Fruit rot control in Hunter Vineyards. Providing growers with tools to stop the rot



Photo by T Somers

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

REGIONAL INNOVATION AND TECHNOLOGY ADOPTION (RITA) PROJECT
Final Report

PROJECT TITLE

Fruit rot control in Hunter Vineyards. Providing growers with tools to stop the rot

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Executive Summary

Bunch rots of grapes, are caused by a range of fungal diseases. They have a serious impact on both berry yield and grape and wine quality. There is a wealth of information on the biology and control of grey mould (*Botrytis cinerea)*, the most commonly encountered fungal bunch rot disease. However there are a range of other fungal pathogens associated with grape berries that are frequently referred to as non-Botrytis bunch rots. The biology of the majority of these is not well understood. This project investigated the incidence of bunch rots other than grey mould in a range of viticultural regions of NSW. The biology of two of the major non-Botrytis bunch rots of grape, ripe rot (*Colletotrichum acutatum*) and bitter rot (*Greeneria uvicola*) was investigated. The project examined the susceptibility of a number of different wine grape varieties to these two fungal pathogens. All of the varieties examined were susceptible at the post-veraison stage, with the possible exception of the *Vitis* hybrid, Chambourcin. Berry susceptibility is most probably linked to Brix levels.

The growth characteristics of Grey Mould, Bitter rot and Ripe rot fungi were compared. Both bitter and ripe rot tended to have optimum growth rates higher than that of Botrytis, consistent with the observation that these organisms have been recorded from vineyards in sub-tropical regions. Furthermore ripe rot appears to tolerate exposure to Ultraviolet B (280 – 320nm) light which supports the observation that this organisms is found predominantly on fruit from open canopies (in contrast to grey mould and bitter rot).

The project has fostered on-going collaborative research between the National Wine and Grape Industry Centre and the two vineyard associations in the Hunter and Hastings Valleys. Furthermore the preliminary findings of this project have provided a framework for further research (GWRDC project CSU03/01) on the management of non-Botrytis bunch rots in vineyards.

Background

Bunch rots of grapes caused by fungi other than *Botrytis cinerea* is frequently a problem in viticulture areas that experience warm and wet periods during the ripening period. Research funded by the Australian Research Council (2000-2002) through the SPIRT scheme identified two serious fungal pathogens of vines from sub-tropical regions of Australia, bitter rot and ripe rot caused by *Greeneria uvicola* and *Colletotrichum* spp. respectively. These diseases have become apparent in the last ten years. A limited survey conducted in the 1998/99 season highlighted the problem, when an absence of the more commonly occurring bunch rot organism, *Botrytis cinerea* was recorded (Castillo-Pando et al, 1999). This tended to suggest that management practices for grey mould were largely effective but that other pathogens were becoming a problem.

Symptoms of bitter rot include the formation of concentric rings of black sporulation around the circumference of the berry surface (Figure 1a) while ripe rot symptoms involve an orange to pink discharge of spores and loss of berry turgor (figure 1b).



Figure 1a. Bitter rot, caused by *Greeneria uvicola* on Chardonnay berries



Figure 1b. Ripe rot, caused by *Colletotricum acutatum* on Chardonnay berries

The names bitter rot and ripe rot are misleading, both diseases result in bitter off-flavours in grapes and wine and both occur when the fruit is ripe. For ripe rot, the threshold level of infection where the disease becomes noticeable in wine, is believed to be less than 1% (Meunier –personal

communication). How widespread non-Botrytis bunch rots are is largely unknown. Bitter rot and ripe rot are known overseas in some vineyards with sub-tropical climates (Daykin and Milholland, 1984; Ullasa and Rawal, 1986; Yamamoto et al, 1999). Both of these diseases were reported on grapevines in Australia for the first time relatively recently (Castillo-Pando et al, 1999; Melksham et al 2002). Management options in the vineyard for these destructive diseases are limited. For instance it is not known which varieties of grapes are more susceptible than others, or how climatic effects influence the level of disease incidence and the infection process. There are no fungicides registered for these diseases on grapes in Australia and there is circumstantial evidence that management practices for the more widely occurring bunch rot fungus, *Botrytis cinerea*, actually favour the development of bitter and ripe rot.

This project investigated these diseases in the Hunter and Hastings regions of NSW and was designed to gather preliminary data on the problem so that further work could be carried out in a three year GWRDC-funded project commencing in July 2003 (GWRDC project number CSU 03/01).

Participants

Name	Position/Role	Organisation
Mr Ian Tinkler	Producer Contact	Hunter Valley Vineyard Association
Mr Jim Mobbs	Producer Contact	North Coast Wine Growers Association
Assoc. Prof. Chris Steel	Project Leader	NWGIC & Charles Sturt University
Mr Tony Somers	Viticulturist	NWGIC & NSW Agriculture
Mrs Lindsay Greer	Technical Assistant	NWGIC & Charles Sturt University
Mr Chris Haywood	Technical Officer	NWGIC & NSW Agriculture
Ms Jo Hatfield	Casual Assistance	NWGIC & Charles Sturt University
Mr Sean Dean	Viticulture Student	NWGIC & Charles Sturt University

Objectives

The aim of this project was to determine the distribution of bunch rots caused by bitter and ripe rot fungi and to determine the susceptibility of wine grape varieties. The project was also initiated to provide a link in funding between two related projects, an ARC SPIRT project that terminated in January 2003 and a GWRDC grant that commenced in July 2003. The grant allowed research activities on non-Botrytis bunch rots to continue during the 2002/3 vintage in the absence of other funds.

Method

1. Survey data

Vineyards were either surveyed by randomly selecting 5 rows and scoring a total of 100 bunches per variety or alternatively growers were invited to send suspected cases of non-Botrytis bunch rots to the NWGIC for identification. Bunches were scored using the method of Emmett, whereby a bunch is given a score from 0 to 4 depending upon the severity of infection (figure 3).

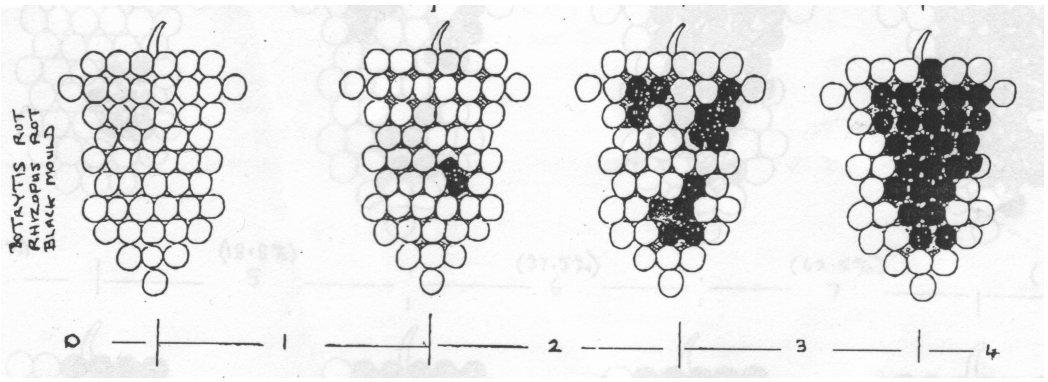


Figure 3. Scoring system used for monitoring bunch rot severity.

For the determination of varietal susceptibility to bunch rots, individual berries were collected from the vineyard at Charles Sturt University, (a region characterised by warm dry summers, with a consequently relative low incidence of bunch rot diseases). The berries were surfaced sterilised, Brix levels determined (from a sub-sample of the berries) and inoculated (no wounding) by placing a 10µl drop of spores (10^4) on the berry surface. The berries were incubated individually in large well ELISA plates (Figure 4), at 25 ^oC and the time taken to the first appearance of symptoms recorded.



Figure 4. Shiraz grape berries artificially inoculated with *B. cinerea* and incubated in an ELISA well plate.

Growth experiments on artificial media where carried out using agar plates of Potato Dextrose Agar (PDA). PDA plates were inoculated by transferring a 5 mm diameter disc of mycelium to the centre of a fresh PDA plate and radial growth determined following incubation at 25°C (Steel, 1996). For experiments on light exposure, UVB radiation was supplied by Fluorescent Lamps (Philips TL 20/12 313nm) and the degree of exposure varied by altering the distance of the culture from the light source. The transmittance of the plastic of the petri dish lid was found to be 100% at 290nm, 50% at 280nm and 0% at 270nm. Thus the plastic material used in the petri dish acted as a screen for short wavelength UV light (ie UVC; < 270nm). UVB exposure was quantified using a Light

radiometer (YSI Model 65 Radiometer, Kettering, Ohio) and the amount of UVB quantified by subtracting the energy of the light source when shielded with a Cellulose acetate film.

Results & Discussion

During February and March 2003 seven vineyards were surveyed for the incidence and severity of bunch rot organisms. The survey was conducted by scoring 100 bunches from the vineyard using a scale of 0 – 4 and the results expressed as a mean score (table 1). Thus the higher the score the greater the bunch rot severity.

Table 1. Survey of 7 vineyards in the Hunter Valley (13 sites) for the incidence of bunch rots in 2003.

Key : U = Unidentifiable, P = *Penicillium* spp.,

Vineyard	Location	Site / Variety	Survey date	Bitter Rot	Ripe Rot	Grey Mould	Other identified rots
1	Upper Hunter	Chard	23rd January	0.38	0	0	0.26U
2	Upper Hunter	Cab. Sav	6th February	0	0	0	0
		Semillon	6th February	0.18	0	0	0.40U
		Shiraz	6th February	0	0	0	0
3	Hunter	Durif	6th February	0	0	0	0
		Merlot	6th February	0	0	0	0
		Shiraz	6th February	0	0	0	0
4	Hunter	Shiraz	28th February	0	0	0.19	0.01U
5	Hunter	Cab. Sav.	28th February	0	0	0.21	0
6	Hunter	Semillon	28th February	0	0	3.05	0
		Shiraz	28th February	0	0.03	0.38	0.05 U
7	Lower Hunter	Cab. Sav	5th March	0	1.67	0	0
		Merlot	5th March	0	1.79	0	0.08 P

In addition to this monitoring exercise, growers from other regions were encouraged to submit suspected cases of bunch rots to the NWGIC laboratories at Wagga Wagga for investigation. This exercise was not particularly successful; material was often in transit for prolonged periods of time and was contaminated with additional unrelated microbial growth upon arrival. A range of fungal rots were isolated (table 2) and these results may not reflect an accurate picture of field conditions. There was a predominance of *Aspergillus, Penicillium* and *Alternaria* spp., fungal genera often associated with post-harvest rots and regarded as secondary invaders or opportunistic pathogens. *Colletotrichum* was only isolated from samples sent from the Hunter and Hastings regions and grey mould and bitter rot were not isolated from any of these samples.

Table 2. Number of fungal isolations made from grape samples submitted to the NWGIC laboratories for bunch rot analysis

Table 2. Isolates per Grape Variety per Region

	Colletotrichum	Aspergillus	Penicillium	Alternaria	Greeneria	Botrytis	Unidentified	Number of Samples
Murrumbateman								
Chardonnay	0	0	0	1	0	0	0	1
Northern Tablelands								
Riesling	0	2	1	0	0	0	0	2
Sauvignon Blanc	0	2	0	0	0	0	0	2
Shoalhaven								
Verdello	0	0	0	0	0	0	1	1
Petit Verdot	0	0	1	0	0	0	0	11
Merlot	0	1	1	0	0	0	0	1
Shiraz	0	1	1	0	0	0	0	1
Chardonnay	0	1	1	0	0	0	0	1
Cowra								
Merlot	0	0	0	3	0	0	1	4
Cabernet	0	1	0	0	0	0	1	1
Shiraz	0	0	0	0	0	0	2	2
Hastings								
Cabernet Sauvignon	4	7	2	1	0	0	14	17
Chambourcin	0	1	2	1	0	0	3	3
Chardonnay	1	1	0	0	0	0	2	2
Variety Undeclared	0	1	0	0	0	0	1	1
Hunter								
Chardonnay (1)	0	1	0	0	0	0	1	1
Chardonnay (2)	0	0	1	2	0	0	0	2
Cabernet Sauvignon (1)	0	1	0	0	0	0	1	1

Table 2. Number of fungal isolations made from grape samples submitted to the NWGIC laboratories for bunch rot analysis – continued.

Cabernet Sauvignon (2)	0	0	0	1	0	0	0	1
Cabernet Sauvignon (3)	0	2	2	2	0	0	1	3
Merlot (1)	1	2	1	0	0	0	1	3
Merlot (2)	0	0	0	1	0	0	2	1
Shiraz (1)	0	1	0	2	0	0	0	2
Shiraz (2)	0	1	0	1	0	0	0	1
Shiraz (3)	0	2	0	0	0	0	1	2
Shiraz (4)	0	0	0	0	0	0	1	1
Shiraz (5)	0	1	0	0	0	0	0	1
Semillon (1)	0	0	0	1	0	0	0	1
Semillon (2)	0	3	0	1	0	0	0	3
Duriff	0	0	1	1	0	0	0	1
Verdello	0	0	1	1	0	0	0	1
Chenin Blanc	0	2	1	0	0	0	0	3
Variety Undeclared	0	1	0	0	0	0	0	1
Total Isolations	6	35	16	19	0	0	33	79

The 2003 vintage was particularly atypical in terms of summer rainfall, and sites surveyed from the end of February onwards were found to have a higher incidence of bunch rots. It is likely that in a more normal year the incidence of these diseases would be greater earlier in the season.

The extent of non-Botrytis bunch rots from the regions examined was much less than that observed in earlier years, probably due to the dry conditions experienced in the first part of 2003. Several of the vineyards appeared to have problems with fungi generally regarded as opportunistic pathogens, or secondary invaders, ie *Aspergillus, Alternaria* and *Penicillium*. However, these results relate to samples of grapes that had been collected by growers (rather than direct isolations made in the field), and so these organisms probably arose as a result of contamination while samples were in transit. Several of the couriers used by growers to deliver material to the NWGIC offered a less than satisfactory service with respect to promptness.

The exercise was useful in that it illustrated the problems of contamination of vine material while in transit. Further survey work is likely to involve field isolations made directly to laboratory media in the field. Another alternatively currently being investigated is the use of ELISA kit for rapid immuno-detection of ripe rot. Such a kit is commercially available and is likely to improve accuracy and speed-up the rate of detection.

2. Varietal susceptibility

Circumstantial evidence indicates that all wine grape varieties are susceptible to bunch rot disease although bitter rot is more easily recognised on white varieties. While there is data to support differences in the susceptibility of different varieties to grey mould, there is no data on susceptibility to bitter rot and ripe rot fungi. Table 3 lists the varieties examined (ranked according to Brix level) and the time taken to the first appearance of symptoms recorded. Because of time restraints around the vintage period, the exercise could not be repeated. This however, is currently being investigated in the 2003/4 growing season using berries at a range of phenological stages from pre-veraison to harvest. This will help growers to target more accurately disease control methods.

Table 3. Susceptibility of a range of grape varieties to grey mould, ripe rot and bitter rot.

		Days post-inoculation to signs of first symptoms		
Variety	^{0}Brix	*B.cinerea*	*C. acutatum*	*G. uvicola*
Shiraz	27.9	<5	<5	<5
Chardonnay	26.2	<5	5	7
Merlot	25.7	<5	5	<5
Traminer	23.5	<5	5	6
Cabernet Sav.	23.3	6	6	7
Chambourcin	23.0	Did not infect	12	12
Sauvignon Blanc	22.5	6	6	7
Riesling	21.0	9	6	8

This preliminary data indicates that all varieties with the possible exception of the *Vitis* hybrid Chambourcin, appear to be equally susceptible. Further work in progress will investigate the effects of temperature, water availability and Brix levels on the susceptibility of varieties. It is anticipated that Brix levels will play a major role in the rate of infection, since non-Botrytis bunch rots are known to develop rapidly close to harvest.

3. Effect of light exposure on disease development

Canopy management practices are widely recognised as factors in the control of plant pathogens. Use of an open canopy in grey mould control in combination with chemical sprays is a standard management option in vineyards susceptible to grey mould (Gubler et al, 1987). However, in field trial sites aimed at managing bitter and ripe rots it has been observed that while an open canopy results in less Botrytis, other diseases are apparent (Steel et al 2003b). It is also known that the ripe rot fungus, *C. acutatum* produces a UV protective mucilaginous sheath when it infects plant tissues. One could hypothesise that *C. acutatum* is more tolerant than other fungi associated with Bunch rots.

To test this hypothesis agar cultures of *B. cinerea*, *G. uvicola* and *C. acutatum* were grown under different levels of UV B (280 – 320nm) light and the radial growth determined. When expressed as a percentage of growth under dark conditions, *C. acutatum* was found to be more tolerant of UV B (table 4). The levels of UV B chosen were those one might expect to encounter in an open canopy in a vineyard in full sunlight in the summer months.

Fungus	% inhibition at UVB 0.2×10^{-4} J/cm^2	% inhibition at UVB 2×10^{-4} J/cm^2
B. cinerea	15.9	37.6
C. acutatum	5.4	12.7
G. uvicola	36.7	51.7

Table 4. Percentage inhibition of radial growth of bunch rot organisms when grown on potato dextrose agar under a UVB light source.

4. Effect of temperature on fungal growth

The rate of growth of *B. cinerea*, *G. uvicola* and *C. acutatum* were compared on PDA plates incubated at a range of temperatures from 10 to 37 ^{o}C and the optimum temperature for growth recorded. Radial growth was recorded at daily intervals and growth rates determined. Only *B. cinerea* showed limited growth at 10 ^{o}C, with an optimum temperature of approximately 22 ^{o}C recorded. Both *G. uvicola* and *C. acutatum* had optimum temperatures of approximately 30 ^{o}C (Table 5), and neither of these fungi grew below 15 ^{o}C.

Fungus	Optimum temperature for Growth
B. cinerea	22 ^{o}C
C. acutatum	30 ^{o}C
G. uvicola	30 ^{o}C

Table 5. Optimum temperature for growth of three bunch rot fungi on PDA

This indicates that ripe rot and bitter rot are more likely to occur in warmer climates, while grey mould is able to grow at cooler temperatures. This finding is consistent with published data for *B.*

cinerea (Nair and Allen, 1993). It should be noted that this exercise was only conducted with one isolate of each fungal species, and it is likely that some isolate variability will occur.

Extension activities arising from this Research

(i) Industry Presentations
Wagga Wagga – June 2003
Pokolbin – 29 August 2003
Wauchope – 31 August 2003

(ii) Publications

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 Grapegrower and Winemaker September Issue 2003, 33 - 36.

Additionally a poster presentation is planned for the 12th Australian wine Industry technical Conference to be held in Melbourne in July 2004.

Conclusions

While the nature of the data reported in this document may appear to be of a preliminary nature, this reflects that this project is part of on-going research that is funded through the GWRDC (project CSU 03/01) until 2006. The investigations initiated in this RITA project are being repeated in 2004 in association with field trials aimed at improving management of non-Botrytis bunch rots. These include canopy management trials, where the degree of light exposure is varied in VSP trained vines and fungicide trials to reduce fungal inoculum in the winter period and improved spray timing during flowering and fruiting.

Although the 2003 vintage was relatively dry in terms of rainfall, a range of bunch rot organisms was recorded from a number of vineyards. The number of vineyards surveyed was limited, but 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 supports earlier observations that *C. acutatum* occurs more frequently in open as opposed to closed canopies (Steel et al 2003b). Furthermore, both ripe rot and bitter rot isolates were able to grow at temperatures higher than what grey mould is able to survive. This suggests that growers will have to consider the balance between these two organisms when planing canopy management in the vineyard. Further work in progress is examining the competition that might occur between different fungal species and other microflora inhabiting the grape berry surface.

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