Control of Bitter Rot or Ripe Rot of grapes caused by *Colletotrichum* spp

FINAL REPORT to
Grape and Wine Research and Development Corporation

Project Number**:** **RT 01/14**

Principal Investigator**: Mr Anthony Somers**

Research Organisation: **Hunter Valley Vineyard Association Inc.**

Date: **11 December 2003**

FINAL REPORT *(for 2001/2002)*

1. Cooperating grower trials conducted

This work has been driven by the Hunter Valley Industry trying to find a practical solution to the control of fruit rots in a region of high disease pressure during the ripening period. Changes in canopy management and better chemical control of *Botrytis cinerea* has significantly reduced the incidence of *Botrytis*. However bunch rots caused by *Colletotrichum acutatum, Colletotrichum gleosporoides* and *Greenaria uvicola* have proved more difficult to control.

There are no fungicides registered in Australia for the control of *Colletotrichum spp* in grapes and no efficacy data on currently registered chemicals. The aim of the field trials was to test the efficacy of a range of chemical control options using existing registered chemicals. It also aimed to assess the impact of these treatments on beneficial mite populations. The On Farm Trial model was used to design and conduct the trials.

Treatments with Mancozeb, Scala, Amistar and copper were assessed and protocols for scoring fruit rot infections at harvest were implemented. Mite populations were also sampled and data on the composition of beneficial mites were assessed for the fungicides being tested.

There is now good evidence that more open canopies provide a more favourable environment for Colletotrichum infection. Other trials have shown that the best control of *Colletotrichum* infection was under vines covered with plastic sheet. The explanation for this seems to be that the fungus is spread in water droplets and free moisture, with more sheltered canopies providing some shingling effect in shedding light rain and therefore reducing the risk of fruit infection. Heavy rain, it is speculated will dilute the spore concentration and therefore reduce the level of infection. There is also the question of sunburn or other environmental factors playing a role in disease incidence. Further trial work in the project by Dr C. Steel will look more closely at these aspects of canopy management. There is good evidence from the literature on the management of this disease in other crops that reducing the inoculum by removing as much of the over-wintering inoculum as possible can reduce infection.

Background

Fruit rot control was investigated at four separate sites using different treatments at each site. Each site had 6 replicates of 2 treatments in a randomised block design. The treatments were applied to 6 whole rows of vines. The middle 2 rows of vines were sampled and 100 locations were randomly chosen. A bunch from each side of the vine (East-West or North-South) was chosen to make 100 bunches per treatment unit.

Measurements

Bunches were rated according to the degree of fruit rot on a 0 - 4 scale. This scale related to the percentage of the bunch affected by the fruit rot. The type of disease was also recorded.

Category	Range % area diseased	Mean score (% area diseased
0	0	0
1	>0-3	1.5
2	>3-25	14
3	>25-50	37.5
4	>50	75

Plant Pathology sampling

Samples were collected from all four sites at three stages and sent to the NSW Agriculture plant Diagnostic Laboratory at the Elizabeth Macarthur Research Institute for diagnosis. Samples were collected according to the protocol outlined in Table 11.

Statistical Methods
Any Disease
The number of bunches in each of the 5 disease categories was summarised for each replicate, treatment and aspect combination. This type of data is referred to as “multinomial” data and was analysed using a generalised linear model with multinomial error distribution and logit link function. Treatments were tested by comparing their deviance values against Chi-square with 1 df. Comparison was made on the logit scale, but average disease scores are presented.

RESULTS

Site Chardonnay
This site contained low vigour Chardonnay vines that were grafted on Ramsey rootstock and had a high incidence of *Colletotrichum* in the previous season. The rows were running in a North South direction. Chemical treatment consisted of a control (treatment 1), the normal spray program and a modification of this program with Mancozeb replacing Copper as the protectant fungicide after flowering (treatment 2).

The overwhelming disease recorded during the harvest assessment was Greeneria uvicola (Figure 1)

Figure 1 *Greeneria uvicola* on Chardonnay grapes

Statistical Analysis
A significant difference in the frequency distribution of the scores between the 2 treatments was found. There was also a significant difference between East and West. The interaction between aspect and treatment was not significant (Table 1).
Treatment 1 had a lower proportion of bunches with low disease scores than treatment 2. Bunches on the eastern side of the vine had more bunches in the lower disease scores that bunches facing west (Table 2). Average disease scores represent this trend.

Table 1: Accumulated analysis of deviance –East vs West

	d.f.	deviance	mean deviance	deviance ratio
+ treat	1	15.7	15.7	15.7
+ Aspect	1	12.9	12.9	12.9
+ treat.Aspect	1	0.5	0.5	0.5
Residual	89	159.0	1.8	
Total	92	188.1	2.0	

Table 2: Treatment and Aspect average disease score

Aspect	Mean East	West
treat		
1	0.7	0.8
2	0.8	1.1

Results of chemical program on mite populations

Leaf samples were collected from each treatment during the growing season and the numbers of rust mites, predatory mites, two spotted mites, thrips and aphid/mealy bug were recorded. The aim was to assess the impact of the changed practice on populations of both pest and beneficial species. It is difficult to draw accurate conclusions without looking in more detail at all the management and chemical inputs. (Figure 2 and 3)

Figure 2

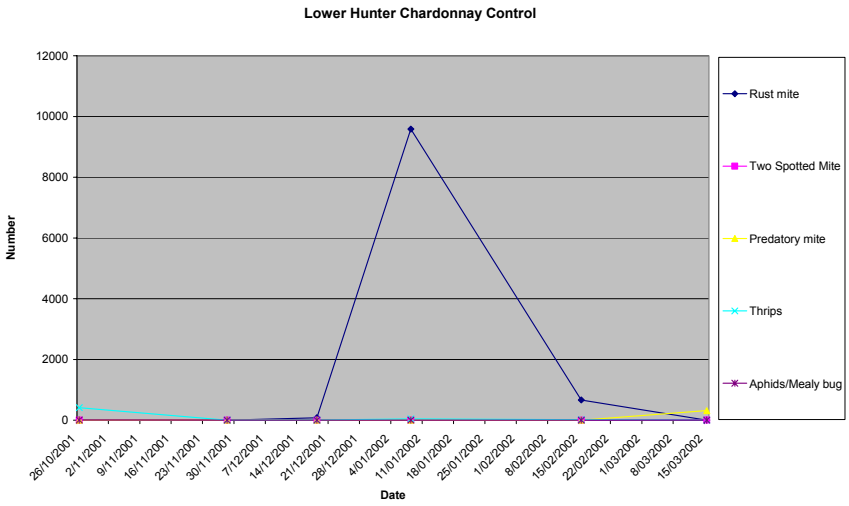
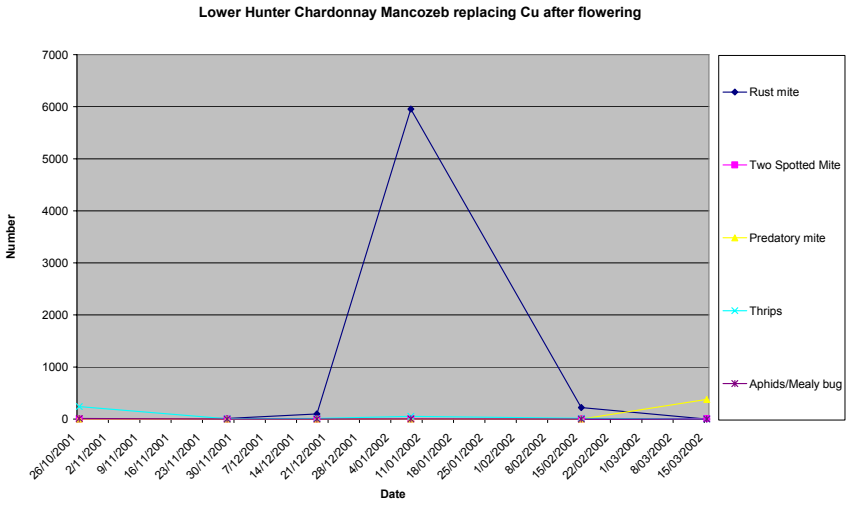
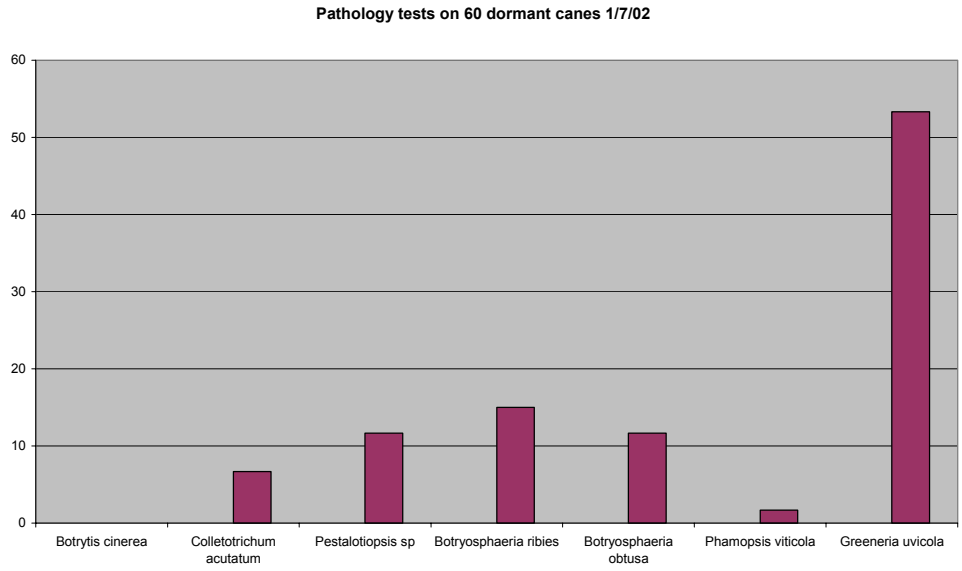


Figure 3



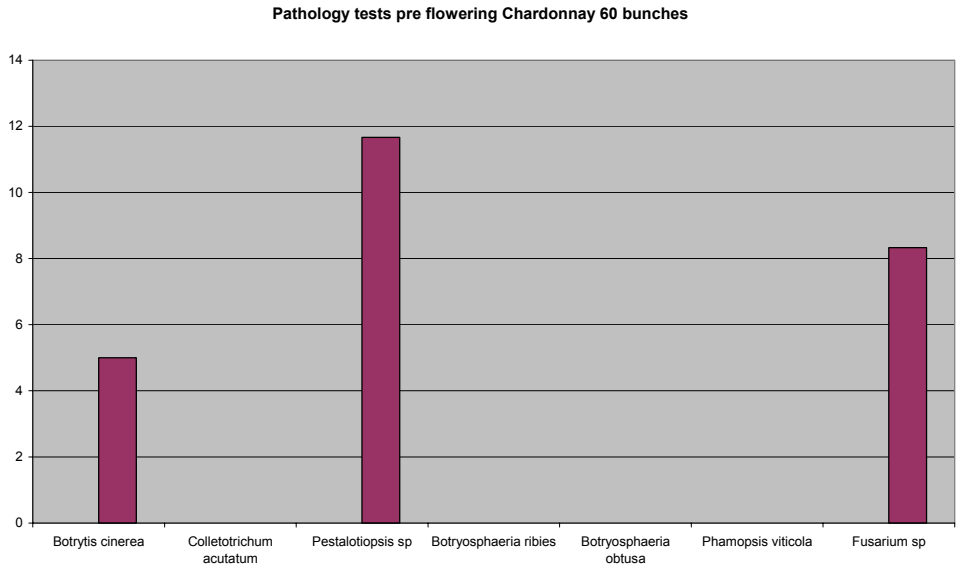
Results of Pathology test samples
Samples of 60 canes or spurs of dormant vines were collected in 15-30 cm sections of dormant shoots with bunch stalk attached send in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen.
The results showed a high carry over of Greeneria uvicola with a lower level of C acutatum as well as other fungi. (Figure 4)

Figure 4



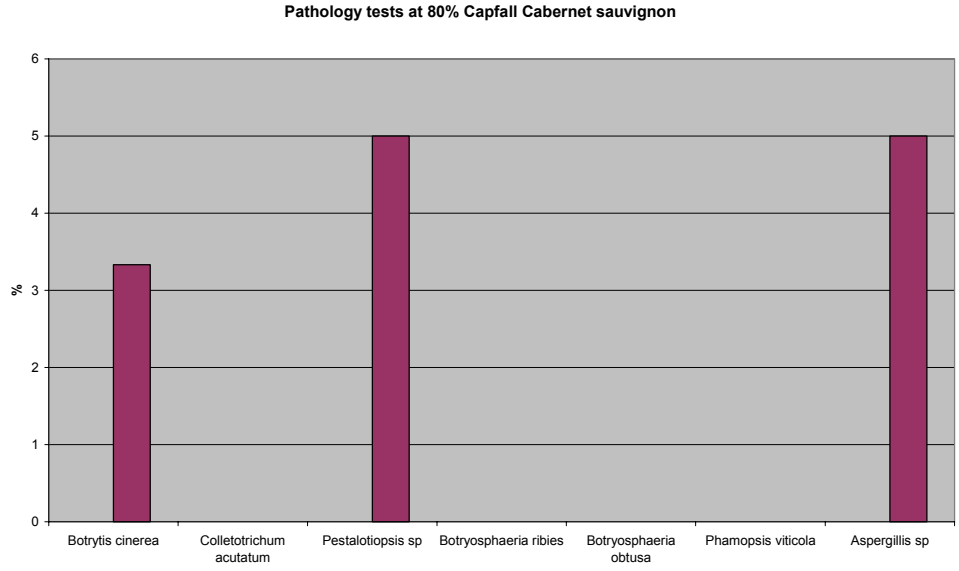
Samples of 60 pre flowering green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. The results are shown in Figure 5.

Figure 5



Samples of 60 80% capfall green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. The results are shown in Figure 6.

Figure 6



Site 2 Shiraz

This site contained moderately vigorous Shiraz vines that had been retrained to a higher canopy configuration and had a history of fruit rot infection.. The control (treatment 1) was the standard spray program and treatment 2 was the replacement of Mancozeb with Acrobat MZ up till 80% capfall. The rows were running in an East-West direction. The main disease recorded on this site was *Botrytis cinerea.*

Statistical Analysis

There was no significant difference in the frequency distribution of the scores between the 2 treatments or between North and South. The interaction between aspect and treatment was also not significant (Table 4).

Table 3: Accumulated analysis of deviance –North vs South

	d.f.	deviance	mean deviance	deviance ratio
+ treat	1	3.4	3.4	3.4
+ Aspect	1	0.1	0.1	0.1
+ treat.Aspect	1	1.0	1.0	1.0
Residual	89	221.7	2.5	
Total	92	226.2	2.5	

Table 4: Treatment and Aspect average disease score

Aspect treat	North	South
1	0.9	0.9
2	1.0	1.0

Results of chemical program on mite populations

Leaf samples were collected from each treatment during the growing season and the numbers of rust mites, predatory mites, two spotted mites, thrips and aphid/mealy bug were recorded. The aim was to assess the impact of the changed practice on populations of both pest and beneficial species. It is difficult to draw accurate conclusions without looking in more detail at all the management and chemical inputs. (Figure 7 and 8)

Figure 7

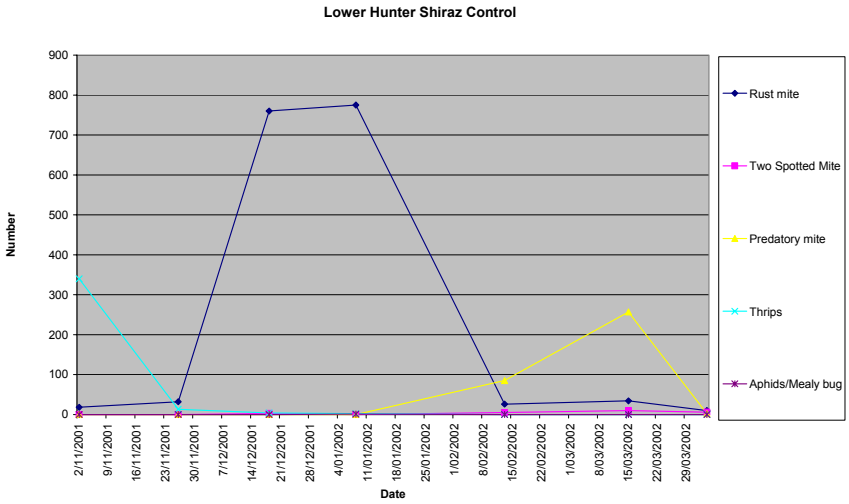
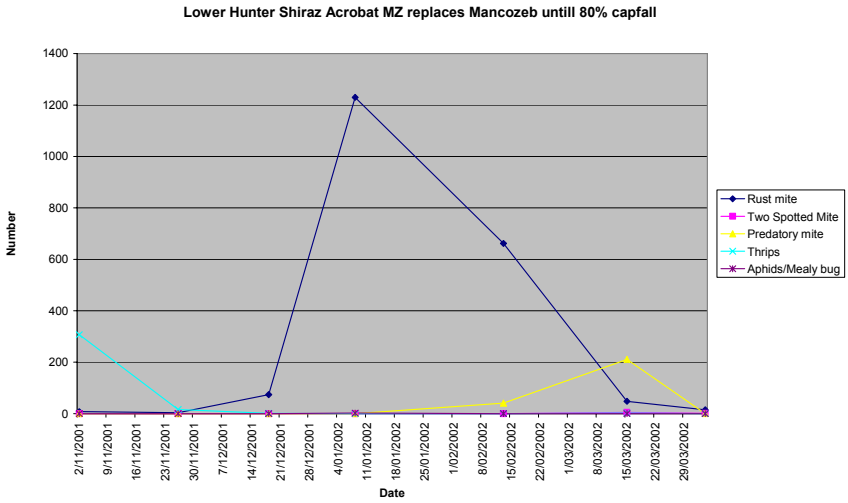


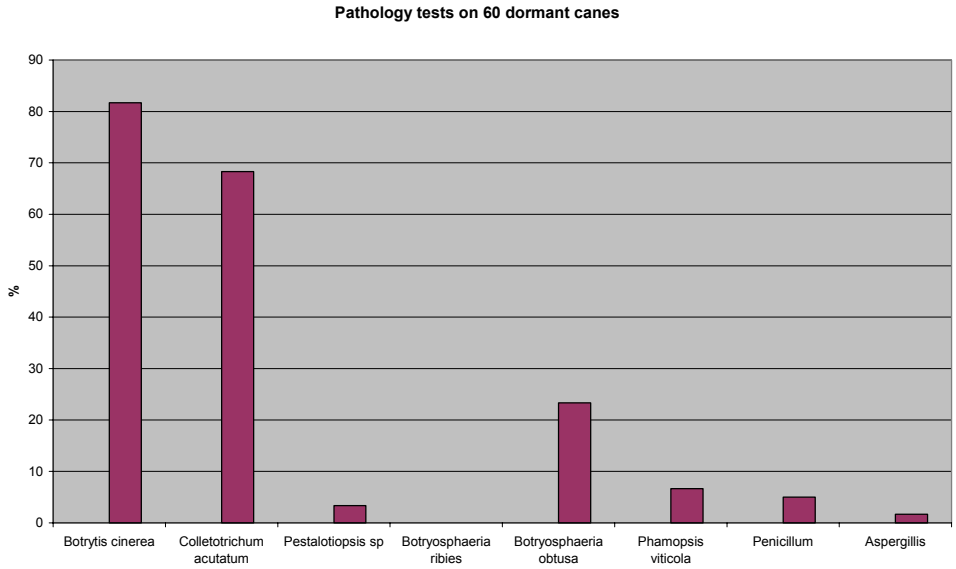
Figure 8



Results of Pathology test samples

Samples of 60 canes or spurs of dormant vines were collected in 15-30 cm sections of dormant shoots with bunch stalk attached send in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen.
The results showed a high carry over of Botrytis and C. acutatum as well as other fungi. (Figure 9)

Figure 9



Site 3 Chardonnay

This site contained moderately vigorous Chardonnay vines on their own roots with the canopy modified by the removal of a double cordon. The control (treatment 1) was again the standard chemical control program treatment 2 was the replacement of Mancozeb with copper after flowering. The rows were running in an East-West direction. Disease incidence was low at this site with the predominant rots being *Greeneria* and *Botrytis.* We combined disease categories 3 and 4 because of the low numbers of bunch in the most severe category.

Statistical Analysis

There was no significant difference in the frequency distribution of the scores between the 2 treatments or between North and South. The interaction between aspect and treatment was also not significant (Table 5).

Table 5: Accumulated analysis of deviance –North vs South

	d.f.	deviance	deviance	ratio
+ treat	1	1.0	1.0	1.0
+ Aspect	1	0.1	0.1	0.1
+ treat.Aspect	1	0.1	0.1	0.1
Residual	66	87.9	1.3	
Total	69	89.2	1.3	

Table 6: Treatment and Aspect average disease score

	Mean	
Aspect	North	South
treat		
1	0.6	0.6
2	0.7	0.6

Results of chemical program on mite populations

Leaf samples were collected from each treatment during the growing season and the numbers of rust mites, predatory mites, two spotted mites, thrips and aphid/mealy bug were recorded. The aim was to assess the impact of the changed practice on populations of both pest and beneficial species. It is difficult to draw accurate conclusions without looking in more detail at all the management and chemical inputs. (Figure 10 and 11)

Figure 10

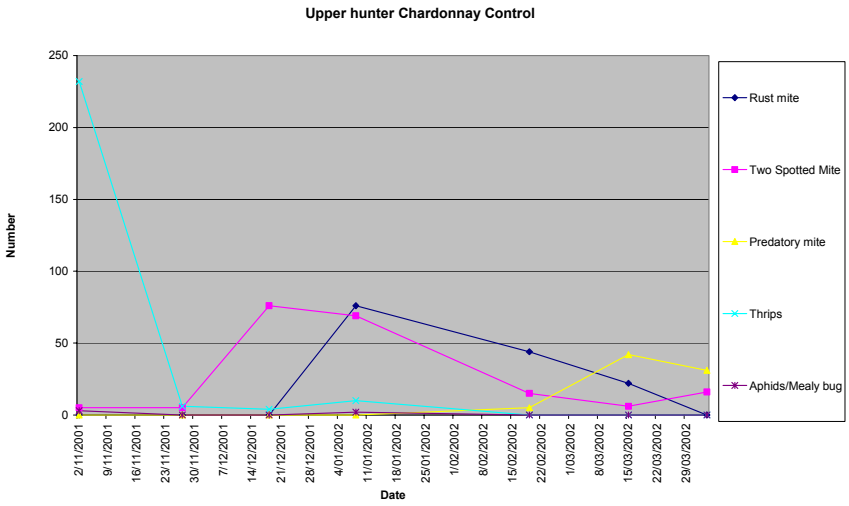
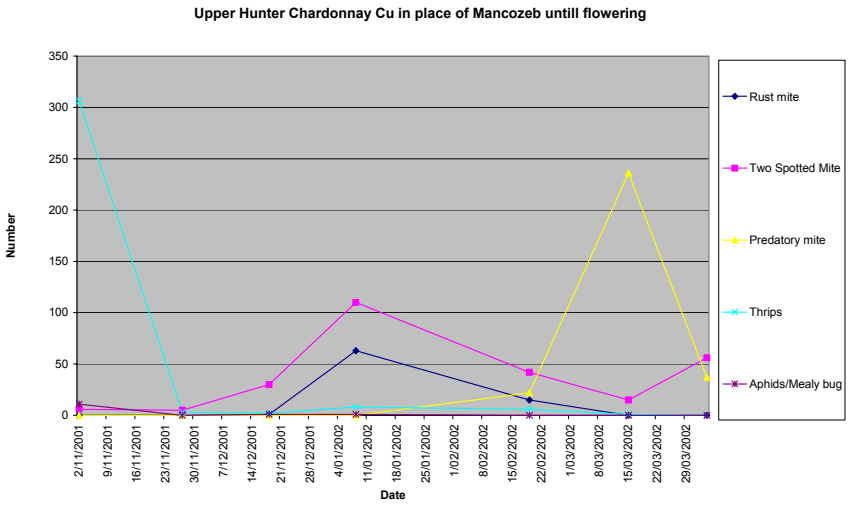


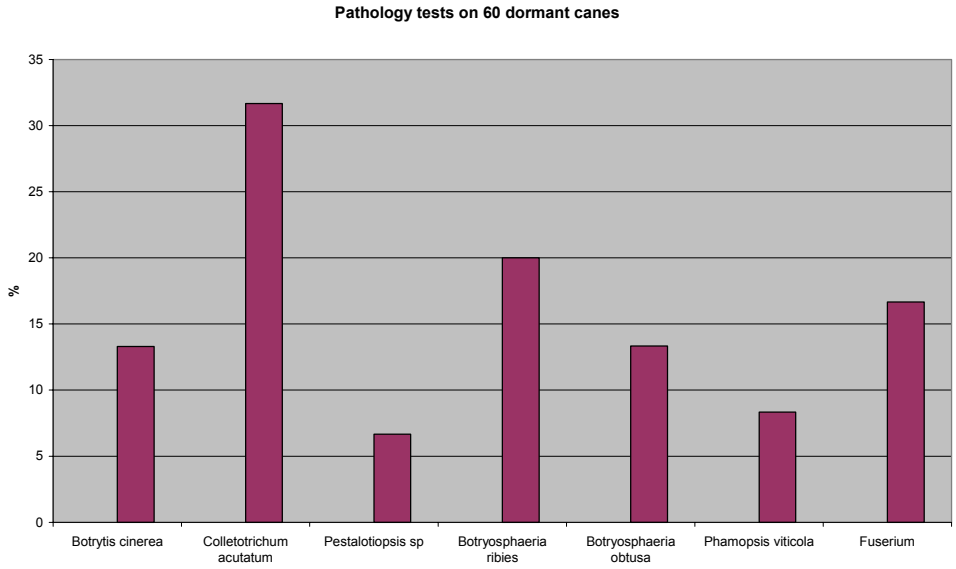
Figure 11



Results of Pathology test samples

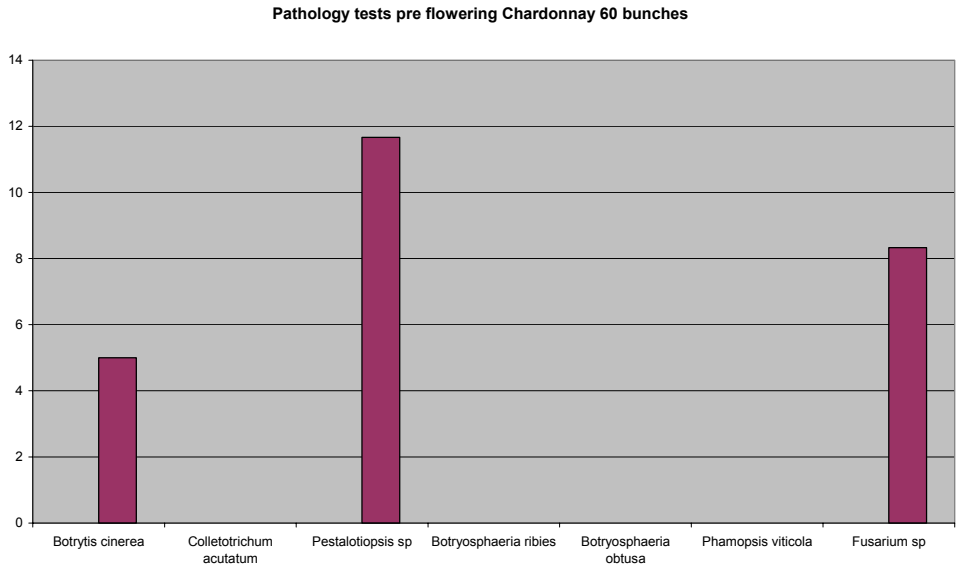
Samples of 60 canes or spurs of dormant vines were collected in 15-30 cm sections of dormant shoots with bunch stalk attached send in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen.
The results showed a high carry over of Botrytis and C. acutatum as well as other fungi. (Figure 12)

Figure 12



Samples of 60 pre flowering green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. The results are shown in Figure13.

Figure 13



Samples of 60 80% capfall green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. No pathogens were detected in this series of samples.

Site 4 Cabernet sauvignon

This site contained moderately vigorous vines trained on Scott Henry trellis. The trial was split into two sections with 2 replicates in one section and 4 in another. The row orientations between the two sections differed. The control (treatment 1) was the standard spray program treatment 2 included an extra spray of Amistar at 5% flowering.
High incidence of *Colletotrichum actutatm* was recorded in this trial (Figure 14). Since all the 4 aspects were not represented in the same replicate – the data was divided into 2 groups and analysed separately. North vs South was tested using data from the first 2 reps and East vs West was tested in the other 4 replicates

Figure 14 *Colletotrichum actutatm* infection on Cabernet sauvignon grapes at harvest

Statistical Analysis

North vs South

There was a significant difference in the frequency distribution of the scores between the 2 treatments. Treatment 1 tended to have significantly more bunches in the higher score categories than treatment 2. Overall there was no difference between North and South, however there was a significant interaction between treatment and aspect (Table 1). This means that the response due to aspect was not consistent between treatments. For north facing bunches there was only a small difference between treatments but for there was a larger treatment difference within south facing bunches (Table 7).

Table 7: Accumulated analysis of deviance –North vs South

	d.f.	deviance	mean deviance	deviance ratio
+ treat	1	6.7	6.7	6.7
+ Aspect	1	0.1	0.1	0.1
+ treat.Aspect	1	4.8	4.8	4.8
Residual	25	54.2	2.2	
Total	28	65.8	2.3	

Table 8: Treatment and Aspect average disease score

Aspect	North	South
treat		
1	2.0	2.3
2	2.0	1.8

East vs West

For this sub-group of data the only significant effect was between the 2 aspects with East facing bunches having a higher proportion of bunches in the lower score categories than west facing bunches.

Table 9: Accumulated analysis of deviance –East vs West

	d.f.	deviance	mean deviance	deviance ratio
+ treat	1	1.5	1.5	1.5
+ Aspect	1	48.2	48.2	48.2
+ treat.Aspect	1	0.1	0.1	0.1
Residual	57	103.8	1.8	
Total	60	153.6	2.6	

Table 10: Treatment and Aspect average disease score

Aspect	East	West
treat		
1	0.8	1.4
2	0.8	1.3

Results of chemical program on mite populations

Leaf samples were collected from each treatment during the growing season and the numbers of rust mites, predatory mites, two spotted mites, thrips and aphid/mealy bug were recorded. The aim was to assess the impact of the changed practice on populations of both pest and beneficial species. It is difficult to draw accurate conclusions without looking in more detail at all the management and chemical inputs. (Figure 15 and 16)

Figure 15

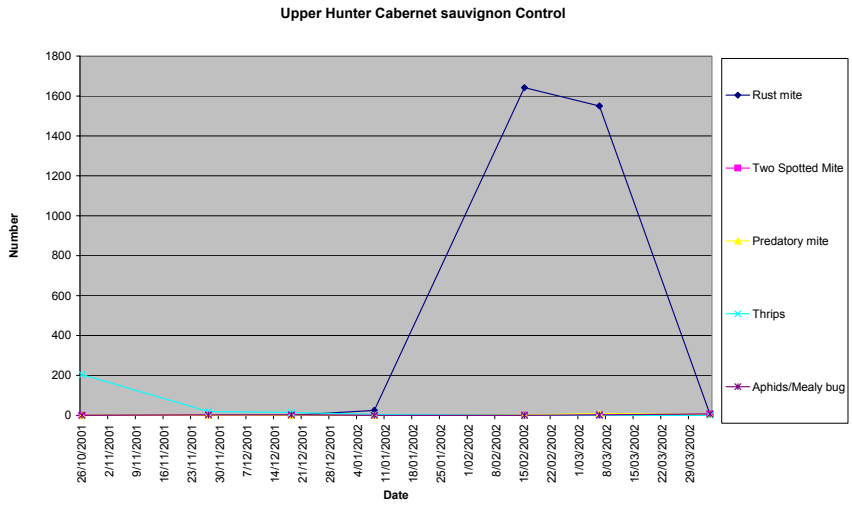
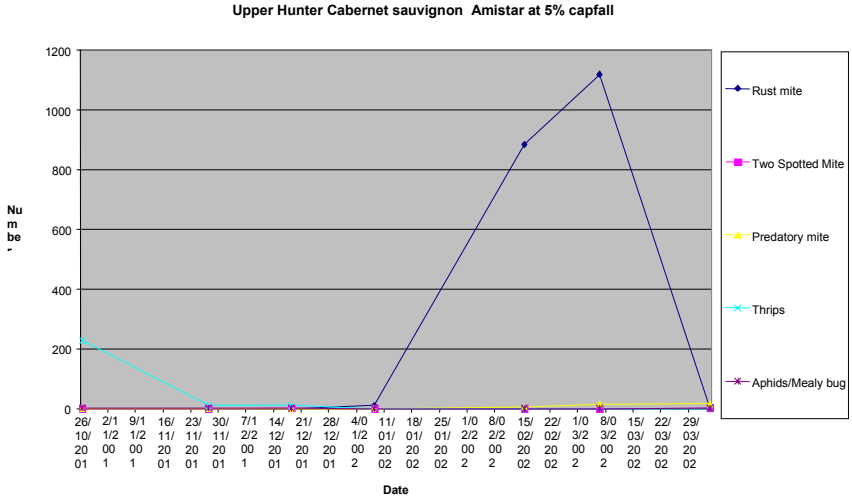


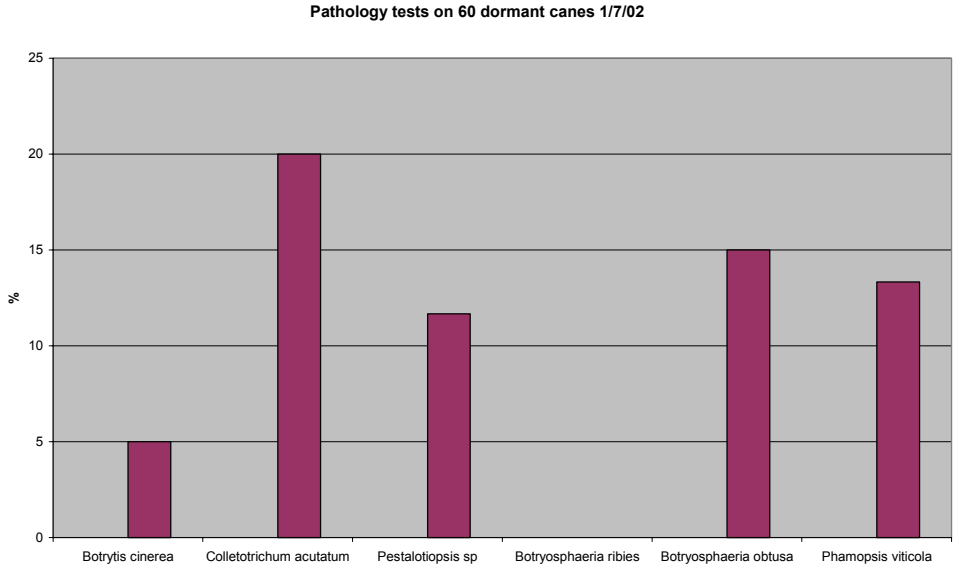
Figure 16



Results of Pathology test samples

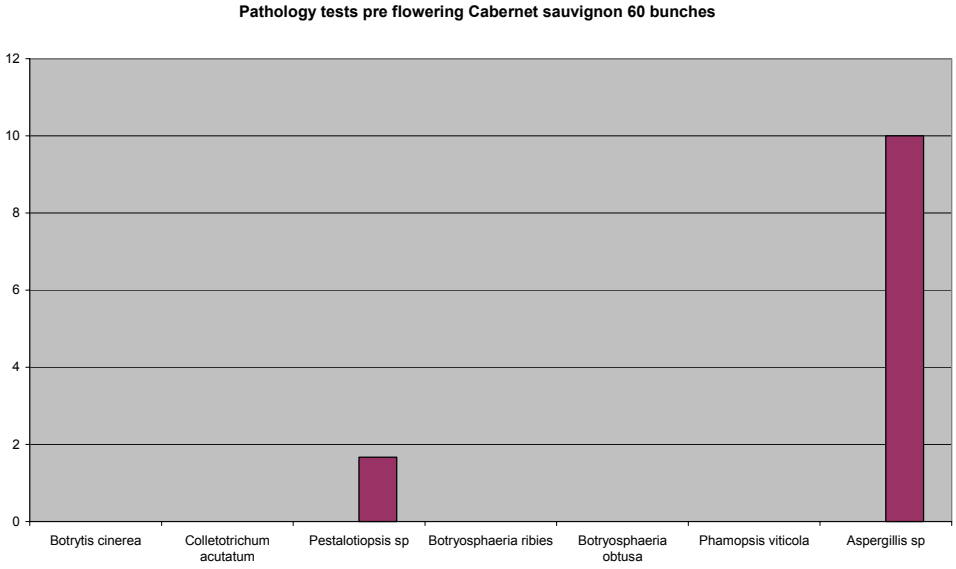
Samples of 60 canes or spurs of dormant vines were collected in 15-30 cm sections of dormant shoots with bunch stalk attached send in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen.
The results showed a high carry over of Botrytis and C. acutatum as well as other fungi. (Figure 17)

Figure 17



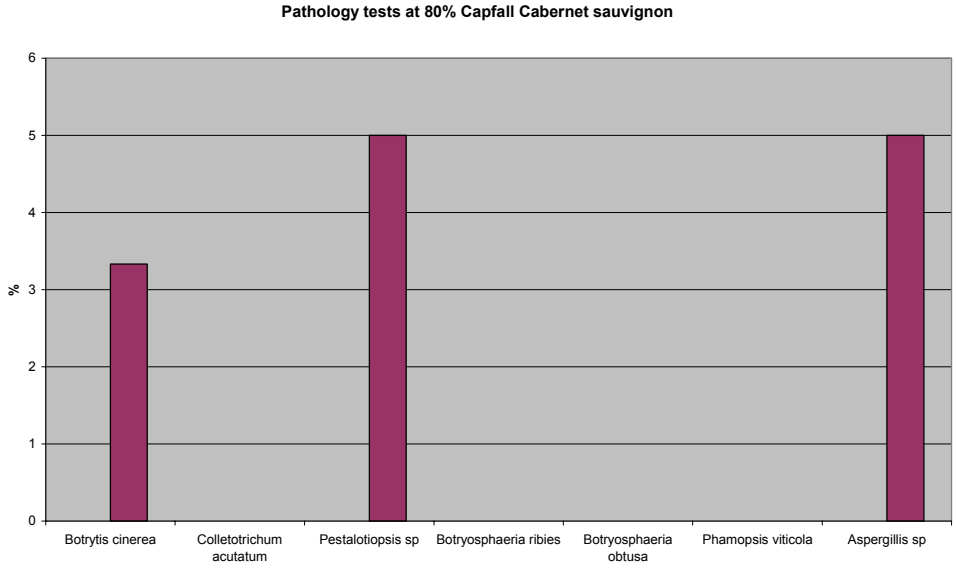
Samples of 60 pre flowering green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. The results are shown in Figure 18.

Figure 18



Samples of 60 80% capfall green shoots with inflorescence and leaf were collected in separate paper bags. The samples were surface sterilised and moist incubated. They were then examined Microscopically and isolated on media for examination of cultures and diagnosis of pathogen. The results are shown in Figure 19.

Figure 19



Industry meetings and communication of results.

A post vintage meeting was organised on the 2nd of May and the results were presented . Wider grower participation is expected as the industry becomes more aware of the losses from these diseases this season and interest grows in finding solutions.

The results and future direction of this work were presented at the HVVA Seminar on 22 May at Kurri TAFE. Over 160 growers and winemakers participated in both formal presentations and workshop sessions Development work is also in progress with NSW Agriculture's Plant Health Diagnostic Service to develop testing procedures for these diseases during the different stages in the vineyard. We believe that Colletotrichum actutatm is more widespread than originally thought and it has now been identified it from vineyards in Mudgee as well as the Hastings Valley.

Describe performance against original targets set for 2001/2002

Outputs (for 2002)	Performance Target (for 2002)
1.Five cooperating grower trials conducted	1.Trial successfully conducted
2.Data collected, analysed and reported	2. Preliminary and Final report to GWRDC
3.Industry seminar conducted	3. 160 growers and industry personnel attend seminar on 22 May 2002. 45 growers attended OFT meeting on 2 May at Broke. Further meeting in Pokolbin Hall attended by 52 growers on 29 July and Cassegrain on 31 July attended by 15 growers.

Conclusion.

These trials further supported the evidence that more open canopies provide a more favourable environment for Colletotrichum infection. A hypothesis could be that the Colletotrichum fungus is spread in water droplets and free moisture, with more sheltered canopies providing some shingling effect in shedding light rain and therefore reducing the risk of fruit infection. Heavy rain, it is speculated will dilute the spore concentration and therefore

reduce the level of infection. The pathology tests on dormant wood clearly show that there is significant inoculum of the disease carrying over in the canopy on dormant wood. This could be the primary source of infection for the developing and maturing fruit. There is also the question of sunburn or other environmental factors playing a role in disease incidence. Further trial work in the project by Dr C. Steel will look more closely at these aspects of canopy management and fruit exposure. There is good evidence from the literature on the management of this disease in other crops that reducing the inoculum by removing as much of the over-wintering inoculum as possible can reduce infection. Practical vineyard management options to achieved this need further investigation.

More detailed epidemiological data on the diseases is needed to better understand and control the rot of grapes caused by Colleteotichum (Ripe rot) and Greenaria (Bitter rot). This project has thrown some light on how these fungi cause infection and how they survive in the vineyard. However more detail on the disease cycle of these fungi and the effect of fungicides on their survival is required. It would appear that infection can occur at any stage of fruit development. Following infection at flowering, the fungus remains latent until veraison and ripening of fruit. The data collected pre flowering and at 80% capfall however did not demonstrate this. The environmental conditions were not favourable for disease development at the start of the growing season in October and November 2002 and disease pressure was low at vintage due to the drought. The disease can spread rapidly to other ripe fruit during rain periods around harvest. There are no fungicides registered in Australia for the control of Colletotrichum or Greenaria in grapes and the trials did not demonstrate commercially effective combinations. New chemistry that is pending registration could prove to be efficacious but needs further research and testing.

More detailed research and field trials are needed to develop control methods for these diseases and prevent the rapid spread and loss of fruit near harvest under climatic conditions that favour infection. In practice, a better understanding of infection levels and the selection of appropriate fungicides to control infection at critical periods of the growing season can be obtained by regular monitoring of the crop during the growing season. A regular check on whether the pathogens are developing fungicide resistance would also help in managing these diseases. A protocol developed for this project (Table 11) could provide a useful tool for testing during the different growth stages in the vineyard. Funding did not permit a full seasons set of data to be collected.

Table 11 Monitoring fruit rots and the diagnostic procedures

Sample time	Grapevine Tissue	Symptom	Sample number & packing	Diagnostic procedure
Dormant	Cane, Spur, Bunch stalk	Bleaching, scaring, Die-back Black dots or streaks. Wood discolouration	Send 30 15-30 cm sections of dormant shoots with bunch stalk attached send in separate paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen
Pre flowering	Green shoot Inflorescence Leaf	Scaring of shoots Leaf spots	Send 30 green shoots with Inflorescences attached (15-30 cm long). Pack individually in paper /plastic bags Leaves individually in paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen ELISA test for Colletotrichum acutatum

80% cap fall	Green shoot Inflorescence Leaf	Scaring of shoots Leaf spots	Send 30 green shoots with Inflorescences attached (15-30 cm long). Pack individually in paper/plastic bags Leaves individually in paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen ELISA test for Colletotrichum acutatum
Pea-size berries	Green shoot Developing bunch Leaf	Scaring of shoots Bunches showing dead tissue Berries not set and showing dark pinkish colour Leaf spot	Send 30 bunches individually in paper/plastic bag. Green shoots individually in paper bags Leaves individually in paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen ELISA test for Colletotrichum acutatum
Verasion	Bunch Cane Tendril	Bunch showing dead tissue. Berries showing discoloured streaks in white varieties. Leaf spots	Send 30 bunches individually in paper/plastic bags. Shoots individually in paper bags Leaves individually in paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen ELISA test for Colletotrichum acutatum
Pre Harvest	Bunches	Bunch showing dead tissue. Berries showing discoloured streaks in white varieties. Leaf spots	Send 30 bunches individually in paper/ plastic bags. Shoots individually in paper bags Leaves in individual paper bags	Surface sterilisation Moist incubation Microscopic examination Isolation on media Examination of cultures and diagnosis of pathogen ELISA test for Colletotrichum acutatum

Budget

Item	GWRDC Funding	Actual expenditure
Technical Assistant (0.2 EFT)	6,967	7176.03
Travel Qfleet	5,100	5200.00
Travel Sustenance	1,500	nil
Plant Health Diagnostic		1572.58
GST	1,357	
Totals	13,567	13948.59*
Total funds requested from GWRDC	14,924	

*Note : The shortfall in funding was met by NSW Agriculture .