from use (Fig. 18). A few instances were observed where benzimidazole and dicarboximde resistant populations of *Botrytis* were present in vineyards that have not used these chemicals.

In general, a fluctuating pattern in fungicide resistance was observed within a vineyard. Cross resistance was observed between the different

Fig. 16 - *In vitro* mycelial growth test to determine benzimidazole and dicarboximide resistance in *Botrytis* on grapevines

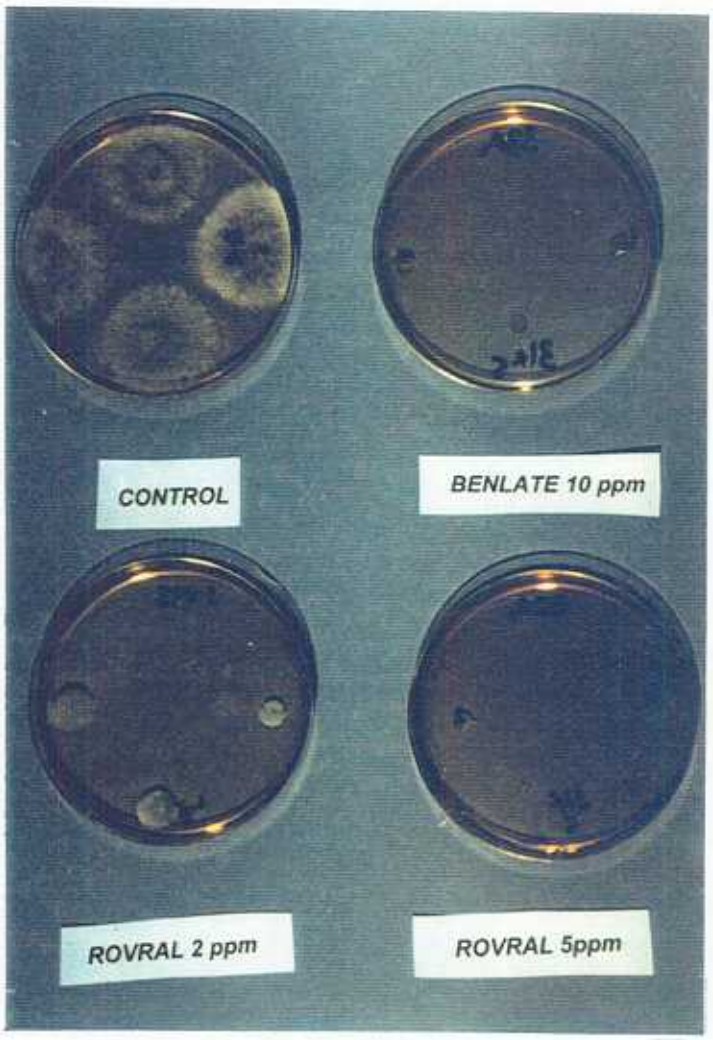

CONTROL
BENLATE 10 ppm
ROVRAL 2 ppm
ROVRAL 5ppm

Fig. 17 - Polymerase chain reaction method for the detection of benzimidazole resistance in *Botrytis* on grapevines

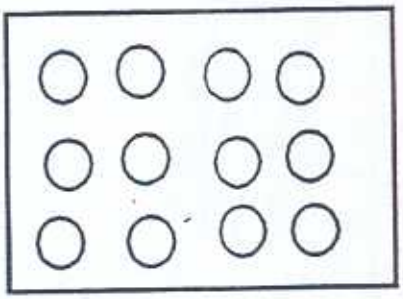
Direct detection of benomyl resistance



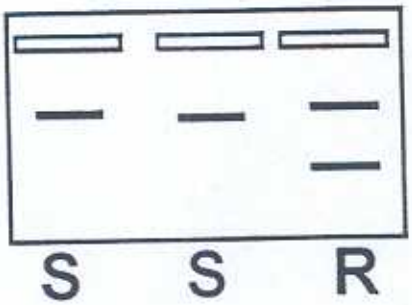
Collect field samples



Mycelial suspension ground in GeneReleaser, microwaved



Add reaction mix and primers, perform PCR



Gel electrophoresis

(adapted from Luck, Gillings and Steel 1995)

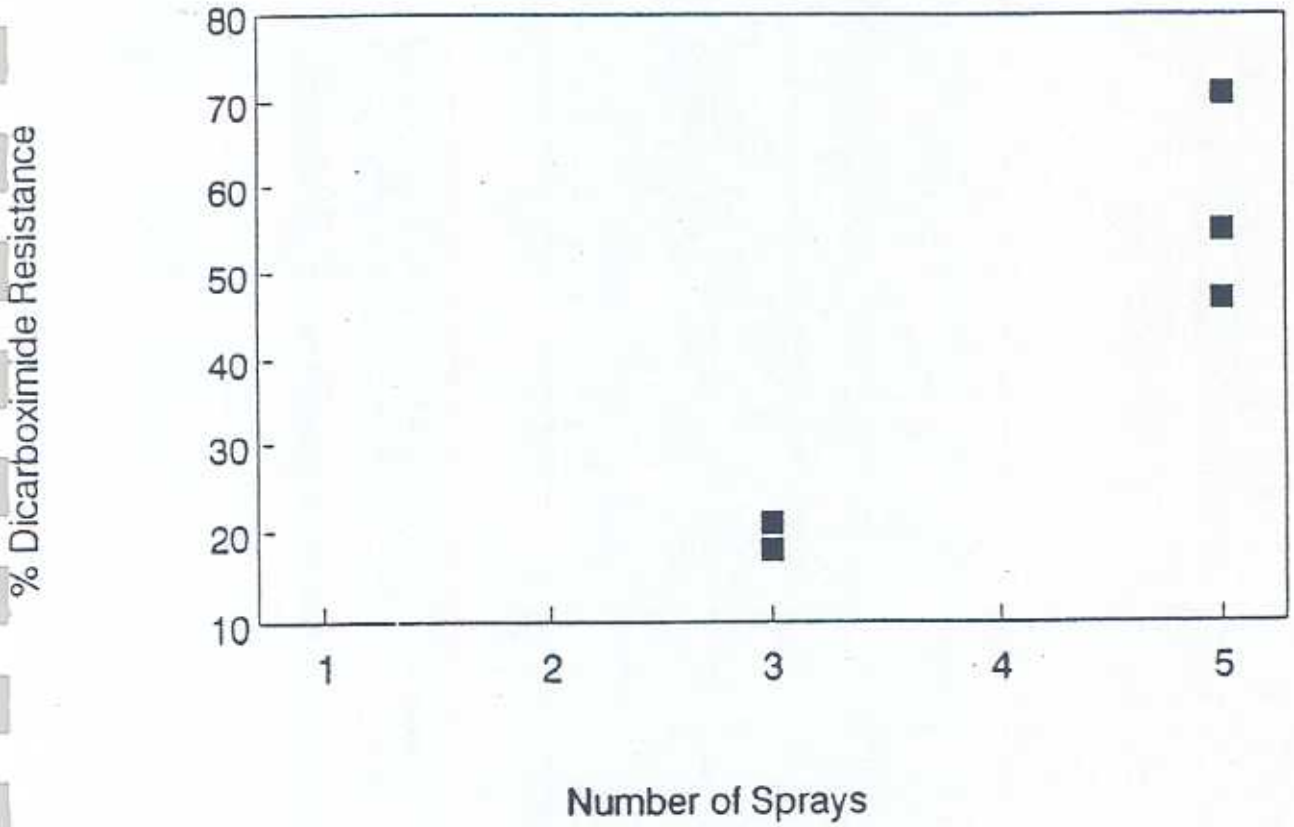
Table 3 - Comparison of three different methods for determining benzimidazole and dicarboximide resistance in *Botrytis cinerea* on grapes

Botrytis isolate	Chemical treatment	Spore germination (%)		Mycelial growth (%)		PCR
a	Benlate (10ppm)	95	R*	100	R	R
	Rovral (5ppm)	87	R	70	R	-
	Rovral (2ppm)	96	R	100	R	-
	Control	99	-	100	-	-
b	Benlate (10ppm)	88	R	100	R	R
	Rovral (5ppm)	84	R	100	R	-
	Rovral (2ppm)	98	R	100	R	-
	Control	94	-	100	-	-
c	Benlate (10ppm)	86	R	100	R	R
	Rovral (5ppm)	72	R	100	R	-
	Rovral (2ppm)	90	R	100	R	-
	Control	97	-	100	-	-
d	Benlate (10ppm)	22	S*	22	S	S
	Rovral (5ppm)	21	S	21	S	-
	Rovral (2ppm)	10	S	10	S	-
	Control	93	-	100	-	-
e	Benlate (10ppm)	68	R	100	R	R
	Rovral (5ppm)	55	?	100	R	-
	Rovral (2ppm)	71	R	80	R	-
	Control	99	-	100	-	-
f	Benlate (10ppm)	74	R	100	R	R
	Rovral (5ppm)	95	R	100	R	-
	Rovral (2ppm)	86	R	100	R	-
	Control	99	-	100	-	-

* R = resistant to benzimidazole S = sensitive to benzimidazoles

** PCR = Polymerase chain reaction method - = not tested

Fig. 18 - Dicarboximide resistance in *Botrytis* in relation to the number of sprays of the chemical in a vineyard


% Dicarboximide Resistance
80
70
60
50
40
30
20
10
1
2
3
4
5
Number of Sprays

dicarboximides. Multiple resistance was common in several isolates of *Botrytis*.

A numbers of factors were seen to influence the changes in dicarboximide resistance frequency during one season. These included :

- ⇒ the number of applications of dicarboximide;
- ⇒ the timing of the initial applications of dicarboximide; and,
- ⇒ the frequency of dicarboximide resistant strains at the beginning of the season.

Although it has not been possible to predict the occurrence of benzimidazole or dicarboximide resistance, the results obtained in this project has helped in managing the resistance levels in the vineyard satisfactorily. A good correlation between resistance level and poor field performance was also seen in several cases.

There are five ways of responding to the presence of fungicide resistance in the vineyard :

- ➲ temporary interruption of the treatment;
- ➲ decrease in the number of treatments;
- ➲ changing to an alternative fungicide;
- ➲ using tank mixes of fungicides; and,
- ➲ taking into account the timing of epidemic development.

The data on the occurrence of fungicide resistance should be used in conjunction with those of *Botrytis* monitoring.

d. *Botrytis* monitoring and fungicide resistance testing service

(i) Introduction : In order to develop an effective management system, disease progress must be measured and appropriate responses taken to reduce or

even nullify the risk. There are two major ways of measuring disease risk. Firstly, the populations of the pathogen at different stages of growth of the grapevine can be monitored and then action taken to lower the level of the population below the threshold by timing the application of the fungicide. This method (which has been described in section 5b of this report) has been in commercial practice during the last seasons (1994/95, 1995/96 and 1996/97). Secondly, disease risk can be measured by the use of an infection model which indicates when an infection period has occurred. Infection model depends on the presence of conducive weather before indicating disease risk. However, we cannot discount the likelihood of high disease risk being present in the vineyard with low levels of the pathogen even if conducive weather was not present; because if the weather became favourable, infection would readily take place. At such times it may be too late to react to control infection. If, on the other hand, population levels were being monitored regularly in the vineyard, the situation would have been rectified by taking measures to manage the activity of the pathogen.

Three main limitations have been observed with regard to the use of fungicides to manage *Botrytis* rot :

1) timing and efficiency of application of fungicides have been inaccurate;
2) the degree of effectiveness of some of these fungicides has been lost through the development of resistance by *Botrytis*; and,
3) the usage of some of these fungicides has been restricted mainly by the imposition of MRL's by countries importing Australian wine.

In order to overcome these limitations, it has become necessary to monitor fungicide resistance levels in the vineyard so that these chemicals can be used effectively and within the restrictions imposed by importing countries.

(ii) Methods : (Protocol 1997/98)

BOTRYTIS MONITORING

Purpose of *Botrytis* monitoring

- To assess disease risk.

Botrytis rot of grapes is influenced by both weather and the inoculum level of *Botrytis*. The incidence of *Botrytis* in the vineyard indicates disease risk; generally, higher incidence resulting in higher disease risk.

Stage for monitoring

At dormancy.

To estimate the carry over of inoculum to new season. The higher the carry over the greater the chances of flower infection, especially if the weather is favourable during the period of flowering.

Method of monitoring

1. Selection of vineyard block

The incidence of *Botrytis* can vary significantly between different blocks in a vineyard; it can also vary within a single block. It would, however, be advisable to test the incidence of *Botrytis* in blocks normally susceptible to the disease from where the highest risk of disease can originate.

2. Number of samples

One hundred samples are required from each block at each time of sampling.

3. Procedure for collecting samples

- Samples should be collected from 100 different sampling points in the block.
- Samples should be representative of the block; therefore, they should be taken randomly from each row within the block.
- Avoid collecting samples from ends of rows.

- Collect samples singly in individual bags.
- Cut the ends of flower bunches or mummified berries or stalks with a clean pair of scissors or secateurs.
- Hold the sample collection bag directly underneath the sample being collected so that it can drop into the bag without being touched.
- The tool used for collecting samples must not come in contact with *Botrytis* visible on the sample.
- Seal the bags immediately after collection.
- Provide the following details on the sample bag :
    - Vineyard name, telephone and fax numbers.
    - Locality
    - Block identification
    - Vine growth stage
    - Spray program of previous and current season
- Dispatch samples soon after collection to reach the following address at the beginning of the week :

    Botrytis Monitoring and Fungicide Resistance Testing Service
    Attention : Dr N. G. (Tan) Nair
    School of Horticulture
    University of Western Sydney
    Locked Bag No. 1, Richmond, NSW 2753

- The Botrytis Monitoring and Resistance Testing Service should be advised that the samples have been sent - Tel - (02) 45701332; Fax - (02) 45701314.

RESISTANCE MONITORING

Purpose of resistance monitoring

- To determine the presence or absence of fungicide resistance and take appropriate management strategy.
- To ascertain whether poor performance of fungicide is due to inefficient methods of spray application and make appropriate changes.

Stages for resistance monitoring

1. At harvest.

To determine presence or absence of resistance to benzimidazoles, dicarboximides and anilinopyrimidines so that a suitable management strategy could be planned for the new season.

2. End of flowering.

(Optional) To determine whether the level of dicarboximide resistance has dropped to a safe level, if it had been high at the previous monitoring (at last season harvest).

Method of monitoring

1. Selection of vineyard block

Level of resistance can vary significantly between vineyard blocks. It is advisable to collect samples from blocks most susceptible to *Botrytis*; however, care should be taken when relating the results of resistance from hot spots to the whole vineyard.

2. Number of samples

(Follow the same procedure as for *Botrytis* monitoring)

3. Procedure for collecting samples

    - Collect individual berries at harvest taking extreme care to avoid cross contamination.

    (For rest of the procedures, follow those of *Botrytis* monitoring)

Laboratory testing

Detailed procedure for assessing the level of *Botrytis*

◊ Before opening the bags check that the bags are fully labelled.

- Label bags that are unmarked.
- Check back to sender if information is incomplete.

◊ Enter sample details, date received and date attended in the register.

◊ Give the sample an accession number.

◊ Fax or send acknowledgment of receipt of sample to the sender.

◊ Mark each bag with service accession number and date received.

⇒ *Botrytis* incidence in flowers and berries :

- Divide the samples into two batches of 50 each if incidence and latent infection are required. For either incidence or latent infection only, work on all the 100 samples.
- Place the bags over night at 0°C (optional).
- Place a small piece of moist paper towel inside the bags and place them on a clean bench at room temperature.
- Check the samples on the fourth day for *Botrytis* and record per cent infection.

⇒ Latent infection of flowers and berries :

- Immerse the samples for 1 minute in 70% ethanol followed by immersion in 1% sodium hypochlorite for 3 minutes.

- Rinse 3 times in sterile water using fresh batches of sterile water every 15 samples.
- Place the sterile samples into new containers with paper towel moistened with sterile water and incubate them at room temperature.
- Check the containers to make sure that the samples do not dry out during the period of incubation.
- Record per cent infection on the 7th day.

Procedure for assessing fungicide resistance

- Prepare axenic cultures of *Botrytis* from infected flowers or berries on malt extract agar (MEA) amended with streptomycin (40 mg/l).
- Incubate the cultures for 3 days at 25° C under alternating light and dark periods (12 hours each).
- Cut out mycelial discs and place on MEA plates (4 discs per plate) amended with different levels of Benlate® (10 ppm) or Rovral® (2 and 5 ppm). A series of plates without the fungicides serve as controls. (After cutting the mycelial discs, keep the original cultures to check for purity).

The inoculated MEA fungicide plates are incubated at 25° C under alternating light and dark periods (12 hours each).

(iii) Results and discussion : The incidence of *Botrytis* and the frequencies of occurrence of benzimidazole and dicarboximide resistance in the different states in Australia during 1994/95, 1996/96 and 1996/97 are given in Table 4. The overall incidence of *Botrytis* as well as dicarboximide resistance were relatively high during the last three seasons. It is interesting to note that although the highest *Botrytis* incidence was recorded in South Australia, the frequencies of occurrences of benzimidazole and dicarboximide resistance were the lowest. New South Wales had the lowest incidence of *Botrytis* and relatively low levels of benzimidazole and dicarboximide resistance.

Table 4 - Incidence of *Botrytis* and frequency of fungicide resistance in Australia over three seasons (1994/95, 1995/96 and 1996/97)

State	Number of vineyards	Number of samples tested	Number of isolates	Botrytis incidence (%)	Frequency of resistance	
					*BR(%)	*DR(%)
NSW	70	6270	1157	18 (1.8)	12 (1.82)	17 (1.91)
Victoria	86	6360	2044	32 (2.18)	26 (2.59)	29 (2.58)
S. Aust	50	5070	1905	37 (2.31)	4 (0.87)	11 (1.50)
Tas/Qld/WA	12	930	240	27 (6.22)	14 (4.90)	12 (3.64)
National	218	19080	5346	28 (1.22)	15 (1.21)	19 (1.24)

*BR = Benzimidazole resistance;
*DR = Dicarboximide resistance
Based on the total number of samples tested during 1994/95, 1995/96 and 1996/97 season.

Figures in parentheses denotes standard error

In general, the range in the frequency of occurrence of benzimidazole resistance (4 - 26%) and of dicarboximide resistance (11 - 29%) in the different viticultural regions in Australia is considered to be relatively low. As far as we know, Australia is one of the few countries in the world where benzimidazole can still be used with success, if enough care is taken in its usage. The regional data on the frequency of occurrence of benzimidazole and dicarboximide resistance obtained in the monitoring service play a significant role in the use of these classes of chemicals in disease management.

There appears to have been a gradual increase in *Botrytis* incidence during the last three seasons in the regions monitored (Fig. 19). In the same period, there has been a decline in the frequency of dicarboximide resistance with no significant shift in the frequency of benzimidazole resistance (Fig. 20). This reflects the rational use of these fungicides by the growers.

The south west region (Wagga Wagga, Walla Walla and Tumbarrumba) of New South Wales had the highest incidence of *Botrytis* as well as the maximum frequencies of occurrence of benzimidazole and dicarboximide resistance during 1996/97 (Table 5). This region includes cool climate viticultural areas where the disease pressure can be relatively high and this would partly explain the disease situation. The incidence of *Botrytis* was also relatively high but not resistance frequency in lower Hunter and Riverina in New South Wales. It is interesting to note that these are warm, inland regions and that they are not immune from *Botrytis* rot.

King Valley, Geelong, Murray Valley and north east of Victoria had high incidence of *Botrytis* and, except King Valley, also had high frequencies of occurrence of benzimidazole and dicarboximide resistance (Table 6). Yarra valley, which is a cool and wet region, had low incidence of *Botrytis* but high frequencies of benzimidazole and dicarboximide resistance.

Fig. 19 - Fluctuations in incidence of *Botrytis* on grapevines on a national basis over three seasons (1994/95, 1995/96, 1996/97)

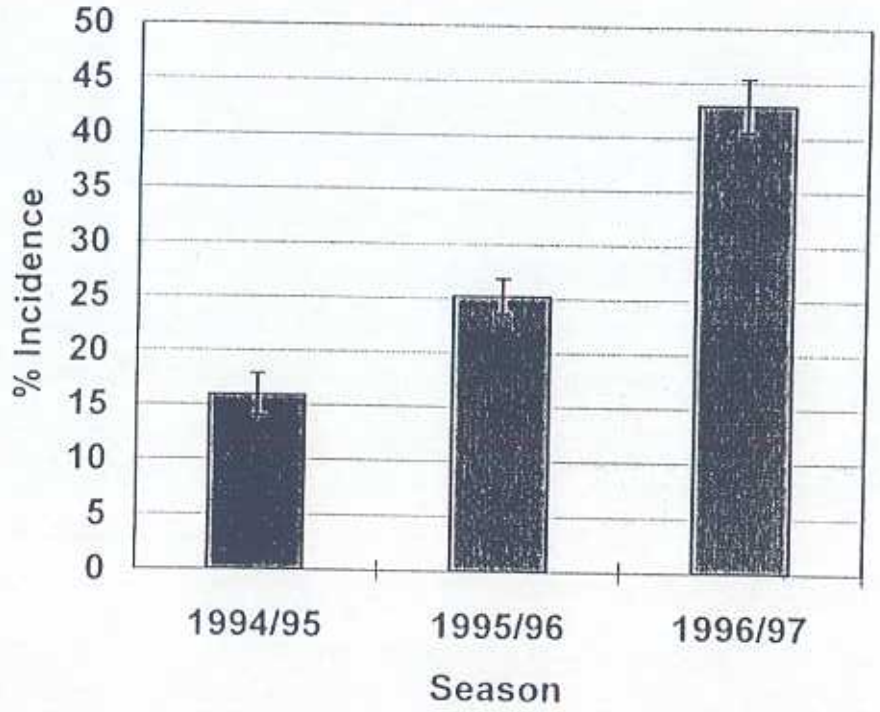

50
45
40
35
30
25
20
15
10
5
0
% Incidence
1994/95
1995/96
1996/97
Season

Fig. 20 - Fluctuations in incidence of benzimidazole and dicarboximide resistance in *Botrytis* on grapevines on a national basis over three seasons (1994/95, 1995/96, 1996/97)

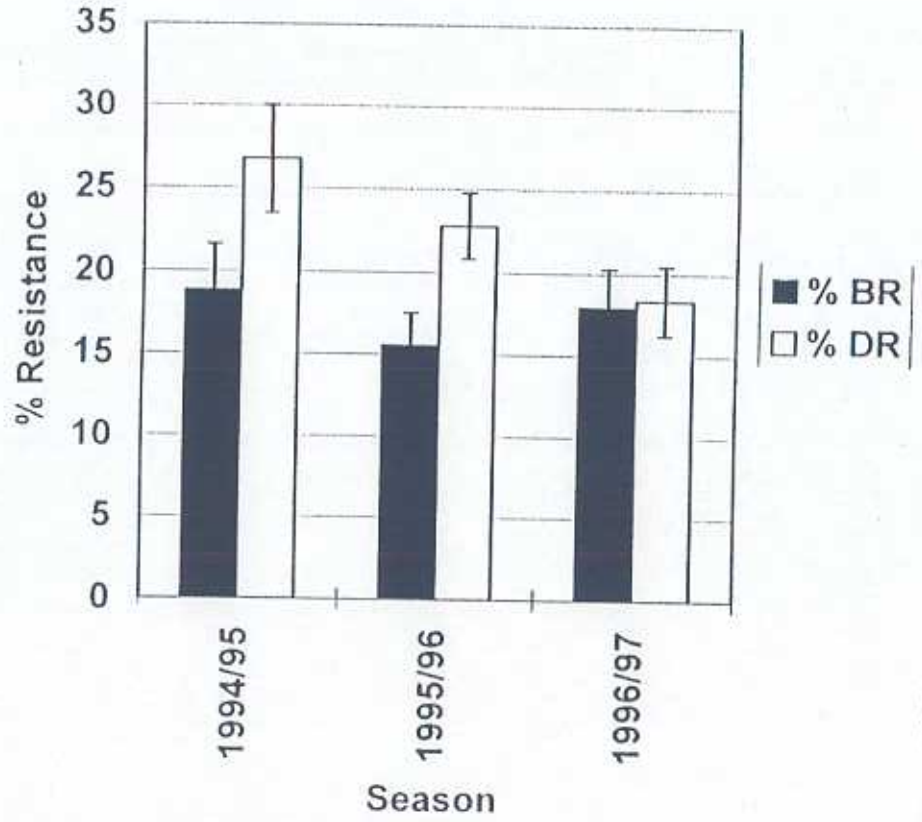

35
30
25
20
15
10
5
0
% Resistance
% BR
% DR
1994/95
1995/96
1996/97
Season

Table 5 - Incidence of *Botrytis* and frequency of fungicide resistance in different regions of New South Wales during 1996/97

Viticultural region	Number of Vineyards	Number of Samples tested	Number of isolates	*Botrytis* incidence %	Frequency of resistance	
					*BR%	*DR%
**South West	3	90	60	67(15.27)	62(19.27)	43 (4.04)
Upper Hunter	4	450	163	41(8.55)	15 (7.19)	25 (6.66)
***Riverina	2	90	35	39 (9.54)	17 (9.53)	27 (6.51)
Mid North Coast	3	420	136	26 (8.26)	35 (11.25)	34(10.46)
Lower Hunter	4	240	48	20 (9.76)	8 (5.92)	8 (5.92)
Mudgee	1	30	1	3 (0)	N/A	N/A
Total NSW	18	1470	465	34 (4.74)	25 (5.27)	26 (4.19)

*BR = Benzimidazole resistance;
*DR = Dicarboximide resistance
Based on the total number of samples tested during 1996/97 season.

Figures in parenthesis denote standard error

**South West region includes Wagga Wagga, Walla Walla and Tumbarumba.
***Riverina region includes Griffith and Hanwood.

Table 6 - Incidence of *Botrytis* and frequency of fungicide resistance in different regions of Victoria during 1996/97

Viticultural region	Number of Vineyards	Number of Samples tested	Number of isolates	*Botrytis* incidence (%)	Frequency of resistance	
					*BR %	*DR %
King Valley	3	210	159	76 (10.43)	21 (19.86)	23 (11.69)
Geelong	1	150	89	59 (19.92)	42 (11.11)	38 (15.80)
North East	6	360	191	58 (13.21)	43 (17.67)	45 (15.87)
Murray Valley	5	480	266	56 (8.35)	48 (10.02)	33 (8.31)
Yarra Valley	4	180	38	21 (10.92)	60 (21.28)	41 (21.27)
Goulburn Valley	5	360	77	21(8.17)	2 (1.67)	32 (29.54)
Mornington Peninsula	1	30	1	3 (0)	N/A	N/A
Total Victoria	26	1950	905	47 (4.66)	35 (5.85)	32 (5.16)

*BR = Benzimidazole resistance;
*DR = Dicarboximide resistance
Based on the total number of samples tested during 1996/97 season.

Standard Errors are given in brackets ( ).

Coonawarra in South Australia had the highest incidence of *Botrytis* followed by Barossa Valley and McLaren Vale (Table 7). The frequencies of occurrence of benzimidazole and resistance were almost negligible in these regions. Coonawarra is a cool, wet region whereas the other two regions are relatively warm and dry. The negligible levels of benzimidazole and dicarboximide resistance in these regions are noteworthy. This could be due to the low numbers of vineyards monitored in these regions.

Observations made during the *Botrytis* monitoring and fungicide resistance testing service have serious implications on the management of *Botrytis* rot of grapes. These observations and guidelines on how to respond are summarised below :

Observations		Response	Cautionary note
Monitoring carry over *Botrytis* levels (before bud burst) quantifies the potential for flower infection.			
>15% carry over	-	Apply a protectant (e.g. chlorothalonil) at pre-flowering (e.g. 10-20 cm shoot length)	Another application at 50% cap fall is necessary if weather is favourable for disease*
<15% or no carry over	-	No action	
Latent infection of *Botrytis* from the previous season can remain in the vineyard and infect flowers in the current season.			

Table 7 - Incidence of *Botrytis* and frequency of fungicide resistance in different regions of South Australia during 1996/97

Viticultural region	Number of Vineyards	Number of Samples tested	Number of isolates	*Botrytis* incidence (%)	Frequency of resistance	
					*BR %	*DR %
Coonawarra	22	1920	934	50 (3.81)	3 (1.19)	4 (1.32)
McLaren Vale	1	90	15	17 (6.89)	0 (0)	8 (8)
Barossa Valley	2	150	53	35 (18.47)	0 (0)	0 (0)
Total South Australia	25	2160	1002	46 (3.66)	3 (1.04)	4 (1.30)

*BR = Benzimidazole resistance;
*DR = Dicarboximide resistance
Based on the total number of samples tested during 1996/97 season.

Figures in parenthesis denotes Standard Error

Spray applications of benzimidazoles and dicarboximides during early flowering (until 80% cap fall) increased resistance frequency and decreased efficacy of these chemicals.

-	Do not apply benzimidazoles until 80% cap fall and dicarboximides until pre-bunch closure	Follow AVCARE guidelines**

Application of eradicant fungicides significantly reduced latent infection established earlier in the flowers.

- If benzimidazole resistance (BR) is low (<10%) or absent, apply this chemical at 80% cap fall. If BR is >10% apply pyrimethanil

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Monitoring *Botrytis* at the end of flowering (e.g. after 100% cap fall) indicated whether the flowering program has been successful in minimising or avoiding infection.

Monitoring fungicide resistance at the end of flowering (e.g. after 100% cap fall) indicated a decrease in the level of dicarboximide resistance from the high level at harvest of previous season.

- Do not apply dicarboximide until pre-bunch closure

Flowers that have failed to set fruit are often caught within the maturing grape bunches. In order to treat these flowers and the infection they may carry sprays must be applied at bunch closure (before berries touch). This must be a compulsory spray irrespective of the location of the vineyard.

- Apply dicarboximide if dicarboximide resistance (DR) is low (<10%) or absent. If DR is present, apply pyrimethanil (check MRLs in US and Canada)

Although the mature berries from fruit set to veraison have a degree of resistance to infection, under favourable weather conditions, infection of set berries could occur.

The population of *Botrytis* can explode after veraison or before harvest from low levels at the previous stages. Factors responsible for this are not known; weather, physical damage to berries, crop vigour etc. can play a part in this explosive stage of the disease cycle.

- Apply dicarboximide after veraison if (DR) dicarboximide resistance is low (<10%) or absent. If DR is present apply pyrimethanil (check for MRLs in US and Canada)

If the season is unusually dry, the amount of *Botrytis* rot of grapes would be less than expected. In cool, wet viticultural regions phenological periods are longer than those in warm, dry regions. It follows, therefore that the period of crop susceptibility is relatively

longer. Under these conditions, assessment of *Botrytis* incidence may show that a greater number of protectant sprays are required to manage the disease.

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* Rainfall in the last 2 weeks, where a rain day = >2 mm in 24 hours

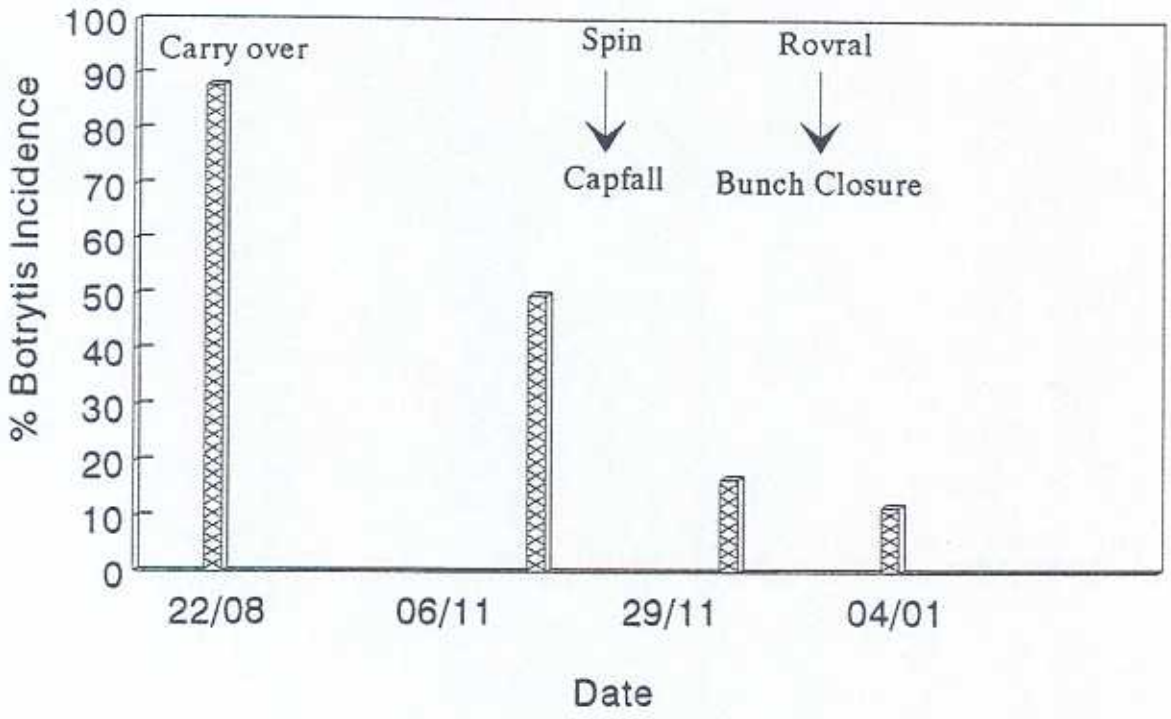
** Do not apply more than 2 dicarboximide or benzimidazole sprays in a season unless tank mixed with a registered protectant fungicide. If 3 or more *Botrytis* rot sprays are applied per season, use only 1 pyrimethanil spray.; if 4 or more *Botrytis* rot sprays are applied per season, use no more than 2 pyrimethanil sprays. Do not apply more than 2 consecutive sprays from the same fungicide group including from the end of one season to the next.

Significance of carry over on *Botrytis* management

Measuring carry over levels of *Botrytis* before budburst quantifies the potential for flower infection in a vineyard block. In some instances it is possible to react to low carry over values by omitting pre-cap fall spray applications. The decision to omit these sprays is currently based on the recording of low levels of *Botrytis* at harvest in the previous season. However, latent *Botrytis* that has infected flowers in the previous season can remain in the vineyard over winter and infect flowers in the current season. This means that carry over levels of *Botrytis* can be high even if a low incidence of *Botrytis* rot was experienced during the previous vintage. For instance, a block of Chardonnay with a carry over of 88% in August 1993 recorded only 33% bunch rot during the 1994 vintage. The same block, however, was harbouring a latent infection level of 93% that originated during flowering in 1993.

It is generally accepted that because *Botrytis* spores are highly mobile (Pak 1992), a vineyard that harbours minimal levels of *Botrytis* can be infected very quickly from sources outside the vineyard. Therefore, it would only be prudent to omit early sprays if *Botrytis* resistance to benzimidazole is low. Fungicides from this group can be used at flowering to reduce infections that have occurred before the sprays were applied (Fig. 21).

Fig. 21 - Effect of responding to monitored disease risk by spray application on incidence of *Botrytis* during the season - Chardonnay at Mudgee, New South Wales, 1994/95


% Botrytis Incidence
100
90
80
70
60
50
40
30
20
10
0
Carry over
Spin
Capfall
Rovral
Bunch Closure
22/08
06/11
29/11
04/01
Date

Latent infection of flowers or berries

Botrytis incidence in a block of Chardonnay that received no botryticide spray showed a high carry over level of 90% in August. Flower samples taken at the end of cap fall indicated that 90% of these flowers were subsequently infected with *Botrytis*. This latent infection level was confirmed at the bunch closure stage.

Botrytis levels measured in a sprayed block of Chardonnay are shown in Fig. 21. The decreasing trend in the disease incidence typified an effective chemical control program aimed at reducing *Botrytis* latent infection to a significantly low level.

Monitoring carried out after cap-fall spray would indicate the success of the flowering spray program and whether latent infection established in the flowers has been managed properly. Samples taken after the bunch closure spray can detect subsequent infections and a relatively small proportion of latent infection that has not been successfully controlled beforehand.

Timing of application of fungicides

The discovery of grape flower infection by *Botrytis* in Australia by Nair (1985) saw the beginning of a rational use of fungicides to manage *Botrytis* rot. Further research (Nair 1990) leading to the establishment of the *Botrytis* monitoring and fungicide resistance testing service (Nair et.al., 1994) has resulted in more precise timing of the application of botryticides with maximum effect on the pathogen.

The development of a disease risk model described in section 5b of this report was intended to be a general guide for the growers in deciding the timing of

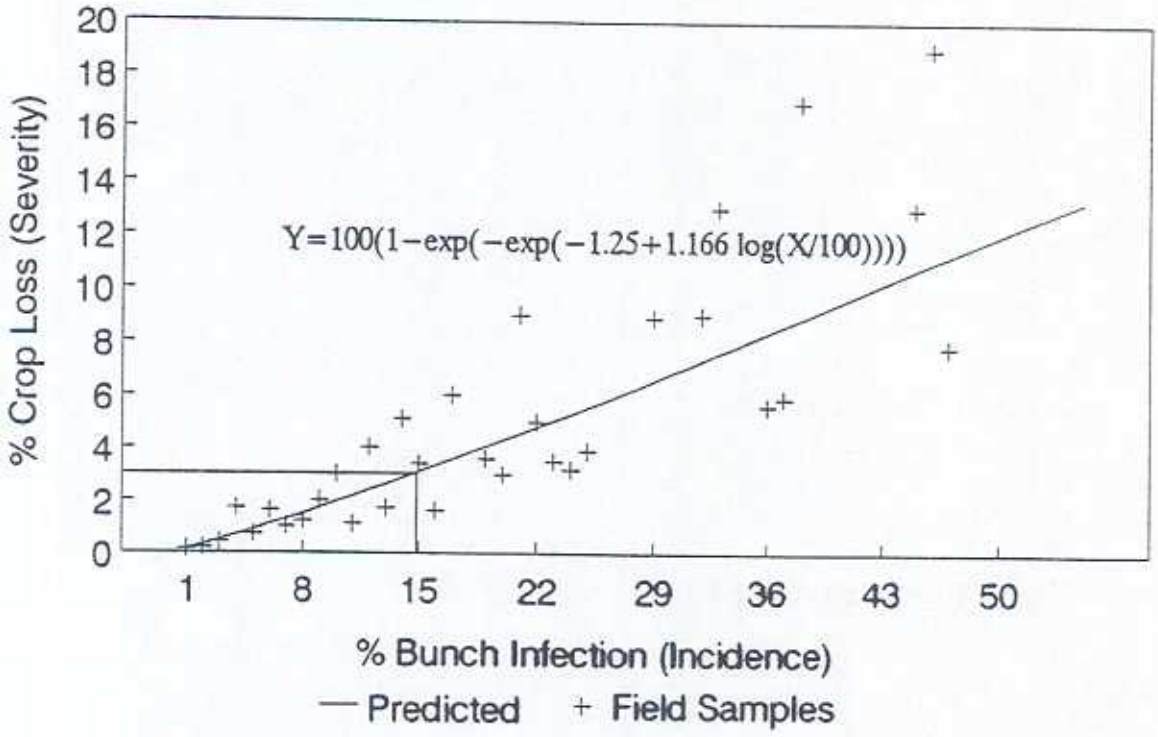
application of fungicides. The model shown in Fig. 14 can also be used to ascertain what level of flower infection can be tolerated. This figure describes the relationship between incidence of flower infection and incidence of berry infection. If, for example, the incidence of flower infection was 50%, the most likely incidence of berry infection would be 26%. As mentioned in the methodology in section 5b of this report, the model was developed from data covering a 10 year period. It should be noted that during this period of study, a range of rainfall conditions was experienced. Further, since the study was undertaken in commercial vineyards, harvest time and the subsequent degree of *Botrytis* were determined to some extent by the amount of *Botrytis* evident. The relationship can thus be considered as a prediction of a likely outcome in an average season.

Most growers, however, measure *Botrytis* rot outcomes as a percentage of the total crop infected. The relationship between the severity (actual crop loss) and the incidence of bunch infection is shown in Fig. 22. This relationship has been calculated using measurements made approaching vintage in the Hunter Valley, Mudgee and Cowra districts of New South Wales during 1994 and 1995. It can be seen from Fig. 22 that the actual values follow closely the values predicted by the model.

Exceptions to successful *Botrytis* management based on latent infection - spray timing

The susceptibility of grapes to infection varies with the stage of growth of the vines. Flowers are considered most susceptible (McClellan and Hewitt 1973; Nair 1990). Berries from fruit-set to veraison have a degree of resistance to infection (Nair and Hill 1992). However, if weather conditions are favourable, infection of berries could occur. This phenomenon has been observed in two of the last ten years in New South Wales; once in 1984 in the Hunter Valley and again in Mudgee, Cowra and the Hunter Valley in the spring of 1993. Therefore,

Fig. 22 - Relationship between severity (actual crop loss) and incidence of *Botrytis* rot disease


20
18
16
14
12
10
8
6
4
2
0
% Crop Loss (Severity)
Y=100(1−exp(−exp(−1.25+1.166 log(X/100))))
1
8
15
22
29
36
43
50
% Bunch Infection (Incidence)
— Predicted + Field Samples

even if flowering has been managed sufficiently, infection could occur later in the season.

All chemicals registered currently for the management of *Botrytis*, apart from those of the benzimidazole group, can be regarded in a practical sense only as protectants, although pyrimethanil is reported to be translaminar in its property. Therefore, *Botrytis* sprays should ideally be applied before an infection event occurs. This can be done on the basis of monitoring *Botrytis* incidence. Data presented in Table 8 show how chlorothalonil and carbendazim sprayed before a likely infection event have been effective in protecting berries from infection. The average level of infection in blocks receiving this treatment was 25% compared with those of unsprayed blocks with 88%. The block in which chlorothalonil was applied after the likely infection period showed an infection level of 83%, differing little from that in unsprayed blocks.

In cool, wet viticultural regions such as north east Victoria, phenological periods are more protracted than those in New South Wales. It follows that the period of grape susceptibility to *Botrytis* infection is relatively longer. Under these circumstances, assessment of *Botrytis* incidence may show that a greater number of protectant sprays are required.

Calculating an acceptable flower infection level

Using the two relationships shown in Figs. 13 and 14, it is possible to calculate flower infection level that can be tolerated. A 12% level of flower infection can be predicted from a carry over level of 15% (Fig. 13). Consequently, if incidence of *Botrytis* rot is not to exceed 15%, the incidence of flower infection should be kept at or below 15%.

Table 8 - Incidence of *Botrytis* In relation to infection period and timing of application of fungicides

Date	Rain (mm)	Unsprayed	Spray application		
			Chlorothalonil before infection period	Chlorothalonil after infection period	Carbendazim before infection period
18 -11 - 94	7	37	37		50
19 -11 - 94					
20 -11 - 94	14				
21 -11 - 94					Carbendazim
22 -11 - 94					
23 -11 - 94			Chlorothalonil		
24 -11 - 94					
25 -11 - 94	41				
26 -11 - 94					
27 -11 - 94					
28 -11 - 94	1				
29 -11 - 94	7 Infection	period →	→	→	→
30 -11 - 94	18				
01 -12 - 94					
02 -12 - 94					
03 -12 - 94					
04 -12 - 94					
05 -12 - 94					
06 -12 - 94		88	28	83	23

Factors affecting actual *Botrytis* rot outcomes

Several factors other than inoculum level affect the actual amount of *Botrytis* rot at harvest; the most significant of these are :

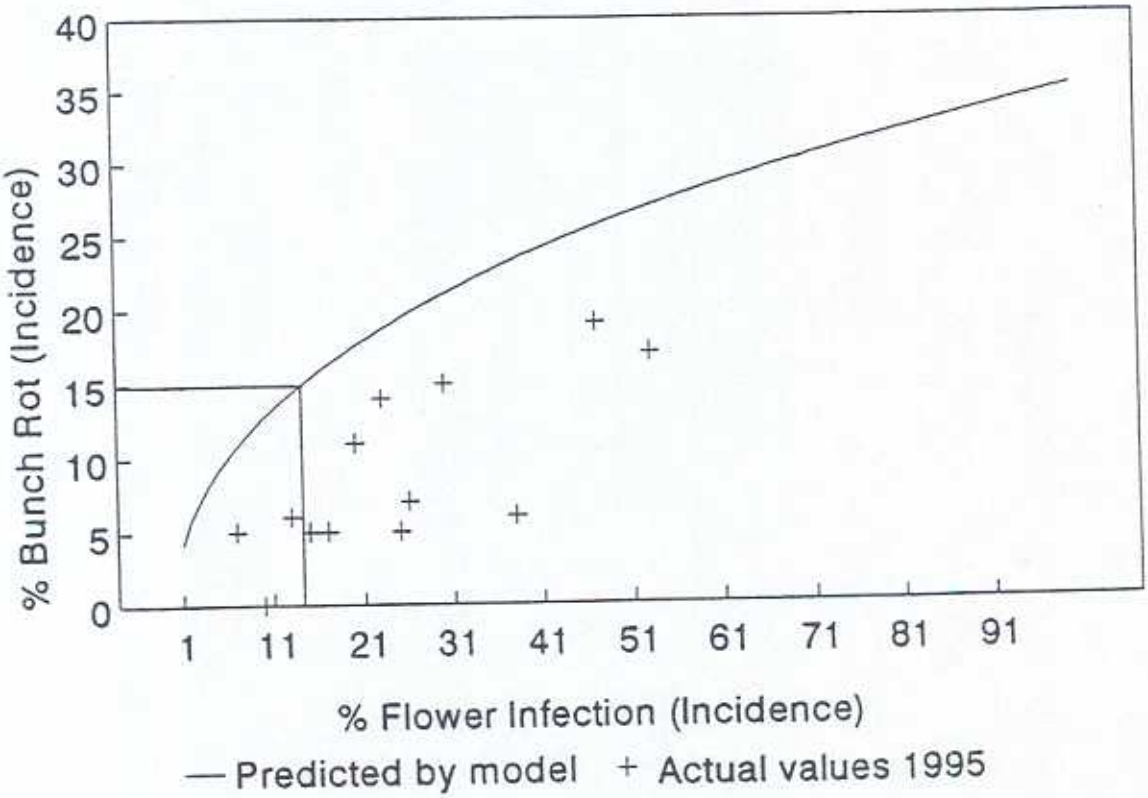
- period of berry wetness; physical damage to berries; and, grape vine variety (Nair *et.al.*1988).

These factors may occur individually or in combination to predispose a block of grapevines to *Botrytis* rot. Thus, predisposition of a vineyard should be taken into account when using the models in Figs. 13,14,15 to predict incidence of *Botrytis* rot. A block of Semillon with a dense canopy, split berries and harbouring a high level of light brown apple moth could be described as having a high predisposition to *Botrytis* rot. In such an instance, a greater incidence of *Botrytis* rot than that predicted by the model may be expected. Conversely, Cabernet Sauvignon with moderate vigour trained to Scott Henry can be described as having a low predisposition to *Botrytis* and, therefore, a lower level of *Botrytis* rot at harvest.

If the season is unusually dry, as it was in New South Wales in 1994/95, the amount of *Botrytis* rot at harvest would be less than expected. The 1995 harvest outcomes were lower than what the flower infection had predicted, although the trend was similar to the predicted values(Fig. 23). It follows therefore that canopy management and other strategies that alter the microclimate of the bunches of grapes will affect the level of *Botrytis* rot.

Tight bunches that have split may result in a build-up of *Botrytis* at harvest to higher levels than those predicted by the models. For instance, two varieties (Chardonnay and Riesling) in one block of vines trained to Scott Henry were evaluated for levels of *Botrytis* in February 1995. The Riesling block was divided into four areas based on clone and vine vigour. One end of the block displayed greater vigour and tighter bunches than the other. Clone 1 had tighter

Fig. 23 - *Botrytis* infection of flowers (at flowering) and mature berries (at harvest) in 1995 of grapevine showing predicted and actual values


40
35
30
25
20
15
10
5
0
% Bunch Rot (Incidence)
1
11
21
31
41
51
61
71
81
91
% Flower Infection (Incidence)
— Predicted by model + Actual values 1995

bunches than clone 2. Splitting was evident in the tighter bunches. No splitting was evident in the Chardonnay block.

The Riesling with a 3% monitored incidence of flower infection would be expected to have a 7% incidence of *Botrytis* rot at harvest according to the model. This is equivalent to 1.3% crop loss. At the low vigour end of the block, crop loss was 0.5% in clone 2 and 1.3% in clone 2. Both outcomes were in the range predicted by the model. The part of the block with vines showing high vigour and tight bunches that have split, had higher levels of *Botrytis* (7.8%) than the remaining part with vines of low vigour (3.5%).

The results obtained in this work have shown that varieties of grapevine that can be considered as inherently predisposed to *Botrytis* rot such as Riesling, Semillon, Chardonnay, Shiraz and Pinot Noir are likely to respond relatively more to the prediction model developed in this project. Varieties that are less susceptible such as Cabernet Sauvignon are unlikely to be found with much *Botrytis* rot at harvest, despite having high incidence of flower infection.

6. Recommendations

- Establish *Botrytis* monitoring and fungicide resistance testing service on a permanent basis so that the growers are given the opportunity to rationalise the use of fungicides to manage *Botrytis* rot.

- The *Botrytis* modules in AusVit and CD on pest and diseases should be an adjunct to the *Botrytis* monitoring and fungicide resistance testing service for managing the disease.

- The infection strategies used by *Botrytis* are not fully understood. For instance, the factors responsible for the explosive nature of the pathogen between veraison and harvest requires more investigation. One of the means of studying this phenomenon is by developing molecular markers capable of distinguishing individual strains of *Botrytis* on grapevines. As a result, population dynamics of virulent strains can be determined and this is likely to provide early warning of disease.

- Refine the disease risk model developed in the project by incorporating weather factors.

- One aspect of *Botrytis* rot that requires urgent investigation is the damage caused by the pathogen to wine production.

- There appears to be a need for testing more botryticides to manage the disease. This is necessary because *Botrytis* is capable of developing resistance to most of the fungicides that are currently available.

7. Technology transfer

Oral presentations :

Progress achieved during the course of the project was presented orally to different regional organisations of grape growers at workshops and seminars in New South Australia, South Australia and Victoria. Additionally, lectures on the epidemiology and management of *Botrytis* rot were given as part of the GWRDC project on IPM in Viticulture - Research to Practice Training Workshops in New South Wales and Victoria.

Lectures on management of *Botrytis* rot were also given to technical support staff of South Corp, Rhone-Poulenc Rural Australia Ltd., Vivco Iama and other horticultural groups.

Formal lectures were given on different aspects of *Botrytis* rot at the annual seminar of the Cooperative Research Centre for Viticulture, Adelaide, Charles Sturt Univerity, Wagga Wagga, an Australian Society of Viticulture and Oenology, Mildura.

Publications :

Research papers were published in refereed international and Australian scientific journals. A list of these papers are included under 'Literature Cited' (section 8) in this report.

AusVit :

The GWRDC project has contributed data required to build the *Botrytis* module in AusVit.

CD on grapevine diseases :

The section on *Botrytis* rot of grapes in the CD on grapevine diseases contains information from the GWRDC project.

8. Literature cited

Akagi, T.; Mitani, S.; Ito, K.; Shigehara, I.; Komyoji, T. and Matsuo, N. (1995). Structure-activity relationships of pyridylcarbamates active against both benzimidazole-sensitive and -resistant isolates of *Botrytis cinerea*. Pesticide Science 44:39-49.

Balasubramaniam, R. and Moore, T. J. (1994). Disease management system for grape. 1993/94, Horticultural Research Client Report No.93/113. Horticulture and Food Institute of New Zealand Ltd., Blenhiem. New Zealand.

Beever, R. E.; Laracy, E. P. and Pak, H. A. (1989). Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealnd vineyards. Plant Pathology 38:427-437.

Beever, R. E.; Pak, H A. and Laracy, E. P. (1991). An hypothesis to account for the behavior of dicarboximide-resistant strains of *Botrytis cinerea* in vineyards. Plant Pathology 40:342-346.

Broome, J. C.; English, J. T.; Marois, J. J.; Latorre, B. A. and Aviles, J. C. (1995). Development of an infection model for Botrytis bunch rot of grapes based on wetness duration and temperature. Phytopathology 85:97-102.

Bulit, J. and Lafon, r. (1970). Quelques aspects de la bilogie du *Botrytis cinerea* Pers. agent de la pourriture grise des raisins. Connaiss. Vigne Vi. 4:159-174.

Bulger, M. A.; Ellis, M. A. and Madden, L. V. (1987). Influence of temperature and wetness duration on infection of strawberry flowers by *Botrytis cinerea* and disease incidence of fruit originating from infected flowers. Phytopathology 77: 1225-1230.

Draper, N. R. and Smith, H. (1981). "Applied Regression Analysis", John Wiley & Sons, Canada.

Feller, W. (1968). "An Introduction to Probability Theory and its Application", #rd. edition, Vol.1, John Wiley & Sons, New York.

Guilbaud-Oulton, S and Nair, N.G. (1995). The meaning and purpose of *Botrytis* and fungicide resistance monitoring. The Australian Grapegrower and Winemaker , September 1995:61-67.

Gullino, M. L. and Garibaldi, A. (1982). Use of mixture or alternation of fungicides with the aim of reducing the risk of appearance of strains of *Botrytis cinerea* resistant to dicarboximides. European and Mediterranean Plant Protection Organisation Bulletin 12:151-156.

Gullino, M. L.; Aloi, C. and Garibaldi, A. (1989). Influence of spray schedules on fungicide resistant populations of *Botrytis cinerea* Pers. on grapevine. Netherlands Journal of Plant Pathology 1:87-94.

Hau, B. (1988). Modeling epidemics of polycyclic foliar diseases and development of simulators, In "Experimental Techniques in Plant Disease Epidemiology", eds. J. Kranz and J. Rotem, Springer Verlag, Berlin.

Luck, J. E.; Gillings, M. R. and Steel, C. C.(1995). Rapid detection of benzimidazole-resistant *Botrytis cinerea* in the vineyards. Australian Grapegrower and Winemaker 378a:122-125.

McClellan, W. D. and Hewitt, W. B. (1973). Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. Phytopathology 63:1151-1157.

Nair, N. G. (1985). Fungi associated with bunch rot of grapes in the Hunter Valley. Australian Journal of Agricultural Research 36:435-442.

Nair, N. G. and Parker, F. E. (1985). Midseason bunch rot of grapes: an unusual phenomenon in the Hunter Valley, Australia. Plant Pathology 34:302-305.

Nair, N. G. and Nadtotchei, A. (1987). Sclerotia of *Botrytis* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. Journal of Phytopathology 119:42-51.

Nair, N. G.; Emmett, R. W. and Parker, F. E. (1987). Programming applications of dicarboximides to control bunch rot of grapes of *Botrytis cinerea*. Plant Pathology 36:175-179.

Nair, N. G.; Emmett, R. W. and Parker, F. E. (1988). Factors predisposing grape berries to infection by *Botrytis cinerea*. New Zealand Journal of Experimental Agriculture 16:257-263.

Nair, N. G. (1990). Strategies for fungicidal control of bunch rot of grapes caused by *Botrytis cinerea* in the Hunter Valley. Wine Industry Journal (May 1990), pp.218-220.

Nair, N. G. and Hill, G. (1992). Bunch rot of grapes caused by *Botrytis cinerea*. In "Plant Diseases of International Importance", eds. J. Kumar, H. S. Chaube, Singh U. S. and A. K. Mukhopadhyay, Prentice Hall, New Jersey, USA pp.147-169.

Nair, N. G.; Green, C. D. and Castillo-Pando, M. (1994). *Botrytis* and resistance monitoring as a fungicide management strategy for the control of bunch rot of grapevines - a service to the industry. The Australian Grapegrower and Winemaker 366a:67-71.

Nair, N. G. and Allen, R. N. (1993). Infection of grape flowers and berries by *Botryis cinerea* as a function of time and temperature. Mycological Research 97:1012-1014.

Nair, N. G.; Guilbaud-Oulton, S.; Barchia, I. and Emmett, R. (1995). Significance of carry over inoculum, flower infection and latency on the incidence of *Botrytis cinerea* in berries of grapevines at harvest in New South Wales. (1995). Australian Journal of Experimental Agriculture 35:1177-1180.

Pak, H. A.; Beever, R. E. and Laracy, E. P. (1990). Population dynamics of dicarboximide resistance strains of *Botrytis cinerea* on grapevine in New Zealand. Plant Pathology 39:301-309.

Pak, H. A. (1992). Das Verhalten fungizidresistenter Stamme des Pilzes *Botrytis cinerea* auf der Weinrebe. Ph. D. Thesis, University of Giesen, Germany.

Pearson, R. C. (1982). Chemical control of Botrytis cinerea on grapes in New York (USA). European and Mediterranean Plant Protection Organisation Bulletin 12:101-104.

Pearson, R. C. and Rigel, D. G. (1983). Control of *Botrytis* bunch rot of ripening grapes: timing of application of dicarboximide fungicides. American Journal of Enology and Viticulture 34:167-172.

Sall, M. A. (1980). Uses of stochastic simulation: Grape powdery mildew example. Zietschrift Pflanzenkrankh. Pflanzenschutz. 87:397-403.

Smyth, G. K. (1992). LOGIST. A Matlab function in Netlib (netlib@research.att.com), a network accessible database. AT&T Bell Laboratory, Murray Hill, NJ.

Stoneham, G. (1995). Strategic management of grape bunch rots - Economic evaluation. Department of Agriculture, Victoria.

Strizyk, S. (1983). Modele d'etat potentiel d'infection application au *Botrytis cinerea*. Version 1983. Association de coordination technique agricole. France.

Vincelli, P. C. and Lorbeer, J. W. (1989). BLIGHT-ALERT A weather-based predicative system for timing fungicide applications on onion before infection periods of *Botrytis squamosa*. Phytopathology 79:493-498.

Zeng, P. G. and Sutton, J. C. (1994). Effects of wetness duration, temperature and light on infection of black spruce seedlings by *Botrytis cinerea*. Canadian Journal of Forest Research 24:707-713.