Management of non-Botrytis bunch rots



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MANAGEMENT OF NON-BOTRYTIS BUNCH ROTS

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Cover illustration: Main picture – Field infection of *Colletotrichum acutatum* on *Vitis vinifera* (cv Shiraz) fruit;
Insert picture – Laboratory infection of *Greeneria uvicola* on *Vitis vinifera* (cv Chardonnay) fruit.

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Abstract

Bunch rot of grapes can be caused by a complex of different organisms depending on vineyard location and characteristics. The relative frequency of organism isolation with phenological growth stage was investigated at a cabernet sauvignon vineyard in the Hastings Valley. The predominant bunch rot pathogens isolated were *Colletotrichum acutatum* (ripe rot) and *Greeneria uvicola* (bitter rot). Isolation of *Botrytis cinerea* was infrequent. Both *C. acutatum* and *G. uvicola* infected post-veraison berries, immature berries resisted infection. *G. uvicola* also infected detached vine leaves. Growth of both organisms on PDA and berry infection was favoured by temperatures relatively higher than that encountered for *B. cinerea*. *B. cinerea* out-competed both *C. acutatum* and *G. uvicola* at 20 ºC but not at 27 ºC. Light exposure, both UVB and white light had no effect on the rate of bunch rot progression. High humidity favoured berry infection and infection was possible at a low relative humidity if the berries were incubated at 100% relative humidity for 24 hours post-inoculation before transfer to a low humidity environment. *C. acutatum* isolates from grapes were not host specific, and were able to infect blueberries, olives and strawberries. A number of fungicide field-based trials were conducted in the Hunter and Hastings Valley. While none of the treatments tested were completely satisfactory for bunch rot management a number of commercially available products did reduce the incidence and severity of bitter rot and ripe rot at harvest. These products included Shirlan (a.i. Fluazinam) applied as a dormant spray and strobilurins (e.g. Amistar, Cabrio) applied at flowering and late in the season.

Non-technical summary

Fungal rots of grape berries close to harvest cause serious economic losses to grape growers particularly in viticulture regions where rainfall during the ripening period is common. The research documented in this report deals with determining the organisms responsible for grape berry bunch rots in two sub-tropical regions of New South Wales, the Hunter Valley and Hastings Valley. Once the pathogens were identified, the project investigated some of the properties of these organisms with respect to their biology and possible disease management options for grape growers.

A complex of fruit rotting fungi were identified from grapevine reproductive and vegetative structures throughout the growing season, of note were two fungi *Colletotrichum acutatum* and *Greeneria uvicola* responsible for ripe rot and bitter of grapes respectively. These two fungi were determined to be the principal organisms associated with bunch rot in the Hunter Valley and Hastings Valley. While both have been recorded on grapevines previously in countries other than Australia, this project is believed to be the first substantial documented study on the behaviour of these diseases on wine grapes in Australia. Other pathogenic fungi recorded from both vegetative and reproductive tissues included organisms more commonly associated with the diseases of the trunk and other woody tissues such as *Phomopsis viticola* and *Botryosphaeria* spp. *Botrytis cinerea* associated with grey mould of grapes was rarely isolated from the vineyards investigated, suggesting that disease control for this disease is largely successful in these vineyards, alternatively that climate does not favour it. In addition to these grapevine pathogens, a number of other organisms were isolated that are regarded as non-pathogenic towards vine tissues. Two of these organisms *Epicoccum* and *Trichoderma* were shown to inhibit the growth of *G. uvicola*. With future research there is thus the potential to develop possible biocontrol agents for sub-tropical bunch rots.

There were seasonal differences in terms of the fungi isolated although the predominant pathogens were *C. acutatum* and *G. uvicola*. These two fungi were therefore selected for further investigation. Both organisms infected mature berries

of all wine grape varieties investigated, immature fruit was not attacked. Infection rates were similar in the 25 – 35°C range, and a humid environment favoured infection. Light exposure, visible and ultra violet B (280 – 320nm) did not influence the rate of infection despite the fact that open canopies in the Hunter Valley appeared to be more prone to attack. Field observations on bunch susceptibility and canopy structure may therefore be explained on the basis of heat as opposed to light stress, although this needs to be proved further.

C. acutatum was found to be non-host specific. Isolates from grapes were able to infect olives and strawberries and blueberries while conversely isolates from non-grape hosts were able to infect grapes. This suggest that there is the possibility that *C. actatum* could spread from nearby non-grape host plants into vineyards.

A range of fungicides were found to offer some level of disease control. These included the strobilurin fungicides such as Amistar (active ingredient azoxystrobin) and Cabrio (active ingredient pyraclostrobin), fungicides applied prior to bud burst, i.e. Shirlan (active ingredient fluazinam) and either Switch (active ingredients fludioxonil and cyprodinil) or Scala (active ingredient anilinopyrimidine) applied prior to bunch closure. Strobilurin fungicides were most effective when applied late in the season (i.e. post-veraison). For Australian wine grapes destined for the export market application of these compounds close to harvest is currently not possible because of nil MRLs for these chemicals set by destination export countries. Management of bitter rot and ripe rot is currently difficult to achieve in sub-tropical vineyards of Australia.

Background

Fungal bunch rots of grapes have a serious impact on both berry yield and grape and wine quality. The principal organism involved in grape bunch rot is *Botrytis cinerea*, which causes grey mould (Figure 1).



Figure 1 *B. cinerea* (grey mould) on a mature *Vitis vinifera* (cv Shiraz) berry

Our knowledge of grey mould of grapes extends over many years, there is a wealth of information on the biology of *B. cinerea* on grapes and other horticultural crops. There are several recent reviews on recent advances in Botrytis research (Elad et al, 2004), including a literature review in a recent GWRDC report, (Cole, 2004). Every four years an international symposium is held on Botrytis and there have been several extension-type activities for grape growers (e.g., Whiting, 2004). *B. cinerea* occurs worldwide, although the disease is generally associated with viticulture regions that are described as cool to mild with wet conditions during the growing season. Management of *B. cinerea* involves a combination of strategically applied fungicide sprays throughout the growing season and management of the canopy to increase fruit exposure. The increased fruit exposure ensures that the fruiting zone isn't exposed to prolonged periods of wetness that will facilitate infection. Despite the extensive knowledge that has been gained on grey mould infection, disease management still fails in some vineyards in some seasons.

There are a number of fungi other than *B. cinerea* that are also capable of rotting *Vitis vinifera* berries that have been collectively termed non-Botrytis bunch rots. The biology and management of these organisms is poorly understood as is the identification. Some of these organisms are true pathogens while a number are

associated with post-harvest rots of grapes and other fruit crops, but under certain environmental situations can infect grape berries close to harvest. These fungi are regarded as opportunistic pathogens, attacking vine tissues when conditions are suitable for infection. Many are saprophytes obtaining nutrients from dead or senescing tissues, while others have been recorded as endophytes in grapevines and other plant species. Of note, are those fungi that have the potential to form mycotoxins, such as the black Aspergilli, including *Aspergillus carbonarius* and *A. niger.* A survey of Australian wine samples was recently completed, fortunately the occurrence of Ochratoxin A was found to be relatively minor (Hocking et al., 2003).

As is the case with *B. cinerea*, many fungi responsible for non-Botrytis bunch rots are also pathogens of other crops. It is not known to what extent host specificity exists with regards to isolates from grapevines. This may be important when management decisions are made with respect to companion or cover crops in vineyards and nearby areas.

A number of fungi, more commonly associated with diseases of the woody tissues of the vine are also reported as potential pathogens of grape berries in some overseas vineyards. These include *Botryosphaeria dothidea* in the USA (Kummuang et al, 1996) *Greeneria uvicola* in India, Taiwan and the USA (Reddy, and Reddy, 1983; Ridings and Clayton, 1970, Wu and Chang, 1993) and *Phomopsis viticola* in India (Lal and Arya, 1982). The climatic regions of these grape growing regions are described as sub-tropical. Similar climatic areas also occur in coastal areas of NSW (e.g. Hunter Valley) and in QLD (e.g. South Burnett). It is conceivable that these pathogens may also occur in Australia as bunch rots, although this has yet to be demonstrated.

The occurrence of non-Botrytis bunch rots in coastal vineyards in NSW was first recorded in 1998 (Somers et al, 1998). This led to an ARC Spirt grant (2000 – 2003) awarded to Charles Sturt University with industry contributions from the Hunter Valley Vineyard Association, Hastings Valley Vignerons Association and the agrochemical company Rhone Poulenc. The major conclusion from this work was that the principal bunch rot disease in the Hastings Valley was ripe rot caused by *Colletotrichum acutatum* (Melksham et al, 2002), however a number of other

fruit rotting fungi were present but were not successfully identified. The work demonstrated that the pathogen had a major impact on grape and wine quality, a 1% level of infection produced a noticeable taint in wine. The work also initiated preliminary studies on canopy management and other control methods. Screening the fruiting zone with plastic sheeting was more successful than any of the fungicide treatments investigated. This result may have been due to weather shielding effects that would in turn limit berry damage. A further hypothesis is that the plastic sheeting altered the canopy micro climate with respect to available water and heat stress. Fungicides that offered some degree of bunch rot control in this work included Scala, Amistar and Mancozeb. The GWRDC continued this funding beyond 2003 firstly through a RITA grant (GWRDC RT 02/39-4) with the Hunter Valley Vineyard Association and with the Mid-North coast wine grape growers and then through the current project.

Results from GWRDC RT 02/39-4 demonstrated further that a range of bunch rot organisms could be recorded from a number of vineyards and that ripe rot and bitter rot appeared to be localised in vineyards in sub-tropical regions. There did not appear to be any great difference in varietal susceptibility, although the *Vitis* hybrid, Chambourcin appeared to be more resistant than the classical *Vitis vinifera* wine grape varieties tested. Compared with grey mould and bitter rot, ripe rot appeared to be more tolerant of UV light when grown in the laboratory. This led to the hypothesis that UV tolerance was a factor that pre-disposed open canopies to ripe rot. If this is the case, then there are important disease management implications in the use of canopy management for bunch rot control.

At the same time studies were in progress to investigate the impact of UVB (280 – 320nm) exposure on vine tissues and *B. cinerea* susceptibility. Field-grown vines shielded using polyester sheeting to reduce UVB exposure had a reduced incidence of grey mould (Steel, 2001). Conversely grapevine callus cultures exposed to elevated levels of UVB (as opposed to the field studies where UVB was filtered out) developed necrosis following *B. cinerea* inoculation at a greater rate than callus cultured under dark conditions, (Steel and Greer, 2005). The impact of climate change and in particular changes in UVB exposure was a topical area of research at the time when the current project commenced, (Schultz, 2000).

Thus at the commencement of this project there was an awareness that bitter rot and ripe rot were important bunch rot pathogens of grapevines, although the biology of these diseases was poorly understood with limited, if any, control measures available to growers. This formed the basis of the current project which had the following objectives.

Objectives

- Determine the incidence of non-Botrytis bunch rots in Australian vineyards and ascertain varietal susceptibility.
- Investigate the role canopy management has on bunch rot incidence with an emphasis on light exposure and free water availability.
- Determine the importance of over wintering structures in bunch rot development.
- Examine the berry microflora, and in particular competition on the berry surface. This will help to elucidate why control of Botrytis can potentially lead to the development of other fungal rots.
- As part of an IPM program, optimise the timing and frequency of fungicide applications for the control of non-Botrytis bunch rots.
- Conduct field-based workshops to assist growers in bunch rot identification and bunch rot management options.

Methodology

1 Vineyard locations and details of field trials

Vineyards were selected in the Hunter and Hastings Valleys regions of NSW (approx 200km and 450km north of Sydney respectively) with known histories of bunch rot problems. These regions typically have a climate characterised as sub-tropical with rainfall during the summer months. Field trials were conducted at commercial vineyards between 2003 and 2006. Fungicides were selected for field trial evaluation based on initially screening efficacy as inhibitors of radial growth on agar plates and current product registration. An inert kaolin-clay particle-film product (Surround WP., Engelhard, NJ, USA) was also evaluated with a number of fungicide groups. Sites 1, 2 and 7 were located in Wauchope and Port Macquarie in the Hastings Valley while site 3 was in the Upper Hunter Valley. Sites 4, 5 and 6 were located in the lower Hunter Valley. Details of the varieties used and further vineyard parameters are presented below. All fungicides were applied at recommended rates as on the fungicide label.

1.1 Site 1

Site 1 in the Hastings Valley consisted of 15-year old Cabernet sauvignon vines (Row spacing 2m, vine spacing 1.5m) trained to a Geneva Double Curtain canopy. Each treatment plot consisted of 3 rows x 8 vines that was replicated five times in a random block design. There was one guard row between plots and two guard vines between treatments (Figure 2).

Row																												
1	Guard Row																											
2			A	A	A		B	B	B		C	C	C		D	D	D		E	E	E		F	F	F		
3			A	A	A		B	B	B		C	C	C		D	D	D		E	E	E		F	F	F		
4			A	A	A		B	B	B		C	C	C		D	D	D		E	E	E		F	F	F		
5	Guard Row																											

Figure 2 Typical section of fungicide field trial design at sites 1, 2 and 7. Letters represent different treatments and cells represent different panels of vines. Treatments were randomised over the block, and only the middle three shaded panels of each treatment were monitored and used for data collection.

Fungicides were applied using a hand-held back-pack sprayer. Standard fungicides applied to all treatments at this site in 2003/4 were Amistar at 5% cap fall and Scala at 80% cap fall to manage grey mould, Flint when berries were pea size and Mancozeb at veraison to control downy mildew. In 2004/5 and 2005/6 standard fungicides applied to all treatments were Shirlan (dormant spray), Scala at 80% cap fall and mancozeb at veraison.

1.2 Site 2

Site 2 was located in the Hastings Valley and consisted of ten-year old shiraz vines (Row spacing 2m, vine spacing 1.5m) trained using vertical shoot positioning (VSP). The design of the treated plot and fungicide application was similar to that used at site 1. In addition to the test treatments, all vines were sprayed with Delan & Tilt when the shoots were 5cm in length to control powdery mildew and with Bravo when the shoots were 10cm in length for grey mould control. Dormex was also applied in early August to synchronise bud burst.

1.3 Site 3

Site 3 was located in the Hunter Valley and consisted of 15 year-old Chardonnay vines (Row spacing 2m, vine spacing 1.5m) trained using VSP. Treatments at this site were applied on a row basis rather than a panel design as described above for sites 1 and 2. Each replicate plot consisted of two rows of sprayed vines with two adjacent buffer rows making a total of six rows per plot (Figure 3). There were six replicates per treatment and treatments were randomised across the block. An example of the layout of a trial is shown in appendix 1 at the end of this report.

Replicate	Row number	Treatment
1	1	Buffer
	2	Buffer
	3	Sprayed & monitored
	4	Sprayed & monitored
	5	Buffer
	6	Buffer

Figure 3 Typical section of fungicide field trial design at sites 3 - 6

Treatments were replicated six times and randomised throughout the vineyard. Standard sprays applied at site 3 for mildew were sulphur, Topas and Dithane. Chlorothalonil was applied after bud burst for Botrytis control. Bunch rots at site 3 were consistently due to bitter rot during the lifetime of this project, so no data is available for ripe rot and other bunch rots at site 3.

1.4 Site 4

Site 4 was located in the Hunter Valley and consisted of Shiraz vines. Part of this site was used to investigate row aspect on bunch rot susceptibility. The vines were orientated in a North-South direction on a level field. The spray design was as described in Figure 3.

1.5 Site 5

Site 5 was located in the Hunter Valley and consisted of Shiraz vines. This site was used to investigate the effect of row orientation on bunch rot susceptibility. The vines were orientated in a North-South direction on a westerly facing slope. This site was also employed to investigate fungicide use for bunch rot management. The spray design was as described in Figure 3.

1.6 Site 6

Site 6 was located in the Hunter Valley and also consisted of Shiraz vines. The vineyard was located on a westerly facing slope with rows running in a north-south direction. This site was used to investigate the effect of row orientation on bunch rot occurrence. The spray design was as described in Figure 3.

1.7 Site 7

This vineyard located in the Hastings Valley consisted of VSP trained Semillon vines. The site had bird control problems and was used only in the 2003/4 growing season. Standard sprays applied at this site were the same as those described for Site 2. The trial design was as described in Figure 2, i.e. individual panels were sprayed.

2 Initial fungicide screening

G. uvicola and *C. acutatum* isolates were collected from a range of vineyards along the eastern coast of Australia and stored as freeze-dried cultures on carnation leaves as described by Fisher *et al*., (1982). Isolates were screened for fungicide sensitivity by determining radial growth rates on PDA as previously described (Steel and Nair, 1993). Active ingredients were obtained from Fluka, and results expressed with respect to the µM concentration, required to inhibit 50% of radial growth, i.e. EC_{50}. The results from this aspect of the project formed the basis of the field trial work described above.

3 Bunch rot monitoring

Berries were scored for visible signs of bunch rot incidence (i.e. disease presence or absence on a single bunch) and severity using a four point scale where a score of 0 equated to a bunch showing no visible signs of bunch rot and scores of 1, 2, 3, and 4 are respectively equivalent with 1.5, 14, 37 and 75% of the berries in a given bunch showing infection (Emmett, 2003). At sites 1, 2 and 7 the fruit was monitored by scoring disease incidence and severity on 20 randomly selected bunches from each replicate and the average scores determined. At other sites twenty bunches of fruit were scored every 25 vines along each row and this was repeated five times per row and results expressed as a mean.

4 Frequency of fungal isolation at different phenological stages

In 2004/5 and 2005/6 the presence or absence of organisms associated with grape bunch rots was monitored on vegetative and reproductive tissues during the growing season from a vineyard in the Hastings Valley, NSW (site 1). Fifty inflorescences or berries were collected from the vineyard at flowering, pre-veraison, early post-veraison berries and at harvest (corresponding to the E-L phenological stages of 23, 31, 35 and 38 as described by Coombe, 1995). Plant material was surface sterilised using sodium hypochlorite (1% v/v, 2 min) and successively washed in three volumes of sterile distilled water before transfer to

DRBC plates and incubation at 25 ºC for 7 days. Any resulting growth was then sub-cultured to PDA plates for subsequent identification.

5 Data analysis for field trials

Data were subjected to analysis of variance (ANOVA) using the CoStat statistical package (CoHort Software, Monterey, CA, USA). The Least Significant Difference (LSD) test was used for comparison of means of disease score at $P = 0.05$.

6 Fungal isolates

C. acutatum and *G. uvicola* were isolated from grapes berries (*Vitis vinifera* L.) from the Hunter and Hastings Valleys. Fungal isolation involved transferring conidia from the berry surface of infected tissue to DRBC, and then subsequently to PDA with incubation at 25°C for four days in darkness. Single spore isolations were sub-cultured to PDA and incubated at 25°C in darkness for seven days. Cultures were stored on PDA slants at 4°C. Five isolates of *C. acutatum* and five isolates of *G. uvicola* (Table 1) were selected for growth rate studies on PDA. Radial growth was measured periodically (Steel and Nair, 1993) and the maximum daily growth rate determined.

Table 1 Fungal isolates used for fungicide screening *in vitro*

Isolates		Geographical origin and date of isolation	Host grape cultivar
DAR Number*	Organism		
75574	*C. acutatum*	Hastings Valley, NSW; 2000	Cabernet Sauvignon
77282	*C. acutatum*	Hastings Valley, NSW; 2000	Pinot noir
77283	*C. acutatum*	Upper Hunter Valley, NSW; 2002	Cabernet Sauvignon
77284	*C. acutatum*	Murgon, QLD; 2002	Semillon
77285	*C. acutatum*	Lower Hunter Valley, NSW; 2000	Semillon
77258	*G. uvicola*	Lower Hunter Valley 2002	Chardonnay
77261	*G. uvicola*	Hastings Valley, NSW; 2004	Chambourcin
77263	*G. uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77271	*G. uvicola*	Upper Hunter Valley, NSW; 2004	Cabernet Sauvignon
77275	*G. uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay

* DAR numbers correspond to isolates deposited with the Agricultural Scientific Collection Unit at NSW DPI in Orange.

7 Infection studies

Spore suspensions were prepared for infection studies by transferring mycelial fragments to a plate of PDA incubated at 27 ºC, under at 12hr light/dark photoperiod (10-14 days *C. acutatum* DAR 75574, 7-10 days for *G. uvicola* DAR 77258) until spores developed. Spores were washed from the plate with 4-5 mL sterile, deionized water and adjusted to a concentration of 10^6 spores per mL. Disease-free grape berries were collected from the Charles Sturt University vineyard at Wagga Wagga. The vineyard at Wagga Wagga was selected for infection studies using detached vine tissues as it is located in a dry inland region of Australia where bunch rot problems are rare. Berries were harvested by cutting the pedicel to ensure that the brush remained intact, and surface sterilised using sodium hypochorite (1% v/v, plus 0.05% v/v Tween 20, 2 mins) followed by three successive washings in sterile distilled water. Berries (24) were placed into the

wells of a microtitre plate (24 well, flat-bottom, Iwaki Microplates) and inoculated by placing a 10 µL drop of a spore suspension (10^6 spores/mL in sterile distilled water) onto the apex of the berry surface without wounding the berry. Control berries were inoculated with 10 µL of sterile distilled water. Twenty millilitres of sterile water were added to the base of each plate, surrounding the wells, giving a 100% relative humidity. Lids were placed on the microtitre plates, stacked up to six high, sealed with cling film and incubated at a range of temperatures and relative humidity conditions. Berries were examined for signs of visible fungal colonisation using a binocular microscope at various time intervals post-inoculation and results were expressed as a percentage of total berries exhibiting infection. Fungi were isolated from a sub-set of berries, and identities confirmed to prove Koch's postulates.

The effect of relative humidity on the infection process was investigated by placing the microtitre plate into a small sealed plastic container (0.8L volume), with 100 mL of a saturated salt solution. Relative humidity (RH) at 27°C in the 23 – 100% range were obtained by using the following saturated salt solutions; $LiCl_2$ (23% RH); $Mg(NO_3)_2$, (50% RH); $CaCl_2$, (33% RH); NH_4Cl, (87% RH); and distilled H_2O, (100% RH). Relative humidity values were confirmed and recorded using a data logger (HOBO® U12, Onset Corporation). Berries were scored by observing the time taken until visible signs of bitter rot or ripe rot were apparent.

Leaf infection studies were based on a modification of the method described by Evans (1996) using leaves with the petioles inserted into water agar. Cabernet sauvignon leaves (first fully expanded leaf) were collected from the CSU vineyard in spring. Leaves were inoculated by placing a 10µL spore suspension (range of concentrations 10^3 – 10^7 spores/ml examined) on the upper side of the leaf. Leaves were incubated at 25°C with a 12hr photo period for up to 21 days. One isolate each of *C. acutatum* (DAR 75574) and *G. uvicola* (DAR 77258) were used for this aspect of the project. Inoculations were carried out in triplicate. Control leaves were inoculated with sterile deionised water.

8 Host Specificity studies

Cabernet Sauvignon grapes (*Vitis vinifera* cv. Cabernet sauvignon) were harvested (13-15^{o} baume) from the Charles Sturt University vineyard and a vineyard at Tumbarumba. Blueberries (*Vaccinium* spp.) were purchased locally from a supermarket and were from Corrindi Blueberries NSW. Olives (*Olea europea*) were picked from the Charles Sturt University olive trees. Grape berries were surfaced sterilized with 1% solution of sodium hypochlorite for two minutes and rinsed with autoclaved deionised water. Blueberries and olives were surface sterilized with 1.5% (1% available Chlorine) solution of sodium hypochlorite for 3 minutes and rinsed as above. Grapes and blueberries were placed in 24-well Elisa plates (Iwaka, Asahi Techno Glass, Japan) and the olives were placed in 30mL plastic disposable cups housed in a sealed 30 x 15cm polyethylene container. Both vessels were filled with sufficient autoclaved deionised water in an attempt to simulate a humid environment. Spore suspensions were prepared from *Colletotrichum* isolates cultured on potato dextrose agar PDA, (Oxoid Ltd., England) and grown at 25^{o}C for 10 days. 3 - 5 mL of deionised water was added to the fungal culture and spores were dislodged by gentle rubbing action of a flame sterilized glass rod. The spore suspension was collected by pipette and resuspended in a McCartney bottle with the spore concentration adjusted to 6 x 10^{6} cells mL^{-1} with sterile deionised water. Aliquots of 10µL of spore solution were pipetted to 48, 4, and 4 replicates of grape, blueberry and olive respectively. All replicates including controls were then incubated at 25^{o}C with a 12 hr dark/light cycle. Observations were made on a daily basis. After 10 days, spores from infected fruit were aseptically transferred and sub-cultured on PDA plates which were incubated at 25^{o}C in darkness. The resultant cultures were visually checked for colony and spore morphology to confirm Koch's postulate.

9 Workshops

Part of this project involved presentations on bunch rot management at regional workshops. The workshops were also an opportunity to survey participants. Participants were asked to use a 7 point scale to mark their response to a series of questions and an average numerical score obtained for each question. A score of

7 indicated that the participant very strongly agreed with the statement/question while a score of 1 indicated that they very strongly disagreed. Details of the surveys used are presented in appendix 2 to this report.

Results

1. Bunch rot occurrence

This aspect of the project was concerned with determining the principal organisms involved in bunch rot of grapes in sub-tropical vineyards and the relationship with phenological stage. Occurrence on structures other than mature fruit was investigated, so that information could be obtained on the occurrence of latent infections and inoculum carry-over. This information was also sought in order to assist with the development of a strategically-timed fungicide spray program.

Vineyards were surveyed in 2003 as part of preliminary funding awarded through the GWRDC Rita scheme (GWRDC RT 02/39-4). This surveyed was conducted by randomly selecting 5 rows and scoring a total of 100 bunches per variety. Part of the data obtained in this project is reproduced here (Table 2), since it formed the basis of the current work. Seven vineyards were surveyed, in most cases the survey related to one variety, although multiple varieties were examined at two of the sites. The 2003 vintage was a dry year, and bunch rot occurrence was therefore expected to be low. However, five sites in four vineyards had ripe rot problems and grey mould was found at three sites. Six of the vineyards had a complex of bunch rotting organisms, which were tentatively identified as bitter rot based on the presence of black rings of sporulation around the circumference of the berry. There was a low incidence in this group of unidentified rots that were blue-green in appearance and were most probably due to either *Penicillium* spp. or *Cladosporium*.

Table 2 Percentage incidence (I) and severity (S) of ripe rot (*C. acutatum*), grey mould (*B. cinerea*) and unidentified bunch rots in vineyards surveyed in the Hunter and Hastings Valleys in 2003. Unidentified bunch rots included black moulds, blue moulds (e.g. *Penicillium* spp.) and sour rots. Results are the means of 100 bunches surveyed. Severity was expressed on a 0 – 4 scale where 0 = no rot symptoms; 1 = one or two berries in a bunch with rot symptoms; 2 = approximately one tenth to one quarter of the berries in a bunch with rot symptoms; 3 = approximately one third to a half of the berries in a bunch with rot symptoms; 4 = greater than half the berries in the bunch with rot symptoms.

Location	Variety	Ripe rot		Grey mould		Unidentified rots	
		I	S	I	S	I	S
Upper Hunter (1)	Chardonnay	0	0	0	0	42	0.64
Upper Hunter (2)	Cabernet Sauvignon	0	0	0	0	0	0
	Shiraz	0	0	0	0	0	0
	Semillon	14	0.18	0	0	33	0.40
Lower Hunter (1)	Shiraz	0	0	15	0.18	2	0.02
Lower Hunter (2)	Cabernet Sauvignon	0	0	16	0.21	0	0
Lower Hunter (3)	Shiraz	1	0.03	30	0.42	2	0.05
Lower Hunter (4)	Merlot	81	1.79	0	0	4	0.05
	Cabernet sauvignon	80	1.67	0	0	0	0
Hastings (1)	Cabernet Sauvignon	5	0.25	0	0	45	0.68

Based on this initial survey data, one vineyard was selected in the 2004/5 and 2005/6 seasons for more detailed analysis of the occurrence of bunch rot organisms on both vegetative and reproductive structures though out the growing season. This approach was taken to determine which bunch rot pathogens were present at what phenological stage.

This site located in the Hastings Valley was selected on the basis that it was in a high summer rainfall area and thus likely to experience bunch rots problems more frequently than the sites located in the Hunter Valley. Tables 3 - 6 show the frequency of fungal isolations from leaf tissues and reproductive structures during the growing season from this vineyard. In 2005/6, dormant buds (i.e. E – L stage 1) were also sampled. The predominant fungal pathogen isolated at flowering from both leaves and inflorescences in both years was *Alternaria*. *C. acutatum* was isolated with a higher frequency from inflorescences in 2004/5 than in 2005/6. Other fungi associated with berry rots at flowering included *Botryosphaeria* spp.,

G. uvicola and *P. viticola.* As the fruit developed *C. acutatum* increased in frequency of isolation. Isolation of *G. uvicola* from fruit only became significant at berry maturity. *Alternaria, Botryosphaeria* spp. and *P. viticola* were consistently isolated from both leaf and reproductive tissues throughout the growing season in both years. Other non-pathogenic fungi isolated at various degrees of frequency were predominantly *Cladosporium* and *Pestalotiopsis*. Representative photographs of ripe rot, bitter rot and Phomopsis bunch rots of mature grapes are presented in Figures 4 – 6.

C. acutatum and *G. uvicola* were selected out of the range of bunch rot organisms isolated for further study because both of these organism are regarded as true pathogens of vines (as opposed to opportunistic pathogens) and because of the high incidence of these two organisms in vineyards that experience wet and warm conditions during vintage. Details of *C. acutatum* and *G. uvicola* isolations made together with representative isolates deposited with the Plant Disease Herbarium Collection of the Agricultural Scientific Collection Unit at NSW DPI in Orange are presented in Appendix 3 and 4. The tables also include details of *Colletotrichum* isolates obtained from non-grape hosts as part of this work.

Table 3 Frequency of fungal isolation from reproductive structures in the 2004/5 growing season

Organism	% Frequency of isolation at E-L growth stages			
	23	31	35	38
Alternaria spp.	82	76	72	44
Botryosphaeria spp.	18	10	10	6
Botrytis cinerea	0	0	0	0
Cladosporium spp.	0	0	0	0
Colletotrichum spp.	32	84	74	84
Epicoccum spp.	18	16	6	8
Fusarium spp.	0	0	0	0
Greeneria uvicola	0	14	10	40
Nigrospora	6	0	4	0
Pestalotia	14	10	30	32
Phomopsis	2	6	10	8
Trichoderma	0	0	0	0
Unknown	68	66	27	28

Table 4 Frequency of fungal isolation from buds and leaves in the 2004/5 growing season

Organism	% Frequency of isolation at E – L growth stages			
	23	31	35	38
Alternaria spp.	66	58	68	100
Botryosphaeria spp.	20	0	0	4
Botrytis cinerea	0	0	0	0
Cladosporium spp.	0	0	0	0
Colletotrichum spp.	34	62	72	96
Epicoccum spp.	16	4	8	32
Fusarium spp.	0	0	0	0
Greeneria uvicola	28	0	4	4
Nigrospora	4	0	4	0
Pestalotia	36	40	36	48
Phomopsis	6	0	6	6
Trichoderma	0	0	0	0
Unknown	84	66	40	18

Table 5 Frequency of fungal isolation from reproductive structures in the 2005/6 growing season

Organism	% Frequency of isolation at E-L growth stages			
	23	31	35	38
Alternaria spp.	54	84	66	70
Botryosphaeria spp.	0	2	26	20
Botrytis cinerea	2	2	0	0
Cladosporium spp.	0	88	54	50
Colletotrichum spp.	10	14	46	86
Epicoccum spp.	10	36	24	40
Fusarium spp.	20	22	18	16
Greeneria uvicola	4	0	4	82
Nigrospora	0	0	26	28
Pestalotia	2	20	56	70
Phomopsis	4	8	12	16
Trichoderma	0	0	2	0
Unknown	26	20	4	4

Table 6 Frequency of fungal isolation from buds and leaves in the 2005/6 growing season

Organism	% Frequency of isolation at E – L growth stages				
	1	23	31	35	38
Alternaria spp.	92	100	56	80	88
Botryosphaeria spp.	22	2	2	2	4
Botrytis cinerea	0	0	0	0	0
Cladosporium spp.	0	0	40	22	54
Colletotrichum spp.	72	14	6	48	70
Epicoccum spp.	8	60	8	6	20
Fusarium spp.	84	38	32	46	14
Greeneria uvicola	0	8	0	0	8
Nigrospora	34	8	10	66	78
Pestalotia	94	62	10	34	92
Phomopsis	0	22	22	28	30
Trichoderma	18	0	0	0	0
Unknown	13	56	26	40	6



Figure 4 Ripe rot (*C. acutatum*) infection of cabernet sauvignon. Orange discharge of spores seen on the berry surface as the berry collapses



Figure 5 Typical symptoms of bitter rot (*G.uvicola*) on Chardonnay fruit. Concentric rings of sporulation may be observed around the circumference of the berry

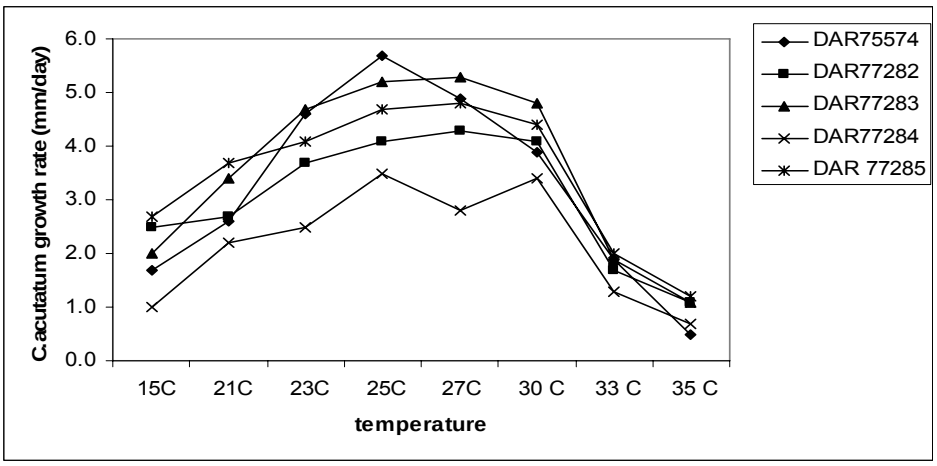


Figure 6 Bunch rot of *Vitis vinifera* (cv. Cabernet Sauvignon) caused by *Phomopsis viticola*. Necrotic rachis and desiccated berries with black pycnidia developing on the surface are observed.

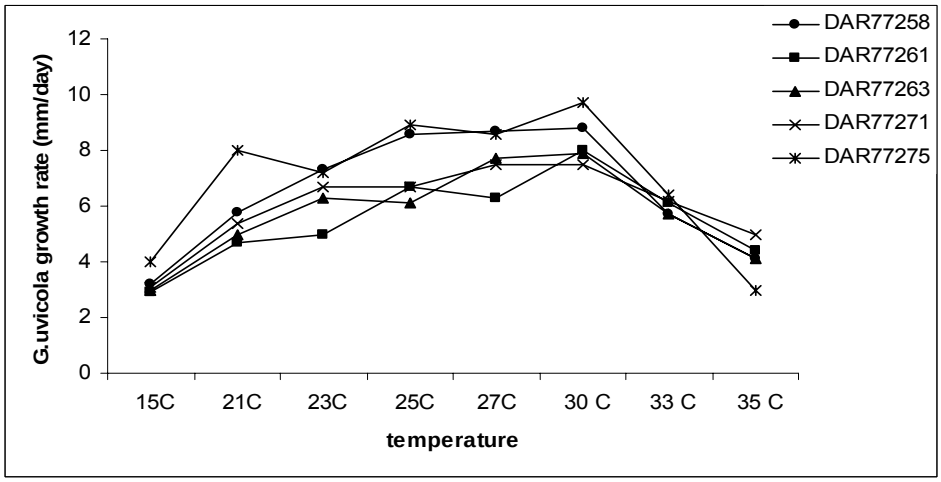
2. Growth characteristics of *C. acutatum* and *G. uvicola* on PDA

The growth characteristics of five isolates of *C. acutatum* and *G. uvicola* were compared on PDA. The growth rate of *G. uvicola* on PDA was faster than that of *C. acutatum*, and although all the isolates examined were able to grow at 35ºC, the optimum temperature for radial growth was comparable with that for *C. acutatum* (Figures 7a and b). In figure 7c the data for the five isolates for each of the organisms is presented as a mean. Both of these organisms appear to grow at temperature optima greater than that recorded for *B. cinerea* (Nair and Allen, 1993).

(a)



(b)



(c)

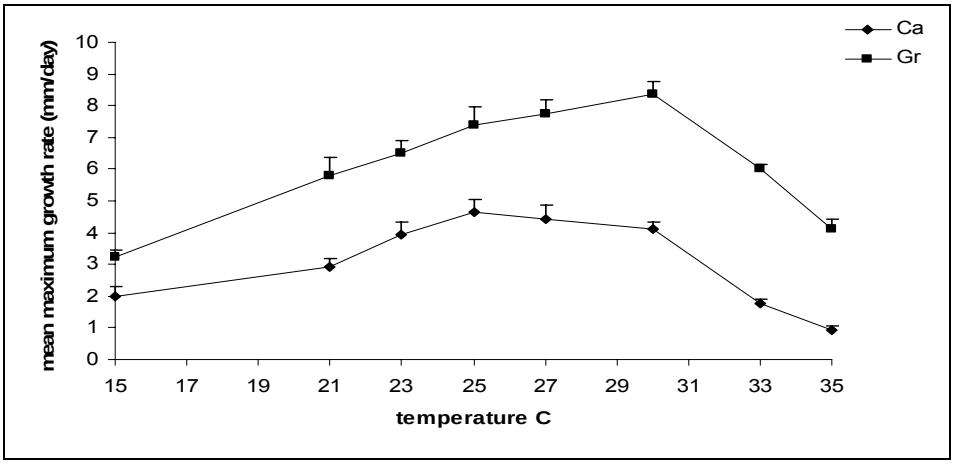


Figure 7 Mean radial growth rates of five isolates of *C. acutatum* (Ca) and *G. uvicola* (Gu) on PDA. Means are of five isolates of each organisms and radial growth was determined in four directions for three plates at each temperature. Data for five (a) *C. acutatum* isolates and (b) *G. uvicola* isolates. Data for all isolates is summarised in (c).

On PDA *C. acutatum* colonies were pale to dark orange, although some isolates had greyish brown pigmentation and the orange colouration was noticeable on the reverse of the plate (Figure 8). *G. uvicola* was pale grey to black when grown on PDA and produced black rings of sporulation (Figure 9).

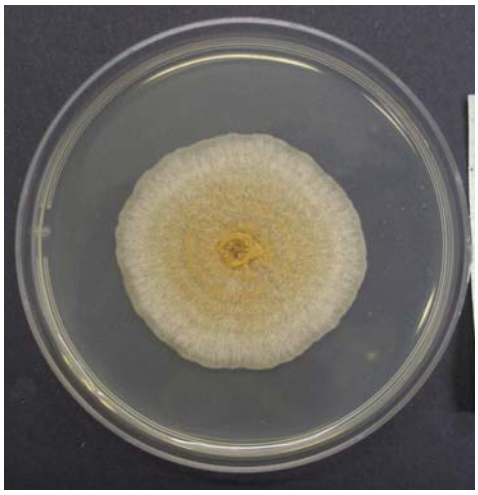


Figure 8 *C.acutatum* culture morphology on PDA.

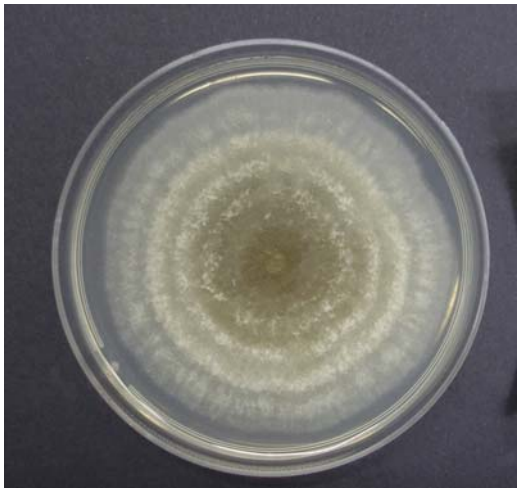


Figure 9 *G.uvicola* culture morphology on PDA.

3. Susceptibility of different varieties and tissues to bunch rot infection

C. acutatum has been recorded as occurring widely on non-Vinifera vines in the USA however there is no information on the susceptibility of *V. vinifera* varieties to *C. acutatum.* Publications on the occurrence of *G. uvicola* largely relate to the susceptibility of fruit and vegetative tissues from table grape varieties. The susceptibility of wine grape cultivars to *C. acutatum* and *G. uvicola* infection was therefore investigated.

Both *C. acutatum* and *G. uvicola* infected post-veraison berries while pre-veraison berries resisted infection (Table 7). There was little difference in varietal susceptibility confirming grower observations. Berries infected with *C. acutatum* initially appeared brown at the infection site, with a sunken lesion. As the infection progressed, an orange exudate of spores appeared at the berry surface (Figure 10).



Figure 10 Shiraz berry infected with *C. acutatum*

Under dry conditions the berry shrivelled. There was little difference in varietal susceptibility, confirming unpublished observations of infection by *C. acutatum* made by grape growers in the vineyard. Berries infected with *G. uvicola* exhibited black acervuli over the berry surface, in some cases concentric rings of sporulation were observed around the circumference of the berry (Figure 11). *G. uvicola* infection did not result in the same level of berry collapse and loss of turgor that was observed following *C. acutatum* infection.

Table 7 Susceptibility of pre- and post-veraison *Vitis vinifera* berries to *G. uvicola* and *C. acutatum*. Berries were incubated at 25ºC and a relative humidity of 100% for 12 days. Berries were then scored for visible signs of *C. acutatum* infection (i.e. orange discharge of spores from berry surface) or *G. uvicola* infection (i.e. black sporulation around the circumference of the berry). Results are the means of 36 individual berries. Control berries (water inoculated) had no visible signs of infection.

	% pre- véraison berries showing visible signs of infection			% post- véraison berries showing visible signs of infection		
Variety	**ºBrix**	***G. uvicola***	***C. acutatum***	**ºBrix**	***G. uvicola***	***C. acutatum***
Chardonnay	15.0	2.8	2.8	26.0	92	69
Merlot	15.2	0	5.6	22.8	39	22
Shiraz	14.1	0	0	23.8	69	39
Cabernet Sauvignon	15.0	2.8	0	21.9	86	61
Traminer	17.5	2.8	0	22.0	81	64
Semillon	16.0	0	0	22.2	47	69
Sauvignon Blanc	13.7	2.8	2.8	22.4	89	61
Riesling	13.7	0	0	19.5	100	75



Figure 11 Sauvignon Blanc berry infected with *G. uvicola*

G. uvicola infection of Cabernet Sauvignon vine leaves led to necrosis around the infection site which expanded with time (Figure 12). Control leaves (water – inoculated) were free of any necrosis (Figure 13). Inoculation of vine leaves with *C. acutatum* did not lead to any necrosis initially, although some chlorosis around the vascular area was observed (Figure 14). When these leaves were incubated for longer periods of time (i.e. 18 days), necrosis did eventually development (Figure 15). With a further extended period of incubation some necrosis and mycelial growth was however observed. Both organisms were re-isolated from the respective inoculated tissues, confirming Koch's postulates.

Attempts were made to inoculate Cabernet Sauvignon flowers with *G. uvicola* and *C. acutatum.* Both fungi infected surface sterilised flowers detached from the vine within 24 hours, further work is warranted on determining the importance of spore concentration and the infection pathways in field-grown vines. Technical difficulties were experienced with the methods used for infection of flowers. Detached influorescences were readily infected. It is anticipated that future work on infection pathways in flowers will employ potted vines that are sprayed with a spore suspension. Influorescence tissue is relatively delicate and senescence is likely to develop soon after detachment which will increase susceptibility to fungal attack. The infection of Cabernet Sauvignon flowers therefore needs to be interpreted with caution.

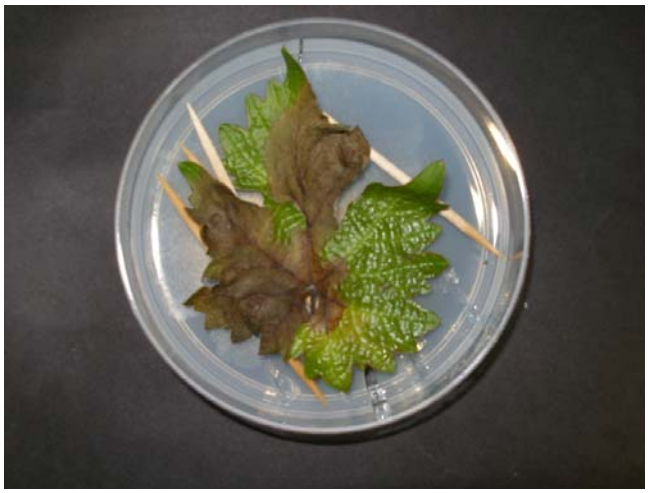


Figure 12 Cabernet sauvignon vine leaf, 11 days post-inoculation with *G. uvicola* (DAR 77258). Leaves were inoculated with 10^6 spores / mL



Figure 13 Control leaf – water inoculated and incubated for 11 days.

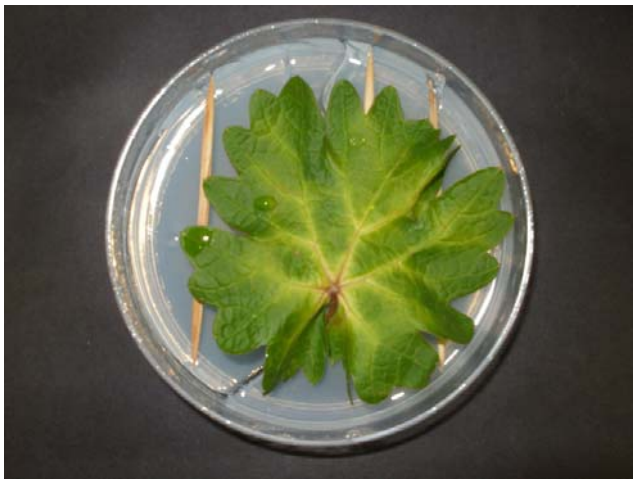


Figure 14 Cabernet sauvignon vine leaf, 11 days post-inoculation with *C. acutatum* (DAR 75574). Leaves were inoculated with 10^6 spores / mL

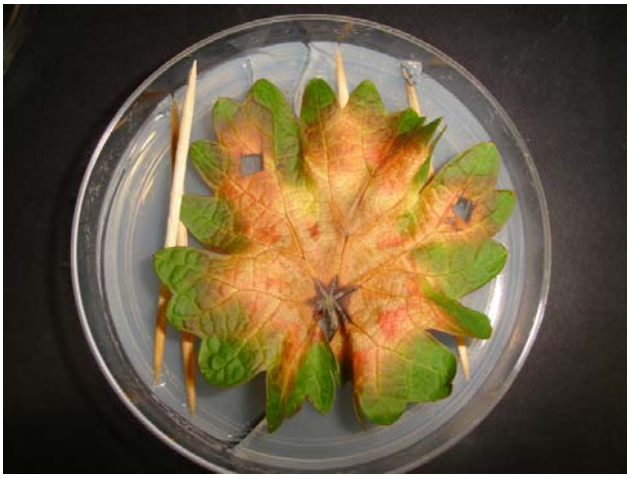


Figure 15 Cabernet sauvignon vine leaf, 18 days post-inoculation with *C. acutatum* (DAR 75574) showing later signs of disease development. Leaves were inoculated with 10^6 spores / mL

4. Host specificity

C. acutatum is known as a pathogen of a range of horticultural crops however the host specificity of isolates from grapes is not known. Infection studies using a number of *C. acutatum* isolates from different hosts were conducted using blueberries, grapes and olives. Also included were other *Colletotrichum* species, *C. gleosporioides* from citrus and *C. musae* from banana.

Two *C. acutatum* isolates from grapes (DAR 75574 and 76886) were able to infect blueberry, grape and olive as did *C. acutatum* isolates from strawberry (DAR 32068), olive (DAR 76921) (Figure 16), blueberry (DAR 76922, 76924, 76926, 76927 and 76930) (Figure 17) and almond (DAR 72407) (Table 8). An isolate of *C. acutatum* from lupin (DAR 76923) failed to infect blueberry and grape but was able to infect olive. *C. gleosporioides* isolates from citrus (Cg50) and avocado (Cg61) infected olives but not grapes or blueberries. *C. musae* (Cm59) which is regarded as host specific for banana failed to infect grape and blueberry but did partially infect olives. A mango isolate of *C. acutatum* (DAR 76925) rapidly infected grape berries, infection of olives and blueberries occurred but at a slower rate. A further isolate of *C. acutatum* from mango (Ca62) weakly infected blueberry but rapidly infected both grapes and olives. While it would be useful to extend this study to include a greater number of isolates from the different host plants, the results demonstrate the cross-infection potential of *C. acutatum* isolates.



Figure 16 *C. acutatum* (DAR 76921) infection of olive.



Figure 17 Blueberry berry infected with *C. acutatum*. Photo: S. Curtin

Table 8 Infection of grapes, olives and blueberries with *Colletotrichum* spp. isolated from a range of host plants

Isolate	Host of Isolate	Region of isolate	Control*	Infection of Olive	Infection of Blueberry	Infection of Grape
75574	Grape	Hastings	--	+	++	++
76886	Grape	Tenterfield	--	+	++	++
32068	Strawberry	Lismore	--	+	++	++
76921	Olive	Hunter	--	++	++	++
76922	Blueberry	Coffs Harbour	--	+	++	+
Cg50	Citrus	South Aust.	--	++	--	--
76923	Lupin	Junee	--	+	--	--
72407	Almond	South Aust	--	++	++	++
Cm59	Banana	Nth. NSW	--	+	--	--
76924	Blueberry	Corrindi	--	++	++	++
Cg61	Avocado	Muswellbrook	--	+	--	--
Ca62	Mango	Nth. NSW	--	+	+	++
76925	Mango	Nth. NSW	--	+	--	++
76923	Blueberry	Corrindi	--	++	++	++
Cg65	Pecan	Muswellbrook	--	+	--	+
76927	Blueberry	Corrindi	--	++	++	++
76929	Chilli	Wagga Wagga	--	--	--	++
76930	Blueberry	Tumbarumba	--	++	++	++

-- denotes no infection; + denotes limited infection; ++ denotes infection; * Control material was inoculated using sterile distilled water.

5. Environmental factors that pre-dispose vine tissue to infection

Bunch rots occur in viticulture regions that experience wet conditions during berry maturation. Table 9 presents a broad summary of our observations on bunch rot occurrence in four contrasting regions of NSW with climatic data for January to March derived from the Australian Bureau of Meteorology web site (www.bom.gov.au/climate/).

Table 9 Summary of observations on bunch rot occurrence in different climatic regions of NSW

Region	Max temp*	Min temp*	Rain (mm)*	Mean RH @ 9am*	Mean RH @ 3pm*	Grey Mould	Ripe rot	Bitter rot
Hastings (Port Macquarie)	27.4	17.9	490	80	75	Rare	Frequent	Frequent
Hunter (Cessnock)	29.0	16.9	278	73	55	Sometimes	Frequent	Frequent
Riverina (Griffith)	30.3	15.4	92	53	35	Noble rot	Never	Never
Tumbarumba	27.0	10.8	182	65	37	Frequent	Never	Never

* = climatic data derived from Australian Bureau of Meteorology web site.

Environmental factors that might influence non-Botrytis bunch rot development include water availability, heat and light. The infection of a given grape berry might also be influenced by biotic factors, such as the profile of the microflora of the berry. It is not uncommon to find more than one bunch rot pathogen on the one bunch and under some circumstances, the one berry. The impact mixed infections have on the expression of bunch rot symptoms is thus a question that remains to be explored. These factors were investigated using detached Cabernet Sauvignon berries at maturity from the CSU vineyard.

5.1 Infection of Cabernet Sauvignon berries at different temps

The infection of Cabernet Sauvignon berries at a range of temperatures is illustrated in Figure 18 at a RH of 100%. Infection occurred at 20 – 35 ºC and was most rapid at 25°C for *C. acutatum* and 35°C for *G. uvicola*. Infection by *G. uvicola* was more rapid than that for *C. acutatum*, five days elapsed before symptoms of ripe rot were visibly evident.

Figure 18a

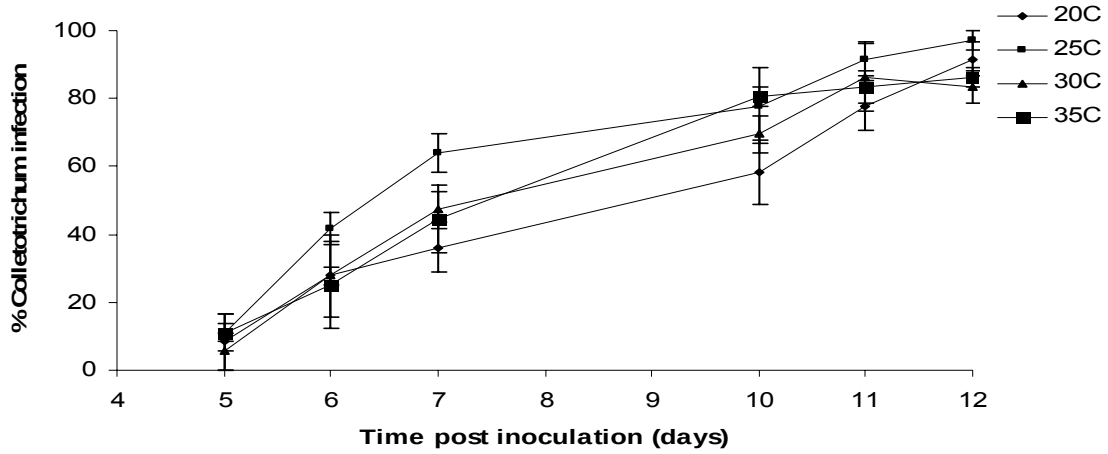


Figure 18b

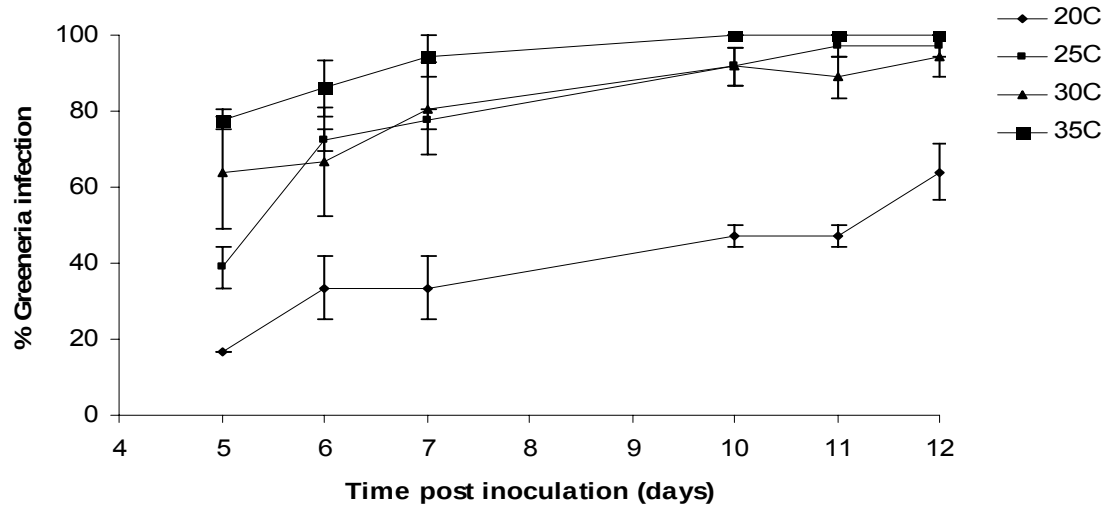


Figure 18 Effect of temperature on infection of *V. vinifera* cv. Cabernet Sauvignon by (a) *C. acutatum* and (b) *G. uvicola*. Berries were incubated at the temperatures indicated under conditions of 100% humidity and periodically assessed for visible signs of *C. acutatum* infection (i.e. orange discharge of spores from berry surface) or *G. uvicola* infection (i.e. black sporulation around the circumference of the berry). Results are the means of 36 individual berries and bars represent standard errors. Control berries (water inoculated) had no visible signs of infection.

5.2 Impact of RH on berry infection

This aspect of the project examined the effect of RH on berry infection at 27°C. The degree of infection was dependant upon the RH, and infection was markedly reduced at RH <87%. There were little if any visible signs of infection at RH of 50% or less (Figure 19). A 24 hr period of incubation at 100% RH before transfer to incubation conditions of lower humidity was sufficient to allow both pathogens to subsequently colonise the berry. Under these conditions *G. uvicola* was able to continue to infect the berries at RH of 23% and *C. acutatum* at RH of 50% (Figure 20).

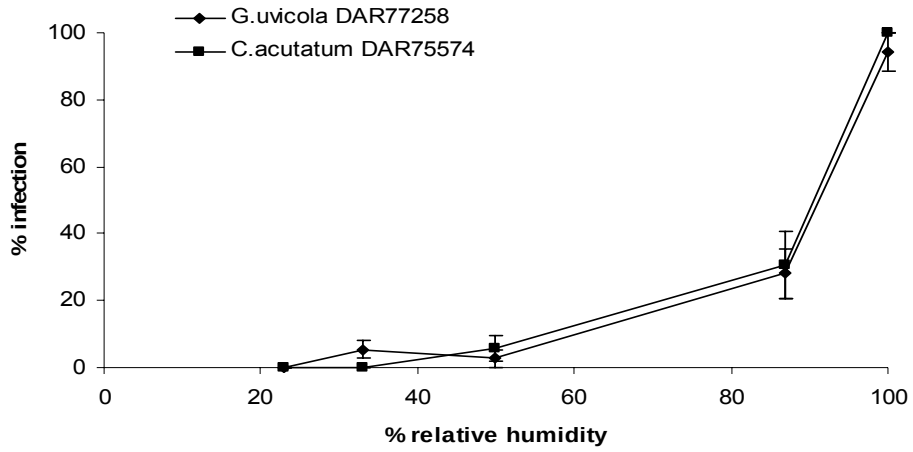


Figure 19 Effect of relative humidity on *C. acutatum* and *G. uvicola* infection of *Vitis vinifera* cv. Cabernet Sauvignon berries. Berries were inoculated and incubated at 27 ºC with relative humidity at 23, 33, 50, 86 and 100% for seven days. Results are the means of 36 individual berries and bars represent standard errors. Control berries (water inoculated) had no visible signs of infection.

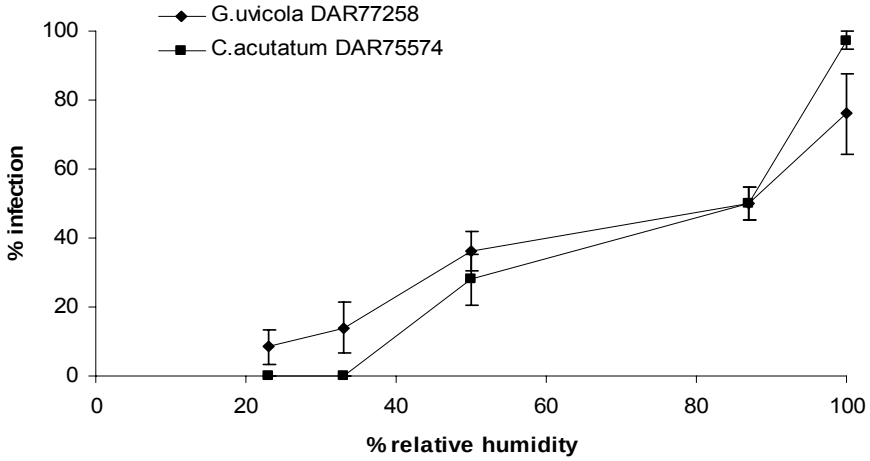


Figure 20 Effect of relative humidity on *C. acutatum* and *G. uvicola* infection on *Vitis vinifera* cv. Cabernet Sauvignon berries following prior incubation at 100% relative humidity. Berries were inoculated and incubated at 100% relative humidity at 27 ºC for 24 hours before transferring to humidity's of 23, 33, 50, 86 and 100% for a further six days. Results are the means of 36 individual berries and bars represent standard errors. Control berries (water inoculated) had no visible signs of infection.

5.3 Effect of light on C. acutatum *and* G. uvicola *infection of* Vitis vinifera *cv. Cabernet Sauvignon*

The infection of Cabernet Sauvignon berries by *C. acutatum* and *G. uvicola* were compared under visible and UVB light because of field observations on the occurrence of these organisms in closed *vs* open canopies. The results of this investigation are presented in Figure 20. There was no statistical difference in the rate of infection under different light conditions.

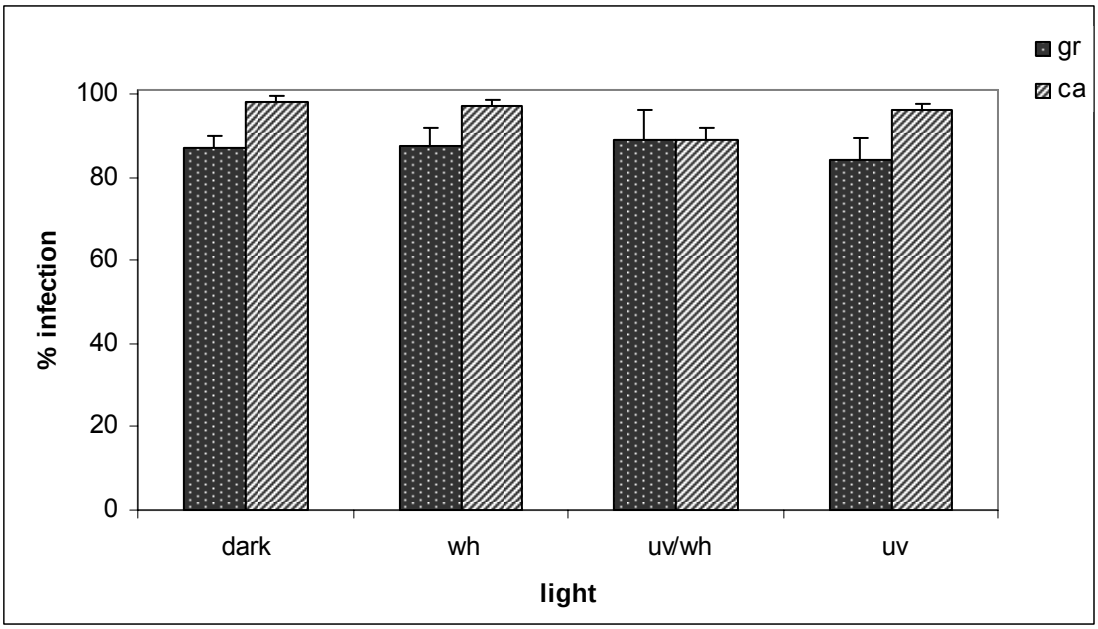


Figure 21 Effect of light on *C. acutatum* (ca) and *G. uvicola* infection of *Vitis vinifera* cv. Cabernet Sauvignon berries. Berries were incubated under dark conditions, visible light (3.1µmol m^{-2} sec^{-1}), a UVB source (0.4 µmol m^{-2} $sec^{-1)}$ or under a combination of the UVB and visible light. Temp 27°C, r/h 100%, incubated 7 days, brix 24.

5.4 Competition on berry surface

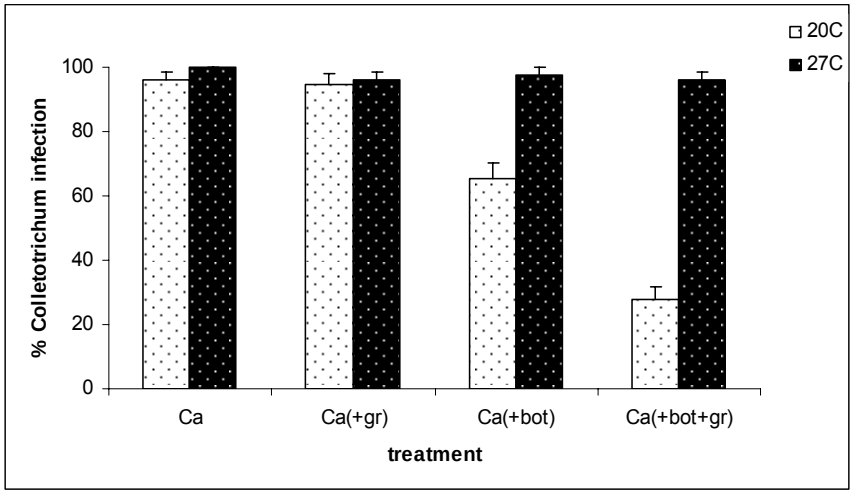
The complete absence of *B. cinerea* from some vineyards prone to bunch rot problems in sub-tropical vineyards is surprising. One possible explanation is that *C. acutatum* and *G. uvicola* are able to out-compete *B. cinerea* under environmental conditions that occur in sub-tropical vineyards. This was first investigated by co-culturing two or more organisms on the one agar plate and observing if there was any antagonism between isolates. Subsequently berry infection experiments were conducted on detached Cabernet Sauvignon berries that were co-inoculated with a combination of *B. cinerea, C. acutatum* and *G. uvicola*. The berry infection experiments were conducted at two temperatures, 20 and 27 ºC.

Figure 22a illustrates *C. acutatum* disease expression with respect to co-inoculation. Co-inoculating *C. acutatum* and *G. uvicola* led to no difference in *C. acutatum* disease expression at either temperature investigated. Co-inoculating *C. acutatum* with either *B. cinerea* or a combination of *B. cinerea* and *G. uvicola* also led to no difference in *C. acutatum* disease expression at 27 ºC, however, when the experiment was conducted at 20 ºC, the level of *C. acutatum* disease expression was significantly reduced. This indicates that *C. acutatum* could not compete as a bunch rot pathogen at the lower temperature investigated.

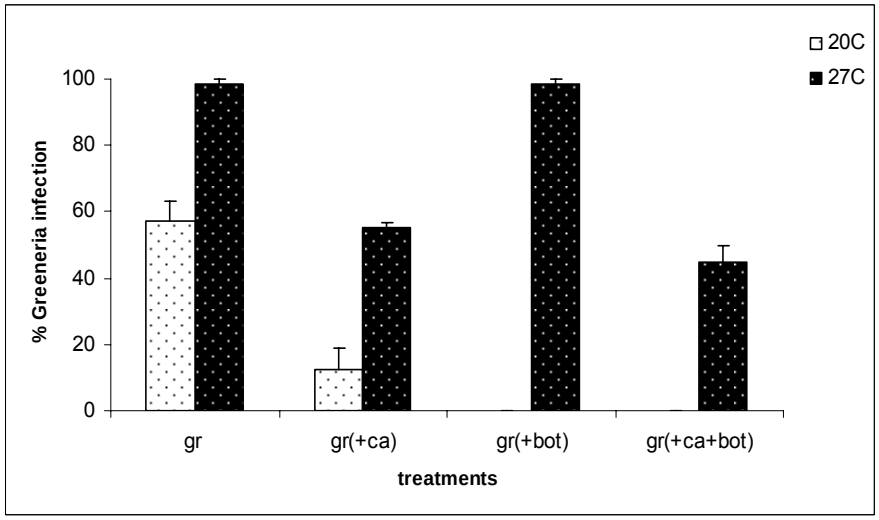
These results are further presented in figure 22b with respect to infection by *G. uvicola*. Disease expression by *G. uvicola* was greatest at 27 ºC and was significantly reduced at both 20 ºC and 27 ºC when co-inoculated with *C. acutatum*. Co-inoculation with *B. cinerea* had no effect on *G. uvicola* expression at 27 ºC but at 20 ºC there was no evidence of *G. uvicola* infection. Similarly a combination of both *C. acutatum* and *B. cinerea* inoculation led to no *G. uvicola* infection at 20 ºC, and a significant reduction in the level of *G. uvicola* disease expression at 27 ºC.

Disease expression in co-inoculation studies with respect to *B. cinerea* disease expression is shown in figure 22c. *B. cinerea* infection was greater at 20 ºC rather than 27 ºC, consistent with published observations that *B. cinerea* is associated with cooler climate viticulture. Co-inoculation of *B. cinerea* with either *G. uvicola* or *C. acutatum* did not lead to any reduction in infection by *B. cinerea* at either of the two temperatures investigated.

(a)



(b)



(c)

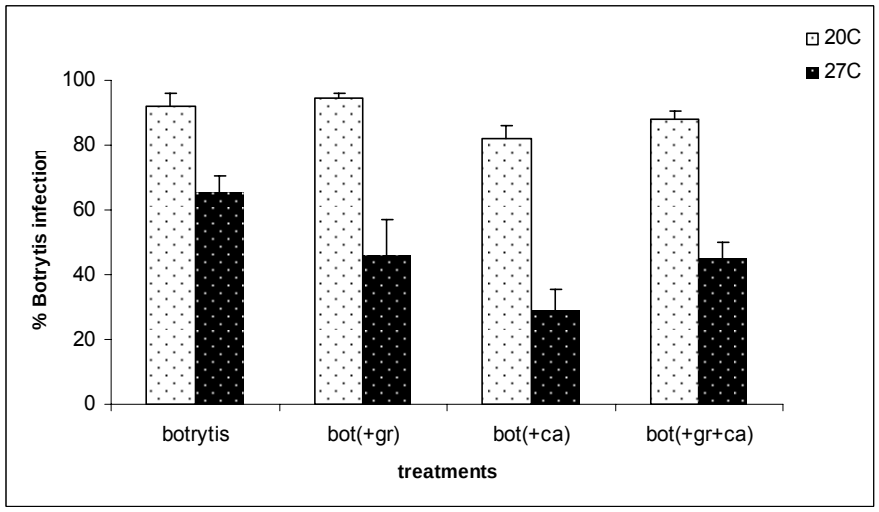


Figure 22 Effect of co-inoculating *C. acutatum* , *G. uvicola* and *B.cinerea* on disease expression in *Vitis vinifera* cv. Cabernet Sauvignon berries (22.4º Brix). Berries were co-inoculated simultaneously, control berries were singularly inoculated with one organism only. Berries were incubated at 100% RH with a 12hr photoperiod. Results expressed with respect to (a*) C. acutatum* (b) *G. uvicola* and (c) *B. cinerea* infection.

5.5 Bio-suppression of bunch rot organisms

A number of saprophytic fungi isolated from vineyards where non-Botrytis bunch rots are common were examined for their potential to suppress *C. acutatum* and *G. uvicola*. Two organisms that exhibited antagonistic properties on agar plates and on whole berries were Epicoccum (Figure 23) and Trichoderma (Figure 24).

Figure 23 illustrates the inhibition of *G.uvicola* radial growth (on PDA) by *Epicoccum*, a common saprophyte isolated from the surface of many plant species while Figure 24 shows the suppression of Bitter rot on grapes by *Trichoderma*. While it is acknowledged that these studies are limited in depth, these observations do demonstrate the bio suppression potential of common berry microflora.

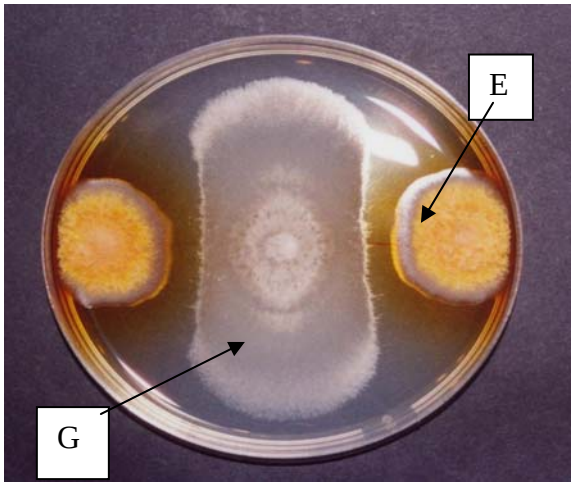


Figure 23 Inhibition of *G. uvicola* (G) by *Epicoccum* (E)

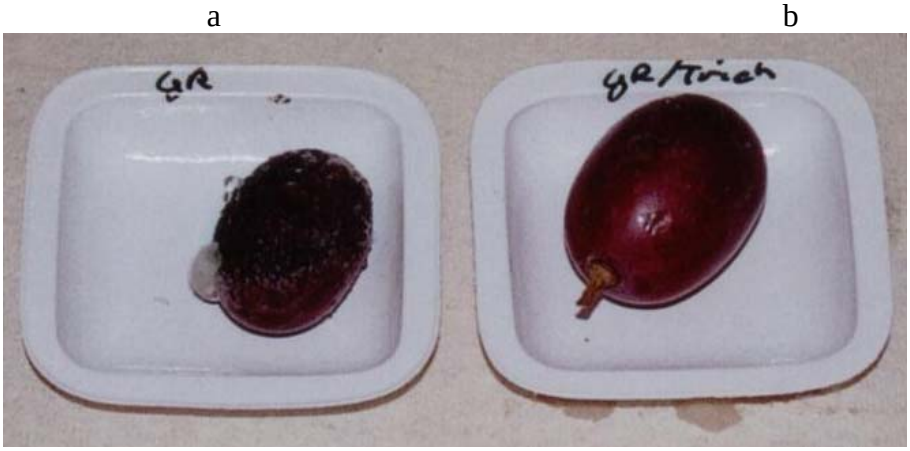


Figure 24 Inhibition of Bitter rot on red globe grapes by *Trichoderma*. (a)- control berry inoculated with *G. uvicola*, (b)- berry co-inoculated with *G. uvicola* and *Trichoderma*

6. Impact of canopy management and fruit exposure on disease occurrence

Management of *B. cinerea* within a vineyard is achieved through an integrated approach, combining both chemical control and cultural practice (English et al., 1993). The incidence of Botrytis in a vineyard can be minimised through canopy management practices that allow a greater aeration of the fruit zone (Gubler et al., 1987). This practice modifies the microclimate of the fruiting zone, allowing bunches to dry more readily after moist periods. However, grower observations indicated that open canopies may, in fact, have a higher incidence of non-Botrytis bunch rots (Castillo-Pando et al., 1999).

The impact of fruit exposure on bunch rot occurrence was investigated in a Chardonnay vineyard (Site 3) in the Upper Hunter in 2004. Shoots were either vertically positioned or left to hang on the canopy. The rows were oriented in a north-south direction so the impact of East versus West aspect were investigated. The results from this trial are presented in Table 10.

Table 10 Effect of bunch exposure on bunch rot severity. Treatments involved altering fruit exposure by lifting wires on east vs. west facing sides of a VSP canopy at Site 3 in 2004. Fruit was scored for bunch rot severity using a 0 – 4 scale as described in the methods, and mean values obtained for 100 bunches

Fruit exposure	Bunch rot severity at harvest			
	G. uvicola	*C. acutatum*	*B. cinerea*	Other rots
VSP on both sides of canopy	0.56	0.02	0	0.03
VSP East facing side only	0.67	0	0.03	0.06
VSP West facing side only	0.66	0.01	0.02	0.07
No VSP	0.67	0.01	0.01	0.05

Disease pressure was low at this site and in this season, and no significant differences could be detected between treatments, although a greater level of fruit exposure where shoots were vertically positioned on both sides of the canopy (i.e. both wires lifted) tended to result in less bitter rot.

Further grower observations suggest that heat stress may also play a role in non-Botrytis bunch rot incidence. Arguably western facing fruit may be exposed to higher temperatures from afternoon sunlight than easterly facing fruit.

We therefore investigated the impact of row orientation on disease occurrence at two different vineyards in 2004/5 and 2005/6. The incidence and severity of non-Botrytis bunch rots was greatest in the west facing side of the canopy at both of the sites investigated (Tables 11 and 12). The west facing side of the canopy is likely to have higher levels of sunburn, light exposure and higher mean daily temperatures. A combination of sunburn and other heat stress events may explain this heightened susceptibility to bunch rot. This finding has practical implications for growers in regions prone to non-Botrytis bunch rots. A solution for bunch rot management in these regions may be to position shoots such that western facing canopies are not over exposed to high temperatures and high light exposures. However, more specific data is required to test this hypothesis.

Table 11 Effect of row aspect on bunch rot occurrence at Site 4 in 2005

Row Orientation	Year	% non-Botrytis bunch rot incidence	non-Botrytis bunch rot severity
East Facing	2005	25	0.76 a
West Facing	2005	38	1.58 b

Table 12 Effect of row aspect on bunch rot occurrence at Site 5 in 2006

Row Orientation	Year	% non-Botrytis bunch rot incidence	non-Botrytis bunch rot severity
East Facing	2006	20	0.23 a
West Facing	2006	55	0.57 b

During the 2005/2006 vintage a series of Hobo Data loggers were placed in bunches at Site 1 prior to bunch closure. The temperature probes were attached to the rachis (Figure 25) such that the bunch encased the probe with bunch closure.



Figure 25 Location of temperature probe in the interior of a Shiraz bunch close to harvest

Despite differences in bunch rot occurrence with fruit aspect (Table 6), there was no significant difference in the internal temperature of the bunch, suggesting that the external fruit acted as a good thermal insulator. Further trial work is in progress as part of the GWRDC-NWGIC Winegrowing Futures program which will evaluate external bunch temperatures.

7. Fungicide sensitivity *in vitro*

A number of fungicides have previously been reported in the literature for the management of *G. uvicola* and *C. acutatum* on a range of crops. However, it is not known if these fungicides have any effect on these fungi isolated from grape tissues. Furthermore there are differences in fungicide registrations from country to country and also crop to crop. There are also several fungicides which have only recently been released on the Australian market. The objective in this part of the project was to screen *in vitro* some of these fungicide groups against bitter rot and ripe rot isolates from grapes in order to obtain an indication of the fungicides that might be effective under field conditions. Additionally one isolate each of *Alternaria alternata*, *B. cinerea* and an unknown species of *Aspergillus* from grape bunches were screened as well. Fungicides were selected based on overseas product registration and product availability within Australia. Table 13 illustrates the EC_{50} (µM) expressed in terms of an inhibition of radial growth on PDA.

Table 13 Sensitivity of a range of bunch rot fungi to fungicides. Results are expressed as EC_{50} (µM) values for an inhibition of radial growth on PDA using active ingredients.

Fungicide	***C. acutatum* isolates**					***G. uvicola* isolates**					***A. alternata***	***Aspergillus***	***B .cinerea***
	75574	**77282**	**77283**	**77284**	**77285**	**77258**	**77261**	**77263**	**77271**	**77275**			
Azoxystrobin	>10	>10	>10	>10	23.2	2.38	>10	>10	27.1	>10	>10	>10	22
Boscalid	>32	>32	>32	>32	>32	>32	>32	>32	>32	>32	ND	ND	40.9
Captan	0.16	3.68	0.78	0.37	0.48	<0.3	<0.3	<0.3	<0.3	<0.3	>10	>10	<0.3
Chlorothalonil	>32	>32	>32	30.2	>32	>100	ND	ND	ND	ND	>100	>10	3.2
Fludioxonil	0.02	0.02	0.02	0.02	0.02	0.03	0.05	0.03	0.04	0.02	0.06	0.14	0.02
Pyraclostrobin	0.11	0.2	0.09	0.02	0.03	0.25	0.11	0.5	0.29	1.79	ND	ND	0.68
Pyrimenthanil	35.7	>32	22.2	30.5	>32	>32	>32	>32	>32	>32	2.13	<0.32	ND
Shirlan	0.13	0.15	0.15	0.08	0.07	0.05	0.05	0.04	0.04	0.05	0.52	1.45	0.02
Triadimenol	>10	>10	>10	8	4.2	ND	ND	ND	ND	ND	ND	ND	ND
Tricop	0.8	0.49	0.8	1.3	0.47	0.47	0.31	0.49	0.46	0.31	4.58	4.89	0.47
Trifluroxystrobin	>32	>32	>32	>32	>32	5.03	15.78	8.04	7.01	10.39	161	ND	38.9

ND = Not Determined

8. Field trials to investigate fungicide efficacy

A number of field trials employing both chemical and non-chemical control methods were conducted during this project. These trials and their objectives are summarised below. While there are no products registered for the management of bitter rot and ripe rot of grapes in Australia, there are a number of fungicide groups that are registered for other diseases of vines that are also reported to control *C. acutatum* and *G. uvicola* on vines and other host plants in overseas countries. In particular the strobilurin group is reported to be effective in the management of late season bunch rots in the southern states of the US (Cline and Sorensen, 2005).

8.1 Timing of strobilurin sprays

The strobilurin fungicides represent a relatively new group of compounds for plant disease control. They are registered overseas for the management of *Colletotrichum* spp. on grapes and other host plants. On grapes they are used post-veraison in the US for ripe rot control. The objective of this trial was to determine the use of strobilurin sprays pre and post-veraison for ripe rot management of grapes. A further objective was to determine if strobilurin sprays applied at flowering could limit bunch rot incidence at berry maturity. These trials were conducted at site 1 in each of the three years of this project and at sites 2, 3 and 4 in 2005/6.

8.1.1 Evaluation of pre- and post-veraison strobilurin sprays

With the exception of the unsprayed treatment, all other treatments in 2003/4 at site 1 involved Amistar (active ingredient Azoxystrobin) (5% cap fall) Scala (active ingredient Pyrimethanil) (80% cap fall), Flint (active ingredient trifloroxystrobin) (berries pea size) and Dithane Rainshield (active ingredient mancozeb) (veraison). There were several aims of this trial site in 2003/4. The first was to compare Switch (active ingredients fludioxonil and cyprodinil) with Flint (trifluoroxystrobin) when the berries were pea size. A second aim was to investigate the use of Shiraln (active ingredient fluazinam) as a dormant spray, while the third aim was to investigate the application of Amistar post-veraison. This site was monitored twice

post-veraison, on 18th February and on 16th March 2004, dates corresponding to approximate brix values of 15 and 19 respectively. Symptoms of bitter rot were seen in the early ripening period, ripe rot became visibly evident as the fruit reached full maturity. At the second monitoring period, this vineyard was suffering from severe bunch rot problems, and although most bunches exhibited a number of bunch rotting symptoms, the predominant diseases were bitter rot and ripe rot. Undoubtedly there were a complex of fruit rots at this late monitoring stage, however, scoring them based on a visual assessment without subculturing to artificial media in the laboratory was not possible (Figure 26). For this reason scoring at this later time point was limited to ripe rot and bitter rot severity.



Figure 26 Late season bunch rot, predominantly caused by *G. uvicola*

The best fungicide regime for bunch rot reduction in this season at this site was Amistar applied as a post-veraison spray (Table 14). Although this isn't practical for Australian growers of wine grapes because of MRL restrictions for export wine, the results do indicate that Amistar had some effect on the severity of ripe rot. Post-veraison strobilurin sprays were investigated further at this site in subsequent years in order to confirm this finding and explore any differences in efficiencies between the different strobilurin fungicides.

Table 14 Evaluation of Shirlan as a dormant spray, Flint vs. Switch applied when berries were pea-size and Amistar as a post-veraison spray for bunch rot management at site 1 in 2003/4. Bunches were scored for disease severity. (a) Fruit monitored at 15° Brix (b) Fruit monitored at 19.2° Brix

(a)

Treatments						**Disease severity on 18th February 2004. Berries at 15 ºbrix**				
Dormant spray	5% cap fall	80% cap fall	Pea size	Veraison	Post-veraison	Bitter Rot	*B. cinerea*	Ripe Rot	unknown rots	Combined all rots
Nil	Amistar	Scala	Flint	Mancozeb	Nil	0.37	0	0	0	0.37
Shirlan	Amistar	Scala	Flint	Mancozeb	Nil	0.32	0	0.03	0.07	0.42
Nil	Nil	Nil	Nil	Nil	Nil	0.69	0	0.03	0.17	0.89
Nil	Amistar	Scala	Flint	Mancozeb	Nil	0.28	0.01	0.01	0.14	0.44
Nil	Amistar	Scala	Flint	Mancozeb	Amistar	0.13	0.02	0.01	0.14	0.30
Nil	Amistar	Scala	Switch	Mancozeb	Nil	0.33	0	0	0.21	0.54

(b)

Treatments						**Disease severity on 16th March 2004, berries at 19.2º Brix**	
Dormant spray	5% cap fall	80% cap fall	Pea size	Veraison	Post-veraison	Bitter Rot	Ripe
Nil	Amistar	Scala	Flint	Mancozeb	Nil	1.33	2.09
Shirlan	Amistar	Scala	Flint	Mancozeb	Nil	1.05	2.19
Nil	Nil	Nil	Nil	Nil	Nil	2.71	1.22
Nil	Amistar	Scala	Flint	Mancozeb	Nil	1.05	2.64
Nil	Amistar	Scala	Flint	Mancozeb	Amistar	0.98	0.84
Nil	Amistar	Scala	Switch	Mancozeb	Nil	2.12	1.29

The occurrence of all bunch rots was high at this vineyard in the second season of this project (2004/5) with a bitter rot incidence approaching 100% irrespective of the fungicides applied. All treatments reduced the severity of bitter rot compared to the unsprayed treatment, and the greatest reduction in disease severity was obtained where strobilurins were applied post-veraison. This level of reduction was not sufficient at this site to make the fruit commercially viable. Periodic monitoring at this site post-verasion revealed that bitter rot occurred in advance of ripe rot in the ripening period. This is consistent with observations made in the 2003/4 season. There was no significant difference between the severity of disease of the three strobilurin fungicides tested (Table 15).

Table 15 Evaluation of Strobilurin sprays applied pre- and post-veraison for bunch rot management at Site 1 in 2004/5. All treatments included shirlan as a dormant spray. With the exception of the unsprayed treatment, other standard sprays applied were Scala at 80% cap fall and mancozeb at veraison. Letters indicate significant differences ($P < 0.05$).

Treatments			Bunch rot monitoring			
5% cap fall spray	Pea size spray	Post veraison	Bitter rot Incidence	Bitter rot Severity	Ripe rot Incidence	Ripe rot Severity
Nil	Nil	Nil	98	3.23 a	68	1.53a
Amistar	Flint	Nil	93	2.19 b	72	1.37b
Cabrio	Amistar	Nil	94	2.01 b	49	0.81c
Flint	Cabrio	Nil	93	2.09 b	64	1.03b
Amistar	Filan	Cabrio	77	1.55 c	69	1.30a,b
Cabrio	Amistar	Flint	89	1.57 c	38	0.5d
Flint	Cabrio	Amistar	88	1.52 c	57	0.92c

Site 1 was investigated again in the 2005/6 growing season in an attempt to evaluate the effect of post-veraison sprays for bunch rot management further. This season was unusual in that *B. cinerea* was also recorded at comparatively high levels (Figure 27). The aims of the trial in this season were to further evaluate Shirlan and strobilurins for bitter rot management. In addition to the sprays listed, the site was sprayed with Amistar at 5% cap fall, Scala at 80% cap fall and Mancozeb at veraison. None of the treatments had a significant impact on ripe rot occurrence and while there were significant differences between treatments, these

did not correlate with the application of post-veraison sprays (Table 16). The results from the trial at this site in 2005/6 were inconclusive, and need to be interpreted with respect to the high disease pressure recorded in this season and the wet weather conditions experienced leading up to vintage.

Table 16 Use of Shirlan in combination with Stobilurin sprays pre- and post-veraison for bunch rot management at Site 1 in 2005/6. (a) Fruit monitored at 17.4° Brix (b) Fruit monitored at 20° Brix. Inc. = incidence, Sev. = severity, Letters indicate significant differences. ($P < 0.05$).

(a)

Treatments			Bunch rots					
Dormant spray	Pea size spray	Post veraison	Bitter rot		Ripe rot		Botrytis	
			Inc.	Sev.	Inc.	Sev.	Inc.	Sev.
Nil	Nil	Nil	36	0.98 a	10	0.14 a	0	0
Shirlan	Cabrio	Filan	45	0.57 b, c	2	0.13 a, b	0	0
Shirlan	Switch	Cabrio	32	0.83 a, b	4	0.02 c	0	0
Nil	Cabrio	Nil	34	0.59 b, c	4	0.04 c	0	0
Shirlan	Nil	Nil	25	0.81 a, b	3	0.05 b, c	0	0
Shirlan	Filan	Cabrio	33	0.47 c	10	0.04 c	0	0
Shirlan	Cabrio	Nil	33	0.58 b, c	10	0.13 a, b	0	0

(b)

Treatments			Bunch rots					
Dormant spray	Pea size spray	Post veraison	Bitter rot		Ripe rot		Botrytis	
			Inc.	Sev.	Inc.	Sev.	Inc.	Sev.
Nil	Nil	Nil	94	2.90 a	66	1.79 a	45	0.39c
Shirlan	Cabrio	Filan	100	2.64a b	70	1.82 a	56	0.96b
Shirlan	Switch	Cabrio	90	2.55 a b	66	1.75 a	25	0.55c
Nil	Cabrio	Nil	94	2.4 b, c	74	1.96 a	69	1.42a
Shirlan	Nil	Nil	91	2.3 b, c	68	1.82 a	26	0.42c
Shirlan	Filan	Cabrio	81	2.15 c	80	1.85 a	46	0.95b
Shirlan	Cabrio	Nil	91	2.06 c	59	1.56 a	64	1.29a



Figure 27 Cabernet Sauvignon from site 1 in 2005/6 showing symptoms of both *B. cinerea* and *C. acutatum* on the one bunch

8.1.2 Strobulurins applied at flowering and pre-veraison

Cabrio was investigated as a spray at flowering and pre-veraison to control bitter rot at site 2 in 2004/5 and in an attempt to reduce the inoculum load in the vineyard early in the season. A single spray of Cabrio at 5% cap fall led to a significant reduction in incidence and severity of bitter rot at berry maturity (Table 17). Inclusion of a second spray when the berries were pea size led to a further reduction in bitter rot although this was not significantly different from the level of disease reduction achieved with a single spray at flowering.

Table 17 Use of Cabrio for bunch rot management at site 2 in 2004/5. Letters indicate significant differences ($P < 0.05$)

Treatment	% Incidence	Severity
Control	39	0.50 a
Cabrio at 5% flowering	23	0.27 b
Cabrio at 5% flowering and at berries pea size	15	0.20 b

8.1.3 Inclusion of Filan into a spray program involving strobilurin sprays at flowering and pre-veraison

Investigations into the efficacy of Cabrio as an early season spray for bitter rot management were extended in 2005/6 with the inclusion of Filan (active ingredient boscalid) at sites 3 and 4. A combination of Cabrio / Filan has been reported to offer disease control against *C. acutatum* of peppers in the USA (Lewis-Ivey et al, 2004). Filan is related to the strobilurin chemical class but differs in the mode of action target site. Strobilurins such as Cabrio bind to the cytochrome b/c1 complex (complex III) of the electron transport chain in mitochondria, while Filan inhibits complex II. A combination of Cabrio and Filan is a potential anti-resistance strategy. In the following two trials the two products were applied singularly at different growth stages.

At site 3 in 2005/6, Cabrio applied when the berries were pea size reduced bitter rot severity. There was a further reduction when Filan was also used in the spray program at flowering, however, this reduction in disease severity was not significantly greater than when Cabrio was applied in the absence of a Filan spray (Table 18). Six possible Filan and Cabrio combinations were compared at site 4 in 2005/6. All treatments with the exception of a single Filan spray applied at 5% flowering led to a significant reduction in bunch rots at harvest (Table 19).

Table 18 Effect of cabrio and filan on the incidence and severity of bitter rot at vineyard site 3 in 2005/6. Letters indicate significant differences ($P < 0.05$).

Treatment number	Details	January 2006		February 2006	
		Incidence	Severity	Incidence	Severity
1	Normal spray program	56	0.95 a	84	1.74 a
2	Normal spray program plus Cabrio at pea size	35	0.52 b	75	1.35 b
3	Normal spray program plus Filan at 5% cap fall & Cabrio pea size	31	0.45 b	72	1.24 b

Table 19 Effect of cabrio and filan on the incidence and severity of bitter rot at vineyard site 4 in 2005/6. Letters indicate significant differences (P < 0.05).

Treatment *			Total bunch rot at harvest
Filan (10% cap fall)	Cabrio (80% cap fall)	Cabrio (pea size)	
-	-	-	0.34 a
+	-	-	0.28 a,b
-	-	+	0.27 b,c
-	+	-	0.258 b,c
+	+	-	0.24 b,c
+	-	+	0.215 c

*+ = spray applied at that phenological stage

- = spray omitted from the trial at that phenological stage

8.2 Use of dormant spray

The life cycles of *G. uvicola* and *C. acutatum* on grape vines are not known. However, it is likely that both fungi overwinter on dormant tissues. *G. uvicola* is a known pathogen of the vegetative tissues (Ridings and Clayton, 1970) so vineyard hygiene in the winter may be important in its control. The objective of this series of trials was to determine if Shirlan could be used as a dormant spray to reduce the incidence of bitter rot later in the season. Part of this data is presented previously in Tables 14 and 16 where Shirlan was evaluated in combination with strategically timed strobulurin sprays.

Shirlan as a dormant spray reduced bunch rot severity at site 1 in 2003/4 when applied in combination with the strobilurins (see Table 14 discussed above). Shirlan was further investigated at site 3 in the Hunter Valley in 2004/5 and found to reduce the incidence of bitter rot (Table 20). A further treatment during this season was an application of Tricop (active ingredient copper octanoate). Tricop is used in the US for the control of bitter rot. Tricop and Shirlan gave a similar level of bitter rot control. Applying both products was no better than applying either product in isolation. Tricop was not investigated further, as there is circumstantial evidence that copper sprays may encourage ripe rot. This hypothesis is based on

the fact that *Colletotrichum gloeosporioides* is copper tolerant (Stirling et al, 1995). Although this is yet to be proved for *C. acutatum* on grapes, it does raise potential concerns with the use of copper sprays in sub-tropical vineyards.

Table 20 Effect of Shirlan and Tricop on the incidence and severity of bitter rot at vineyard site 3 in 2004/5. Letters indicate significant difference (P < 0.05).

Treatment	% Incidence	Severity
Control	41	0.68 a
Shilan applied as a dormant spray	36	0.47 b
Tricop applied 30 days prior to harvest	34	0.49 b
Shilan applied as a dormant spray plus tricop applied 30 days prior to harvest	37	0.53 b

8.3 Use of Flint, Switch and Clay surrounds

Circumstantial evidence suggests that sunburnt fruit and fruit in an open canopy is more susceptible to ripe rot attack (Figure 28).



Figure 28 Sunburn on chardonnay grapes associated with bitter rot in the Hunter Valley 2006.

The purpose of these trials in 2003/4 was to investigate the use of an inert kaolin-clay particle-film product (Surround™ WP – Engelhard, NJ, USA) sprayed onto grape bunches and vines, as a method of preventing sunburn and possibly ripe rot attack. Also examined at this site was a comparison of Flint and Switch for the

management of bunch rots. The results are presented below in Tables 21 and 22. This site suffered extensive bird damage close to harvest which led to the loss of several replicates. The predominant bunch rot problem was sour rot. While the use of clay surrounds did appear to reduce the severity of all rots, the differences between treatments were not statistically significant. There was also little difference between using Switch or Flint when applied prior to bunch closure. As the site had a history of bird problems and other non-bunch rot disorders the site was not used in subsequent years.

Table 21 Comparison of Flint and Switch applied at pea size at site 7 for the management of mixed bunch rots in 2003/4. Predominant rots were due to sour rot. There were severe bird damage problems at this site.

Treatment	Severity of bunch rots at harvest				
	Bitter rot	Ripe rot	Grey Mould	Unknown rots	Combined all rots
Flint	0.05	0.14	0.06	0.75	0.99
Switch	0.04	0.15	0.02	0.73	0.93

Table 22 Use of clay surrounds applies post-veraison at Site 7 for the management of mixed bunch rots in 2003/4. Predominant rots were due to sour rot. There were severe bird damage problems at this site.

Treatment	Severity of bunch rots at harvest				
	Bitter rot	Ripe rot	Grey Mould	Unknown rots	Combined all rots
No surround	0.05	0.17	0.05	0.76	1.02
Plus surround	0.04	0.12	0.03	0.71	0.90

9 Field based workshops for bunch rot identification.

Three workshops were held on bunch rot identification as part of this project. Details of the workshops are presented in Table 23.

Table 23 - Workshops held on bunch rot identification

Venue	Date	Presenters	Number of participants
McLaren Vale, SA	7th Sept. 2005	A/Prof C C Steel	37
Toowoomba, QLD	10th Nov. 2005	A/Prof C C Steel Dr S Savocchia Mr B Cleaves Mrs L Greer	27
Hunter Valley, NSW	2nd May 2006	A/Prof C C Steel Dr M A Weckert Dr K Evans (TIAR) Ms R Huang Mr A Somers	30

The workshops in Toowoomba and Kurri Kurri involved a series of lecture type presentations followed by workshop exhibits. These included grape berries and other vine tissues inoculated with representative bunch rot isolates. Participants were able to view these live specimens with the aid of microscopes (Figure 29).



Figure 29 Participants at the Kurri Kurri workshop examine bunch rot exhibits

Live specimens were supplemented with large colour photographs to illustrate the different stages of disease development. Other demonstrations included the

biocontrol potential and growth characteristics of different berry microflora organisms. The workshop at Toowoomba also included exhibits of trunk disease pathogens, given that these organisms also occur as berry pathogens. Research experts were stationed adjacent to exhibits which facilitated discussion with growers and other participants. Dr Kathy Evans (Tasmanian Institute of Agricultural Research / University of Tasmania), a guest speaker, presented an update on Botrytis research at the Kurri Kurri workshop. Participants at the Toowoomba and Kurri Kurri workshops were surveyed using a questionnaire to gauge the value of the workshop and to what extent bunch rots were regarded as a problem in the district. A summary of the findings from the questionnaire are presented below (Table 24). Results from the two workshops were similar, and the participants indicated that they found the day useful. Findings from this simple questionnaire will be used to improve future workshop presentations.

Table 24 Workshop survey results. Participants at the Toowoomba and Kurri Kurri workshops were asked to respond to a series of questions using a 7 point scale where a score of 7 equated to very strongly agree and a score of 1 equated to very strongly disagree. Results are the means of the responses.

Question	**Mean score**	
	Toowoomba	**Kurri Kurri**
My attendance at the workshop was valuable	**6.0**	**6.5**
As a result of my attendance at this workshop my knowledge of bunch rot disease of grapes has improved	**6.1**	**6.6**
I would recommend colleagues working in the wine industry to attend a similar workshop if run in the future	**6.2**	**6.1**
The duration of the workshop, ie half a day was appropriate	**5.7**	**5.8**
The exhibits at the end of the seminar presentations were useful	**5.9**	**5.6**
Bunch rot diseases are not a problem and are easily managed in my grape growing region	**2.5**	**2.5**
Ripe and/or bitter rot frequently occur in my vineyard	**4.0**	**4.1**
Botrytis (grey mould) frequently occurs in my vineyard	**4.0**	**4.1**
Trunk disease and other disease of the wood are a problem in my vineyard	**3.5**	**5.1**
Disease management in general, is largely successful in my vineyard	**4.7**	**4.9**

Analysis of outcomes and discussion

Vineyards in northern NSW were surveyed for non-Botrytis bunch rot and two regions, the Hunter Valley and Hastings Valley were selected for a more detailed investigation into bunch occurrence. *B. cinerea* was present in four sites at three vineyards in the Hunter Valley during the 2003 vintage. Several vineyards had bunch rot symptoms that could not be attributed to *B. cinerea*. Four of the vineyards exhibited symptoms that were due to ripe rot (*Colletotrichum* spp.), but the nature of the other organisms involved was not clear. A culture collection was initiated at this point in the project, and *Colletotrichum* isolates were collected from various viticulture regions. Based on culture morphology and appearance on PDA all of these isolates were believed to be *C. acutatum*. However, two species of *Colletotrichum* are associated with ripe rot of grapes, *C. acutatum* and *C. gloeosporioides*. *C. gloeosporioides* has been reported on grapes in the Philippines (Quimio and Quimio 1975) and Taiwan (Wu and Chang 1993) while *C. acutatum* has been reported from Japan (Yamamoto et al. 1999). Both species are reported to occur in Southern states of the USA (Daykin and Milholland 1984, Kummuang et al. 1996). Isolates of *Colletotrichum* spp. from non-grape hosts are reported to produce similar disease symptoms and vary in their culture morphology (Adaskaveg and Forster 2000). In research associated with this project, and part funded through the GWRDC honours scholarship scheme a range of morphological and molecular methods (RAPD-PCR and sequencing of parts of the 5.8S-ITS regions and the β- tubulin 2 gene) were used to confirm that all ripe rot isolates examined to date from Australia are *C. acutatum* rather than *C. gloeosporioides* (Whitelaw-Weckert et al. 2007). This work further revealed that *C. acutatum* isolates from vineyards in mid to north NSW and in QLD belonged to a new phenologically distinct group. It may of course be that *C. gloeosporiodes* is present in Australian vineyards, but to date this species is yet to be isolated from grape berries in the Hunter Valley and Hastings Valley. Our limited observations on host specifity using three *C. gloeosporiodes* isolates from non-grape hosts, indicate that this species, should it naturally occur in Australian vineyards, is less pathogenic than *C. acutatum.*

Based on the survey study conducted in 2003 a vineyard in the Hastings Valley was selected for a more detailed investigation into the organisms responsible for bunch rot diseases during the 2004/5 and 2005/6 growing seasons. A complex of bunch rotting fungi were isolated; two of these fungi were *C. acutatum* and *G. uvicola. C. acutatum* has been previously reported as occurring on wine grapes in Australia (Melksham et al, 2002) however to our knowledge *G. uvicola* has not been recorded previously as a pathogen of wine grape berries in Australia. The biology and behaviour of these fungi on *V. vinifera* is not understood, and both fungi are reported as occurring in countries other than Australia and simultaneously in the one vineyard (Kummuang et al, 1996). The predominant pathogen is likely to be influenced by environmental factors such as seasonal variations in climate. In the Hastings Valley in 2004/5 and 2005/6, *C. acutatum* predominated on berry samples. *G. uvicola* was isolated with greater frequency in 2005/6 than in 2004/5, although *C. acutatum* predominated from all tissues examined in both seasons. Differences in the fungal profile between seasons may be due to climatic differences.

Although bitter rot and ripe rot symptoms were not visibly evident on either inflorescences or pre-veraison berries, both *C. acutatum* and *G. uvicola* were isolated from asymptomatic tissue, indicative of latent infection. Pre-veraison berries of all the wine grape cultivars examined resisted artificial inoculation with either *C. acutatum* or *G. uvicola* suggesting that latent infections become established at flowering. This is consistent with observations made on the pathogenesis of *B. cinerea* on grapes (Elmer and Michailides, 2004). Our inability to infect pre-veraison berries with *G. uvicola* and the absence of disease symptoms on field-grown vines at this phenological stage, despite the presence of latent infections, contrasts with earlier published work on non-vinifera vines in the USA (Kummuang et al, 1996, Ridings and Clayton, 1970).

A similar rage of organisms was also isolated from leaf tissue in both seasons. The frequency of *G. uvicola* isolation from vine leaves was relatively low in both seasons investigated despite reports that this organism is frequently isolated from leaves of bunch rot affected vines (Reddy and Reddy,1983). The predominant fungi isolated from leaf tissues were *Alternaria* spp. and *C. acutatum*. A greater

range of fungi were isolated from both reproductive and vegetative tissues in the 2005/6 season, corresponding to a higher disease pressure in that year. These organisms included *Cladosporium* spp. and *Phomopsis viticola*.

B. cinerea which has an optimum temperature for berry infection of 20.8 ºC (Nair and Allen, 1993) and is more commonly associated with cool climate viticulture, was rarely isolated from any of the tissue samples analysed from the vineyard in 2004/5 and 2005/6. This suggests that the programs of fungicide application were effective in managing grey mould. Alternatively the relatively warm climatic conditions of the Hastings Valley during berry ripening (mean minimum temperature of 17.9 ºC, see Table 9) may not be conducive to infection by *B. cinerea*. *Alternaria* spp. was frequently isolated and there was a high incidence of *Cladosporium* spp. in 2005/6. Both of these fungi are associated with vine tissues as endophytes, secondary invaders or quiescent infections (Dugan et al, 2002, Mostert et al. 2000). While these fungi can infect wounded grape berries they are commonly recognised as opportunistic pathogens that are weakly pathogenic to *V. vinifera*. *Botryosphaeria* spp. and *P. viticola* were isolated from the reproductive structures with low frequency throughout the growing season. Although rarely associated with bunch rot of grapes these two organisms were also contributing to the bunch rot complex, and it is likely that these fungi were infecting berries as secondary pathogens (Lal and Arya, 1982). The isolation of *P. viticola* from bunch rot affected fruit is the first report of this pathogen on wine grape berries in Australia (Savocchia et al, 2007). Other organisms isolated from the vineyard investigated, included *Epicoccum* spp., *Fusarium* spp., *Nigrospora* spp., *Pestalotia* spp. and *Trichoderma* spp. These organisms are saprophytes that are frequently recovered from plant surfaces, (Beasley et al, 2001) some of them have also been implicated as potential biocontrol agents against *B. cinerea* (Szandala and Backhouse, 2001).

Our observation that an isolate of *Epicoccum* could also inhibit *C. acutatum* is indicative that these organisms also play a role in determining berry competition and disease expression (Cleaves et al, 2004). Both *Epicoccum* and *Trichoderma* are known antagonists of fungal growth and both organisms are frequently isolated from the surface of plant tissues. Use of these organisms in disease management

in the field as part of a commercially released product, however would require further work involving a feasibility study.

Inoculation of berries in the laboratory with either *C. acutatum* or *G. uvicola* led to identical symptoms observed in the field for ripe rot and bitter rot respectively. Re-isolation of the fungi from inoculated berries confirmed Koch's postulates. All varieties of wine grape berries examined in this study were susceptible to bitter rot and ripe rot infection at post-veraison without any wounding of the berry surface. There are limited reports on the varietal susceptibility of table grapes grown in India to bitter rot (Reddy and Reddy 1983) however, as far as we are aware there are no reports on the varietal susceptibility of wine grapes to either bitter rot or ripe rot. Our observations that *G. uvicola* was in particular an aggressive pathogen of vine leaves indicates that foliar infections may be a source of inoculum for subsequent bunch rot.

No literature is available on the dynamics of bitter rot and ripe rot on *V. vinifera* tissues throughout the growing season. Kummuang et al. (1996) found a higher frequency of bitter rot occurrence on immature berries of *V. rotundifolia* (berry size 1 – 3 mm) than on berries later in the season, but noted a high degree of berry drop later in the season. At the sites and in the seasons when Kummuang et al. (1996) conducted their work, ripe rot was of minor significance however they noted that there were seasonal and site differences in the relative occurrences of bitter rot and ripe rot on Muscadine vines. These authors also indicate that *G. uvicola* was frequently associated with russet-like symptoms on pre-veraison berries, possibly associated with some secondary organism(s). Russet-like symptoms were not observed on *V. vinifera* vines in this study or on berries that were inoculated under laboratory conditions. Other pathogens found in association with *G. uvicola* on Muscadine vines in Mississippi were black rot caused by *Guignardia bidwellii* (not reported as present in Australia) and Macrophoma rot (*Botryosphaeria dothidea*) (Kummuang et al. 1996).

There was little difference in the rate of berry infection over the 25 to 35 ºC range for both *C. acutatum* and *G. uvicola*, however at 20 ºC the rate of infection by *G. uvicola* was markedly reduced. The optimum temperature for post-veraison berry

colonisation for *C. acutatum* and *G. uvicola* was 25 and 35 ºC respectively, significantly higher than that reported for *B. cinerea* (Nair and Allen, 1993). While there is published data in the literature on growth rates on artificial media for *C. acutatum* isolated from non-grapevine hosts (Adaskaveg and Hartin, 1997, Freeman et al. 1998), to our knowledge nothing has been published on the optimum temperature for colonisation of either *C. acutatum* or *G. uvicola* on *V. vinifera* berries. Incubation under high humidity conditions facilitated berry colonisation by both pathogens. Pathogen colonisation under lower humidity conditions was possible if the berries were incubated for a 24 hr period at 100% relative humidity immediately post-inoculation. This indicates that high humidity conditions are not required for subsequent infection once *C. acutaum* and *G. uvicola* have invaded the grape tissue.

Cross infection of bunch organisms from companion crops has yet to be demonstrated. While this project has demonstrated the lack of host specificity of *C. acutatum* isolates, the movement of isolates in and out of vineyards is not known. *G. uvicola* is also a pathogen of other fruit crops, but to date no work has been done on the host specificity of this organism. Further studies involving spore trapping and other techniques are warranted in an attempt to elucidate the epidemiology of *C. acutatum* and *G. uvicola*.

Bunch rot of grapes can be due to a complex of organisms. Which disease is expressed depends upon the environmental conditions. Under high temperature conditions (27 ºC in this study) *C. acutatum* and *G. uvicola* can compete with *B. cinerea* on the berry surface and can occupy the same ecological niche. At lower temperatures (e.g. 20 ºC) *B. cinerea* out competes *C. acutatum* and *G. uvicola*. Both *C. acutatum* and *G. uvicola* grow readily on PDA and infect Cabernet Sauvignon berries at >30 ºC. These observations may explain the higher incidence of these organisms in open canopies that may be prone to heat stress and sun burn effects. An original hypothesis developed at the commencement of this project that UV tolerance could explain difference in bunch rot occurrence in canopies appears to be incorrect. Under the experimental conditions reported here, there was no difference in berry infection under different light conditions.

Management of *B. cinerea* is largely effective through existing fungicide programs and canopy management. Products registered for use in Australian vineyards include the dicarboximides, anilinopyrimidines and strobilurin fungicides. Fungicides are targeted at flowering and other crucial times of Botrytis susceptibility. These well established disease management strategies, may partially explains why control of Botrytis grey mould is largely successful and the almost absence of this pathogen from vineyards in the Hunter and Hastings Valleys. A further explanation may be the different temperature optima for infection. *B. cinerea* generally occurs in vineyards at lower temperatures, and although the pathogen is able to infect at a range of temperatures, the optimum for berry infection has been reported as 20.3ºC (Nair and Allen, 1993).

As *G. uvicola* is likely to over-winter on dormant canes and debris from the previous season's harvest (Kummuang et al., 1996), we investigated the potential of Shirlan applied prior to bud burst to limit inoculum levels in the growing season. Our results indicated that this strategy did lead to a reduction in the level of *G. uvicola*; however, there was little impact on *C. acutatum*. While *C. acutatum* can be isolated from woody tissues of the vine, it is more likely that mummified berries from the previous season are a more important inoculum source. Similarly use of Tricop also reduced the incidence of bitter rot. However, because *Colletotrichum* spp. may potentially be copper tolerant, Tricop and other copper based sprays should be used with caution.

A number of strobilurin fungicides were also investigated since they appear to have a wide spectrum of activity against a range of fungal pathogens and is recommended for the control of both bitter rot and ripe rot on Muscadine grapes in North Carolina (Cline and Sorensen, 2005). Strobilurin fungicides can be divided into two separate groupings depending on the step in the electron transport chain that they bind to. The first of these two groupings include Amistar and Cabrio which act by binding to complex III of the mitochondrial electron transport chain. Filan on the other hand binds to a different site in the electron transport chain, Complex II (succinate-ubiquinone reductase). Filan alternated with either Amistar or Cabrio could therefore be considered as an acceptable fungicide resistance strategy. For this reason we investigated Filan in combination with strobilurins that

inhibit complex III. Furthermore, Cabrio and Filan have been reported as effective in the management of *C. acutatum* on bell peppers (Lewis Ivey, 2004) while azoxystrobin (i.e. Amistar) has been reported to control ripe rot of grapes in Japan (Fukaya and Takahashi, 1999).

Application of Cabrio at flowering reduced bitter rot at vineyard 2, highlighting the importance of flowering sprays. Data from site 1 indicated that prior to full maturity of the fruit, bitter rot was the most prevalent bunch disease but once the fruit had matured, ripe rot predominated.

Both bitter rot and ripe rot problems were encountered in all treatments at vineyard 1, and at levels generally regarded as unacceptable for harvest (a severity index of > 1.0 is sufficient to cause a noticeable taint in wine – unpublished observations). Application of all strobilurins post-veraison led to a slight reduction in bitter rot in the fruit prior to full maturity, although under high disease pressure as existed in the Hastings Valley at site 1 in 2005/6, strobilurin sprays had minimal impact when the occurrence of disease was assessed at vintage. All strobilurin fungicides used in this study can be applied up to four weeks before harvest, however they must not be used on wine grapes destined for export either beyond flowering (Amistar and Filan) or shortly after berry set (Cabrio) (Bell and Daniel, 2006).

Results from this project indicate that strobilurin sprays, and in particular Cabrio, applied at flowering and up to veraison do offer some level of bitter rot control. Shirlan applied as a dormant spray offered some bitter rot control, although growers are reluctant to use this product because of the cost and restrictions on re-entry to the vineyard post spraying. These limitations need to be evaluated against the possible loss of fruit to bitter rot in the vineyard under question. The advantage of using shirlan is that the product is also effective against *P. viticola* and other wood infecting fungi. This is of particular note since we have demonstrated that *P. viticola* and *Botryosphaeria* are both associated with bunch rots under sub-tropical conditions.

The extension activities of this project included running three interstate workshops on bunch rot biology and management for grape growers. Feed back from

participants indicated that the workshops were of value, it is anticipated that future workshops will be run as part of the GWRDC - NWGIC Winegrowing Futures extension program. The workshops were also an opportunity to gather information on grower perceptions regarding bunch rot problems in their vineyards.

Conclusions and Recommendations for Growers

The successful management of bitter rot and ripe rot under Australian conditions needs further investigation. Based on our observations of latent infection, any chemical management strategy that might be developed in the future will need to take into account sprays targeted at both the flower and berry stages of development. While fungicides such as those belonging to the strobilurin chemical class are reportedly effective in controlling ripe rot on table grapes (Yamamoto et al. 1999) they need to be applied post-veraison. For Australian wine grapes destined for the export market, this is currently not possible because of nil MRLs for these chemicals set by destination export countries (Bell and Daniel, 2006). This limits the use of chemical control options for bunch rots in high disease pressure areas. Later maturing varieties such as Cabernet Sauvignon, which was investigated at site 1 in the Hastings Valley should be avoided since the bulk of the summer rainfall in the Hastings Valley falls in February and March. Through the communication and extension component of this project, growers have become more aware of this fact and many growers in the Hunter Valley have now removed Cabernet Sauvignon vines and planted earlier maturing varieties. The use of biological control agents has not been investigated to any great extent in this project, and may form an alternative to chemical control options which requires further investigation

Results from the project bring into question the use of canopy management for bunch rot control. An open canopy is a well established management technique for *B. cinerea* and is also recommended for other bunch rots such as *Aspergillus* (Emmett, 2003, pers. comm.) Under tropical conditions and situations where heat stress may occur, growers will need to balance canopy management with bunch rot control. A broad suggestion for regions where ripe rot is a serious problem is to partially keep the fruiting zone screened on westerly facing rows. This suggestion must be evaluated with respect to the overall vigour of the vine and previous history of bunch rot problems.

Communication and extension activities

Peer-reviewed papers

(Copies of the publications that have arisen from this work are available from the principal investigator, csteel@csu.edu.au)

Steel, C C and Greer, D H 2006
Effect of climate on vine and bunch characteristics: Bunch rot disease susceptibility. Acta Horticulturae – Submitted.

Whitelaw-Weckert, M A; Curtin, S; Huang, R; Steel, C C; Blanchard, C L and Roffey, P. 2007. Phylogenetic relationships and pathogenicity of grape *Colletotrichum acutatum* isolates in sub-tropical Australia. Plant Pathology –In Press. (doi: 10.1111/j.1365-3059.2007.01569.x)

Savocchia, S, Greer, L A and Steel, C C 2007
First report of *Phomopsis viticola* causing bunch rot of grapes in Australia
Plant Pathology – In Press (Doi: 10,111/j.1365-3059.2007.01586.x)

Steel, C C, Greer, L A and Savocchia, S 2007
Bunch rot of grapes in sub-tropical Australia is caused by a complex of fruit rotting fungi, principally *Colletotrichum acutatum* and *Greeneria uvicola*
Australian Journal of Grape and Wine Research **13**, 23-29

Industry Articles

Greer, D, Greer, L, Steel, C C and Rogiers, S 2003.
Sunburn in grapes contributes to disease infection. Australian and New Zealand Grapegrower and Winemaker – April 2003.

Steel, C C, Somers, T, Greer, L, Hatfield, J, Hayward, C. and Radovanovich-Tesic M 2003. Aspects of IPM for the control of Ripe rot and Bitter rot diseases of grapes. Australian and New Zealand Grapegrower and Winemaker September Issue 2003, No 476, 33 - 36.

Cleaves, B W, Whitelaw-Weckert, M A, Steel, C C and Greer, L A 2004
Colletotrichum acutatum in the South Burnett Region, Queensland: Interaction with Grapevine Microflora. Australian and New Zealand Grapegrower and Winemaker September Issue 2004, pp 62 – 66.

Steel, C C, Greer, L A and Savocchia, S 2005
Biology and control of late season bunch rots of grapes in sub-tropical vineyards of Australia.
Abstract 2B-2, Proceedings of the 2nd Asian Conference on Plant Pathology, Page 27, 25 – 28th June 2005, Singapore

Steel, C C 2005. Ripe rot fungal pathogen of grapes is also a pathogen of non-grape host plants. Australian and New Zealand Grapegrower and Winemaker October 2005, Issue 501, pp 13 – 16.

Industry and conference presentations

C C Steel
Bunch rot update. A progress report for 2003/4.
Hunter Valley Vineyard Association Autumn Seminar, Kurri Kurri TAFE, May 2004.

C C Steel
Biology and management of non-Botrytis bunch rots.
Canberra and Southern NSW grape growers Association Seminar, Murrumbateman, November 2004.

C.C. Steel, L. Greer, S. Savocchia, A.J. Somers, M. A. Weckert-Whitelaw, and K.J. Melksham 2005
Management of non-Botrytis bunch rots in sub-tropical vineyards
Proceedings of the 12th Australian Wine Industry Technical Conference, pp 282-3, Melbourne, July 2004.

B.W. Cleaves, M.A. Whitelaw-Weckart, C.C. Steel and L.A. Greer 2005
Colletotrichum acutatum: its detection and responses to light and climate in the South Burnett Geographical Region, Queensland
Proceedings of the 12th Australian Wine Industry Technical Conference, pp 273-4, Melbourne, July 2004.

A Somers (for C C Steel)
Recent research on non-Botrytis bunch rot fungi
Hunter Valley Vineyard Association Autumn Seminar and Mudgee Winegrape Growers June 2005

C C Steel, L A Greer and S Savocchia 2005
Biology and control of late season bunch rots of grapes in sub-tropical vineyards of Australia.
Abstract 2B-2, Proceedings of the 2nd Asian Conference on Plant Pathology, Page 27, 25 – 28th June 2005, Singapore

C C Steel and D H Greer 2006
Effect of climate on vine and bunch characteristics: bunch rot disease susceptibility.
Abstract SS4 IL 2, International Symposium on grape production and processing, 6 – 11 February 2006, Baramati, India. Page 35.

Workshops

C C Steel
Current best management practices for the control of bitter rot and ripe rot of grapes Hunter Valley Vineyard Association Autumn Seminar, Kurri Kurri TAFE, 3rd May 2006.

C C Steel 2005
Advances in the management and biology of ripe rot and bitter rot of grapes University of Southern Queensland, Toowoomba, 10th November 2005 and McLaren Vale, SA, September 2005.

Theses produced

B Cleaves 2003. *Colletotrichum acutatum* in the South Burnett region, Queensland: Effect of light exposure and interaction with grapevine microflora. BAppSc Viticulture Honours Thesis, Charles Sturt University, 2003

S Curtin 2004. Molecular variation and host specificity of the fungal pathogen *Colletotrichum acutatum*.
BAppSc Viticulture Honours Thesis, Charles Sturt University, 2004

Staff involved with the project

Name	Role	Affiliation	% Involvement
Chris Steel	Project Leader & Principal Investigator	NWGIC /CSU	40%
Lindsay Greer	Technical Officer	NWGIC/CSU	100%
Sandra Savocchia	Researcher	NWGIC/CSU	10%
Melanie Weckert	Researcher & Honours student supervisor	NWGIC/NSW DPI	5%
Tony Somers	Extension Officer	NWGIC/NSW DPI	10%
Shaun Curtin	GWRDC Funded honours student	NWGIC/ CSU	5%
Bruce Cleaves	Honours Student	NWGIC/CSU	5%

Statement of Receipts

GRAPE & WINE RESEARCH & DEVELOPMENT CORPORATION

Statement of Receipts and Expenditure - Form B
1 July 2003 to 31 December 2006 - Final

Trust Fund:	**RESEARCH TRUST FUND**	FUNDING	$
CSU Ledger Code:	**R779-482**		
Project No:	**CSU 03/01**	Salaries	**136,187.00**
Grantee:	**CHARLES STURT UNIVERSITY**	Travel	**12,000.00**
Project Coordinator:	**Chris Steel**	Operating	**11,000.00**
Title of Project:	**Management of non-Botrytis bunch rots**	Capital	**4,441.00**
		Total Funding	**163,628.00**

	EXPENDITURE				
	Salaries	**Travel**	**Operating**	**Capital**	**Total**
A Uncommitted (c/f 1st July)					
B Outstanding Commitments (c/f 1st July)					
C Refunds of funding					
D Cash received from the Trust Fund	**130,380.00**	**12,000.00**	**8,511.70**	**4,441.00**	**155,332.70**
E Approved Transfers (from Form C)					
F Cash available (A+B-C+D+E)	**130,380.00**	**12,000.00**	**8,511.70**	**4,441.00**	**155,332.70**
G Expenditure	**139,124.08**	**26,414.00**	**25,955.34**	**0.00**	**191,493.42**
H Outstanding Commitments (30 June)					
I Total funds Committed (G+H)	**139,124.08**	**26,414.00**	**25,955.34**	**0.00**	**191,493.42**
J Uncommitted (30 June) (F-I)	**(8,744.08)**	**(14,414.00)**	**(17,443.64)**	**4,441.00**	**(36,160.72)**
K Other Income (Paid to Trust Funds)		**1,336.09**	**27,272.70**		**28,608.79**

Note: *Row B should be the same as Row H from the previous year and Row A the same as Row J from the previous year.*

Certification
I hereby certify that this statement of expenditure is correct.

Signature	Adrian Lindner, Director Division of Financial Services Printed Name	26/2/07 Date

References

Adaskaveg, J. E. and Foster, H. (2000) Occurrence and management of anthracnose epidemics caused by *Colletotrichum* species on tree fruit crops in California. In: '*Colletotrichum*. Host specificity, pathology and host-pathogen interaction'. Eds D. Prusky, S. Freeman and M. B. Dickman (APS Press: Minnesota) pp 317-336.

Adaskaveg, J.E. and Hartin, R.J. (1997) Characterization of *Colletotrichum acutatum* isolates causing anthracnose of almond and peach in California. Phytopathology **87**, 979-987.

Barnett, H.L. and Hunter, B.B. (1998) Illustrated genera of imperfect fungi. (APS Press, St. Paul, Minnesota, USA).

Beasley, D.R., Joyce, D.C., Coates L.M. and Wearing, A.H. (2001) Saprophytic microorganisms with potential for biological control of *Botrytis cinerea* on Geraldton waxflower flowers. Australian Journal of Experimental Agriculture **41**, 697-703.

Bell, S-J. and Daniel, D. (2006) Agrochemicals registered for use in Australian viticulture 2006/2007. http://www.awri.com.au/agrochemicals/agrochemical_booklet/booklet.pdf

Bureau of Meteorology (2006) www.bom.gov.au/climate/

Castillo-Pando, M., Somers, A., Green, C.D., Priest, M. and Sriskanthades, M. (2001) Fungi associated with dieback of Semillon grapevines in the Hunter Valley of New South Wales. Australasian Plant Pathology **30,** 59-63.

Castillo-Pando M, Steel C C and Somers T. (1999). Fungal bunch rots of grapes in the Hunter and Hastings Valleys. Australian Grapegrower and Winemaker No 429 September 1999 pp33-38.

Cleaves, B. W., Whitelaw-Weckert, M. A., Steel, C. C. and Greer, L. A. (2004) *Colletotrichum acutatum* in the South Burnett Region, Queensland: Interaction with Grapevine Microflora. Australian and New Zealand Grapegrower and Winemaker September Issue 2004, pp 62–66.

Cline, W. O. and Sorensen, K. A. (2005) Muscadine grape spray program.
In; 2005 North Carolina Agricultural Chemical Manual, Chapter 7 Insect and disease control of fruits, http://ipm.ncsu.edu/agchem/chptr7/707.pdf, Publ: North Carolina State University, Raleigh, North Carolina 27695-7603, USA.

Cole, M. (2004) Ensuring optimal grape quality through management strategies for *Botrytis cinerea*. MOU 01/02 Report to Grape and wine Research Development Corporation, 18 November 2004.

Coombe, B.G. (1995) Adoption of a system for identifying grapevine growth stages. Australian Journal of Grape and Wine Research **1**, 100-110.

Daykin, M. E. and Milholland, R. D. (1984) Ripe rot of Muscadine grape caused by *Colletotrichum gloeosporioides* and its control. Phytopathology **74**, 710-714.

Dugan, F.M., Lupien, S.L. and Grove, G.G. (2002) Incidence, aggressiveness and *in planta* interactions of *Botrytis cinerea* and other filamentous fungi quiescent in grape berries and dormant buds in Central Washington State. Journal of Phytopathology **150**, 375-381.

Dyko, B.J. and Mordue, J.E.M. (1979) *Colletotrichum acutatum*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, No. 630.

Elad, Y., Williamson, B., Tudzynski, P. and Delan, N. (2004) *Botrytis*: Biology, pathology and control. Publ: Kluwer Academic Publishers, Dordrecht 2004.

Elmer, P.A.G. and Michailides, T.J. (2004) Epidemiology of *Botrytis cinerea* in orchard and vine crops. In: *Botrytis*: Biology, pathology and control. Eds Y. Elad, B. Williamson, P. Tudzynski and N. Delen (Kluwer Academic Publishers, Dordrecht) pp 243-272.

Emmett, R.W. (2003). Standardized protocols for conducting grapevine improvement trials in Australia. In: *Final report to the Grape and Wine Research Development Corporation for Project No. SAR 99-6.* Ed. P.R. Nicholas. Pp. 20-26

English, J.T., Kaps, M.L., Moore, J.F., Hill, J. and Nakova, M. (1993) Leaf removal for control of Botrytis bunch rot of wine grapes in the Midwestern United States Plant Disease **77**, 1224-1227.

Evans, K.J. (1996). Characterisation of *Uncinula necator*, the grapevine powdery mildew fungus. *PhD Thesis, The University of Adelaide*, South Australia

Farr, D.F., Castlebury, L.A., Rossman, A.Y. and Erincik, O. (2001) *Greeneria uvicola*, cause of bitter rot of grapes, belongs in the Diaporthales. Sydowia **53**, 185-199.

Fisher, N.L., Burgess, L.W., Tousson, T.A. and Nelson, P.E. (1982). Carnation leaves as a substrate and for preserving cultures of Fusarium species. Phytopathology 72, 151

Freeman, S. (2000) Genetic diversity and host specificity of *Colletotrichum* species on various fruits. In: *Colletotrichum* host specificity, pathology and host-pathogen interaction. Eds D. Prusky, S. Freeman and M. B. Dickman (APS Press, Minnesota) pp. 131-144.

Freeman, S. Katan, T. and Shabi, E. (1998) Characterization of Colletotrichum species responsible for anthracnose diseases of various fruits. Phytopathology **82**, 596-605.

Fukaya, M., and Takahashi, I., (1999). Effect and optimum application time of strobilurins for the control of grape ripe rot. Ann. Rept. Plant Prot. North Japan **50**, 100–103.

Gubler, W. D., Marois, J. J., Bledsoe, A. M. and Bettiga, L. J. (1987). Control of Botrytis bunch rot of grapes with canopy management.
Plant Disease **7**, 599 – 601.

Hocking, A.D., Varelis, P., Pitt, J.I., Cameron, S.F. and Leong, S.L. (2003) Occurrence of ochratoxin A in Australian Wine. Australian Journal of Grape and Wine Research, **9**, 72-78.

Kummuang, N., Diehl, S.V., Smith, B.J. and Graves, C.H., Jr. (1996) Muscadine grape berry rot diseases in Mississippi: Disease epidemiology and crop reduction. Plant Disease **80,** 244-247.

Lal, B. and Arya, A. (1982) A soft rot of grapes caused by *Phomopsis viticola*. Indian Phytopathology **35**, 261-264.

Lewis-Ivey, M.L., Nava-Diaz, C., and Miller, S.A., (2004). Identification and management of *Colletotrichum acutatum* on immature bell peppers. Plant Dis. 88, 1198-1204.

Melksham, K.J., Weckert, M.A. and Steel, C.C. (2002) An unusual bunch rot of grapes in subtropical regions of Australia caused by *Colletotrichum acutatum.* Australasian Plant Pathology **31,** 193-194.

Merrin, S.J., Nair, N.G. and Tarran, J. (1995) Variation in *Phomopsis* recorded on grapevine in Australia and its taxonomic and biological implications. Australasian Plant Pathology **24**, 44-56.

Mostert, L., Crous, P.W. and Petrini, O. (2000) Endophytic fungi associated with shoots and leaves of *Vitis vinifera*, with specific reference to the *Phomopsis viticola* complex. Sydowia **52**, 46-58.

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

Phillips, A.J.L. (1998) *Botryosphaeria dothidea* and other fungi associated with Excoriose and dieback of grapevines in Portugal. Journal of Phytopathology **146**, 327-332

Prusky, D., Freeman, S. and Dickman, M. B. (2000) '*Colletotrichum*. Host specificity, pathology and host-pathogen interaction'. (APS Press: Minnesota).

Quimo, T. H. and Quimo, A. J. (1975) Notes of Philippine grape and guava anthracnose. Plant Disease Reporter **59**, 221 - 224.

Reddy, M.S. and Reddy, K.R.C. (1983) Greeneria fruit rot – an endemic disease of grape in India. Indian Phytopathology **36**, 110-114.

Ridings, W.H. and Clayton, C.N. (1970) *Melanconium fuligineum* and the bitter rot disease of grape. Phytopathology **60**, 1203-1211.

Savocchia, S, Greer, L A and Steel, C C (2007). First report of *Phomopsis viticola* causing bunch rot of grapes in Australia. Plant Pathology – In Press (Doi: 10,111/j.1365-3059.2007.01586.x)

Schultz, H.R. (2000) Climate change and viticulture: A European perspective on climatology, carbon dioxide and UV-B effects. Australian Journal of Grape and Wine Research **6**, 2-12.

Somers, A., Castillo-Pando, M., Steel, C.C. and Tesoriero, L. (1998)
Grape berry rot diseases in NSW : Disease identification and incidence.
Proc. 10th Australian Wine industry Technical Conference, Sydney 1998. Publ. WineTitles, Adelaide. p285.

Stirling, A.M., Coates, L.M., Pegg, K.G. and Hayward, A.C. (1995) Isolation and selection of bacteria and yeasts antagonistic to preharvest infection of avocado by *Colletotrichum gloeosporides*. Australian Journal of Agricultural Research **46**, 985-995.

Steel, C. C. (2001) Effects of altered UV light and climate change on the susceptibility of grapevines to fungal diseases. Australian Grapegrower and Winemaker – **No. 449,** June 2001, pp 13 – 15.

Steel C. C. and Greer, L. A. (2005)
Catalase activity and susceptibility of grapevine callus culture (cv. Cabernet Sauvignon) to *Botrytis cinerea* infection: Effect of UV-B exposure. Vitis **44,** 149 – 150

Steel, C. C. and Nair, N. G. (1993)
Physiological basis of resistance to the dicarboximide fungicide, iprodione in *Botrytis cinerea*. Pesticide Biochemistry and Physiology **47**, 60 - 68.

Steel, C. C., Somers, T., Greer, L., Hatfield, J., Hayward, C. and Radovanovich-Tesic, M. (2003). Aspects of IPM for the control of Ripe rot and Bitter rot diseases of grapes. Australian and New Zealand Grapegrower and Winemaker September Issue 2003, No 476, 33 - 36.

Sutton, B.C. and Gibson, I.A.S. (1977) *Greeneria uvicola*. C.M.I. Descriptions of Pathogenic Fungi and Bacteria, No. 538.

Swart, A.E. and Holz, G. (1994) Colonization of table grape bunches by *Alternaria alternata* and rot of cold-stored grapes. South African Journal of Enology and Viticulture **15**, 19-25.

Szandala, E.S. and Backhouse, D. (2001) Suppression of sporulation of *Botrytis cinerea* by antagonists applied after infection. Australasian Plant Pathology **30,** 165-170.

Tournas, V.H. and Katsoudas, E. (2005) Mould and yeast flora in fresh berries, grapes and citrus fruits. International Journal of Food Microbiology **105**, 11-17.

Ullasa, B.A. and Rawal, R.D. (1986) Studies on American rot of grapes due to *Greenaria uvicola* from Bangalore, India. Indian Journal of Plant Pathology **4**, 154-161.

Whitelaw-Weckert, M.A; Curtin, S; Huang, R; Steel, C.C; Blanchard, C.L and Roffey, P. (2007) Phylogenetic relationships and pathogenicity of grape *Colletotrichum acutatum* isolates in sub-tropical Australia. Plant Pathology –**In Press.**

Whiting, J. (2004) Botrytis control – a best practice approach. Regional Workshops. Final report to Greater Victoria Wine and Grape Industry Development Committee.

Wu, W. and Chang, L. (1993) Biological control of grape ripe rot and bitter rot. Plant Pathology Bulletin **2**, 20-25.

Yamamoto J., Sato T. and Tomioka K. (1999) Occurrence of ripe rot of grape (*Vitis vinifera* L.) caused by *Colletotrichum acutatum* Simmonds ex Simmonds. Annals of the Phytopathology Society of Japan **65**, 83-86.

Appendix 1

A typical field trial design showing six replicates where two rows are sprayed with two adjacent unsprayed buffer rows. Colours relate to flagging tape placed at the end of the rows, where each colour relates to a different treatment. A minimum of six replicates were used for each treatment.

Replicate	Row id / number on end post	**Rows to be sprayed**	Treatment	Colour Code
1	31-36	**33 & 34**	3	Blue
	37-42	**39 & 40**	1	Unmarked
	43-48	**45 & 46**	2	Pink
2	49-54	**51 & 52**	3	Blue
	55-60	**57 & 58**	2	Pink
	61-66	**63 & 64**	1	Unmarked
3	67-72	**68 & 69**	3	Blue
	73-78	**75 & 76**	1	Unmarked
	79-84	**81 & 82**	2	Pink
4	85-90	**87 & 88**	1	Unmarked
	91-96	**93 & 94**	3	Blue
	97-102	**99 & 100**	2	Pink
5	103-108	**105 & 106**	3	Blue
	109-114	**111 & 112**	2	Pink
	115-120	**117 & 118**	1	Unmarked
6	121 - 126	**123 & 124**	3	Blue
	127 - 134	**129 & 130**	2	Pink
	135 - 140	**137 & 138**	1	Unmarked

Appendix 2

Vine Disease Workshop Questionnaire – Hunter 2006

Your assistance in completing this brief questionnaire will help us to improve any future workshops that we might run. The questionnaire is also an opportunity to gauge the incidence of non-Botrytis bunch rots and trunk diseases in your region. You are under no obligation to complete the questionnaire. Please return the questionnaire to Chris Steel at the conclusion of the workshop.

Instructions: Please tick your response to each item on the 7-point scale. Very Strongly Agree = 7, Strongly Agree = 6, Agree = 5, Uncertain = 4, Disagree = 3, Strongly Disagree = 2, and Very Strongly Disagree = 1. Where an item does not apply to you, please mark Not Applicable = 0.

Questions 1 -5 relate to the workshop, questions 6 – 10 relate to your practical experience of vine diseases. It is recognised that questions 6 – 10 may not be relevant to all participants. These questions have been designed to survey the practical experience of grape growers and others with a direct involvement in the wine industry.

		VSA	SA	A	U	D	SD	VSD	NA
		7	6	5	4	3	2	1	0
1	My attendance at the workshop was valuable	O	O	O	O	O	O	O	O
2	As a result of my attendance at this workshop my knowledge of bunch rot disease of grapes has improved	O	O	O	O	O	O	O	O
3	I would recommend colleagues working in the wine industry to attend a similar workshop if run in the future	O	O	O	O	O	O	O	O
4	The duration of the workshop, ie half a day was appropriate	O	O	O	O	O	O	O	O
5	The exhibits at the end of the seminar presentations were useful	O	O	O	O	O	O	O	O
6	bunch rot diseases are not a problem and are easily managed in my grape growing region	O	O	O	O	O	O	O	O
7	Ripe and/or bitter rot frequently occur in my vineyard	O	O	O	O	O	O	O	O
8	Botrytis (grey mould) frequently occurs in my vineyard	O	O	O	O	O	O	O	O
9	Trunk disease and other disease of the wood are a problem in my vineyard	O	O	O	O	O	O	O	O
10	Disease management in general, is largely successful in my vineyard	O	O	O	O	O	O	O	O

Appendix 3

Colletotrichum isolates deposited with the Agricultural Scientific Collection Unit at NSW DPI in Orange

DAR#	organism	Geographical origin and date of isloation	Host cultivar
75574	*C.acutatum*	Hastings Valley, NSW; 2000	Cabernet Sauvignon
76883	*C.acutatum*	Hastings Valley, NSW; 2000	Cabernet Sauvignon
77282	*C.acutatum*	Hastings Valley, NSW; 2000	Pinot Noir
76884	*C.acutatum*	Hastings Valley, NSW; 2000	Pinot Noir
76885	*C.acutatum*	Hastings Valley, NSW; 2000	Chambourcin
76886	*C.acutatum*	Tenterfield, NSW: 2001	Merlot
76887	*C.acutatum*	Lower Hunter Valley, NSW; 2000	Semillon
76888	*C.acutatum*	Hastings Valley, NSW; 2000	Cabernet Sauvignon
76890	*C.acutatum*	Hastings Valley, NSW; 2002	Shiraz
76890	*C.acutatum*	Hastings Valley, NSW; 2002	Verdelho
76891	*C.acutatum*	Hastings Valley, NSW; 2000	Verdelho
76892	*C.acutatum*	Hastings Valley, NSW; 2002	Verdelho
76893	*C.acutatum*	Kingaroy, Queensland; 2002	Verdelho
76894	*C.acutatum*	Kingaroy, Queensland; 2002	Verdelho
76895	*C.acutatum*	Hastings Valley, NSW; 2002	Verdelho
76896	*C.acutatum*	Hastings Valley, NSW; 2002	Verdelho
77283	*C.acutatum*	Upper Hunter Valley, NSW; 2002	Cabernet Sauvignon
76897	*C.acutatum*	Murgon, QLD; 2002	Semillon
76898	*C.acutatum*	Murgon, QLD; 2002	Semillon
76899	*C.acutatum*	Murgon, QLD; 2002	Semillon
77284	*C.acutatum*	Murgon, QLD; 2002	Semillon
76900	*C.acutatum*	Murgon, QLD; 2002	Semillon
76901	*C.acutatum*	Hastings Valley, NSW; 2002	Cabernet Sauvignon
76902	*C.acutatum*	Hastings Valley, NSW; 2002	Cabernet Sauvignon
76903	*C.acutatum*	Hastings Valley, NSW; 2002	Cabernet Sauvignon
76906	*C.acutatum*	Kingaroy, Queensland; 2002	Chardonnay
76907	*C.acutatum*	Shoalhaven, NSW; 2002	Cabernet Sauvignon
76908	*C.acutatum*	Shoalhaven, NSW; 2002	Cabernet Sauvignon
76909	*C.acutatum*	Shoalhaven, NSW; 2002	Cabernet Sauvignon
76911	*C.acutatum*	Hastings Valley, NSW; 2002	Verdelho
76912	*C.acutatum*	Hastings Valley, NSW; 2000	Cabernet Sauvignon
76913	*C.acutatum*	Tenterfield, NSW: 2001	Chardonnay
76914	*C.acutatum*	Hastings Valley, NSW; 2002	Chambourcin
77285	*C.acutatum*	Lower Hunter Valley, NSW; 2000	Semillon
76915	*C.acutatum*	Hastings Valley, NSW; 2001	Chambourcin

76916	*C.acutatum*	Hastings Valley, NSW; 2001	Chambourcin
76917	*C.acutatum*	Hastings Valley, NSW; 2001	Semillon
76918	*C.acutatum*	Hastings Valley, NSW; 2001	Chambourcin
76919	*C.acutatum*	Hastings Valley, NSW; 2001	Chambourcin
76920	*C.acutatum*	Hastings Valley, NSW; 2001	Chambourcin
24831a	*C.acutatum*	Hastings Valley, NSW; 2000	Chambourcin
28076	*C.acutatum*	Shoalhaven, NSW; 2000	Chambourcin
76934	*C.gloeosporiodes*	Tamworth, NSW; 2002	Pistachio
76925	*C.acutatum*	Corrindi, NSW; 2004	blueberry
76935	*C.acutatum*	Upper Hunter Valley, NSW; 2004	avocado
76926	*C.acutatum*	North Coast, NSW; 2004	mango
76927	*C.acutatum*	Corrindi, NSW; 2004	blueberry
76936	*C.gloeosporiodes*	Upper Hunter Valley, NSW; 2004	pecan
76928	*C.acutatum*	Corrindi, NSW; 2004	blueberry
76929	*C.acutatum*	Corrindi, NSW; 2004	blueberry
76930	*C.acutatum*	Wagga Wagga, NSW; 2004	chilli
76931	*C.acutatum*	Tumbarumba, NSW; 2004	blueberry
76932	*C.acutatum*	Tumbarumba, NSW; 2004	blueberry
76933	*C.acutatum*	Wagga Wagga, NSW; 2004	chilli

(This table continued from previous page)

Appendix 4

G. uvicola isolates deposited with the Agricultural Scientific Collection Unit at NSW DPI in Orange

DAR#	organism	Geographical origin and date of isloation	Host cultivar
77258	*G.uvicola*	Upper Hunter Valley, NSW; 2002	Chardonnay
77259	*G.uvicola*	Hastings Valley, NSW; 2004	Chambourcin
77260	*G.uvicola*	Hastings Valley, NSW; 2004	Chardonnay
77261	*G.uvicola*	Hastings Valley, NSW; 2004	Chambourcin
77262	*G.uvicola*	Hastings Valley, NSW; 2004	Cabernet Sauvignon
77263	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77264	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77265	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77266	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77267	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77268	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77269	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77270	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77271	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Cabernet Sauvignon
77272	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Cabernet Sauvignon
77273	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Cabernet Sauvignon
77274	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Cabernet Sauvignon
77275	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77276	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77277	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77278	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77279	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77280	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay
77281	*G.uvicola*	Upper Hunter Valley, NSW; 2004	Chardonnay