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Department of Primary Industries, Water and Environment, Tasmania, and industry

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Planning research toward sustainable management of bunch rot in grapes



A workshop for researchers
Hobart, November 2-3, 2005.

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Planning research toward sustainable management of bunch rot in grapes

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GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Planning research toward sustainable management of bunch rot in grapes

Executive summary

Bunch rot of grapevine, caused by *Botrytis cinerea* and other opportunistic plant pathogens, continues to be managed by the wine industry with mixed success. Management of this disease is complex requiring multiple, integrated approaches. Effective application of protective fungicides during flowering depends on appropriate spray dose, timing and coverage in relation to the incidence of moisture and establishment of latent infection. For most sites and years, it is not known if applications of protective fungicide during flowering contributed significantly to a reduction in the severity of bunch rot at harvest. A false sense of security can develop when protective fungicides appear to work well in years that are unfavourable for the expression of bunch rot. Hence, this form of crop insurance continues, despite the potential for more strategic applications. A major constraint in bunch rot management is the lack of tolerance for chemical residues in wine destined for various export markets. This has restricted options for late-season control of bunch rot. Integrated management, based on pathogen activity and reduced reliance on synthetic fungicides, has become paramount.

Outcomes of Botrytis research in the last 5-10 years are being adopted by industry through mechanisms such as the Botrytis Checklist. In order to maintain the momentum, twenty researchers and industry proponents from Australia and New Zealand met in Hobart on November 2-3, 2005, to develop a coordinated research strategy for bunch rot of grapevine. The workshop aims, process and outcomes are described in this document.

After an opening address by Mr Tony Scherer, an organic grape grower from the Coal River Valley, Tasmania, the biological scene for the workshop was set by Dr Philip Elmer, HortResearch, New Zealand (NZ). The messages of both the scientist and practitioner were consistent, in that the potential and importance of reducing reliance on synthetic fungicides was illustrated. Dr Elmer, a developer of BOTRY-Zen® in NZ, demonstrated that knowledge of pathogen infection pathways and disease epidemiology is essential for integrated management that utilises a biosuppressant.

Dr Bob Emmett, DPI Victoria, began the formal process of the meeting by reviewing research conducted in Australia since 1999. He then highlighted recommendations for future research arising from recent projects. Group sessions involved identifying gaps in current knowledge through a systematic process, akin to the headings that appear in a review of the literature. Knowledge gaps were recorded and grouped according to one of nine theme areas identified before the workshop. Two formal presentations, on the second day of the meeting, also stimulated 'brainstorming' and shifted the focus from the applied and practical to basic, 'blue sky' research. Dr Ian Dry of CSIRO explained the potential of genetic approaches for obtaining disease resistant varieties and Dr Tony Reglinski, HortResearch, NZ, described induced disease resistance and practical issues associated with utilising this phenomenon.

The final group session involved synthesising preliminary research proposals using the gaps in knowledge generated during the workshop. A total of 18 topics for research were proposed across discipline areas including host-pathogen interactions, disease epidemiology and management, technology transfer and method development. Topics and research objectives were prepared without a specific funding body in mind, so that each proposal reflected the knowledge gap transparently and without reference to the strategic plan of any one institution. This outcome will enable individuals to use the separate proposals as modules in project development. That is, the information can be used in different ways, including selection of multiple subsets of research objectives for larger projects.

By the end of November 2005, participants will have indicated to each other how their host institution can contribute to the research proposed. This process will allow initiation of collaborative research and project development over the next 12 months.

Background

Bunch rot, caused by *Botrytis cinerea* and other opportunistic pathogens, continues to be managed by grape growers with mixed success. *Botrytis cinerea*, in particular, has a biology and epidemiology on grape that is unlike that of any other fruit rot caused by this pathogen. Strategies for sustainable disease management are based on specific knowledge of how bunch rot develops in grapes.

A workshop was held at Knoxfield, Victoria, in May 1999, for researchers studying *Botrytis* and grape pathologists involved in development of bunch rot management strategies. The workshop provided a mechanism for coordinated planning of research and development. For example, a final GWRDC report entitled '*Strategic management of Botrytis bunch rot and light brown apple moth on grapevines*' is now available and details outcomes of research planning initiated in 1999 and earlier.

Given events since the Knoxfield workshop, it was deemed an appropriate time to review research outcomes and, most importantly, to dissect recent studies and tease out components of the host-pathogen interaction, disease epidemiology and/or environmental factors where knowledge gaps remain. Defining specific questions for research requires input from a range of scientists in a multidisciplinary approach, especially if novel and/or improved approaches to integrated disease management are to be realised. Involvement of experienced grape pathologists with researchers from other disciplines will build critical mass for continuous generation of knowledge necessary for managing bunch rot sustainably.

Aims

1. To summarise outcomes of relevant and recent research programs. Participants were given selected literature for reading prior to the workshop.
2. To identify knowledge gaps, in a facilitated, workshop environment.
3. To create specific research questions for each knowledge gap.
4. To 'brainstorm' novel and/or integrated approaches to disease management based on developments in science.
5. To set priorities for research proposed based on industry needs and their likely contribution to sustainable disease management.
6. To identify research groups in Australia or elsewhere that are capable of addressing the research proposed.
7. To nominate individuals at the workshop who can facilitate development of each 'high priority' research proposal.

Statement of Purpose

To develop a coordinated research strategy for *Botrytis* and non-*Botrytis* bunch rot detailing critical knowledge gaps and future research direction in the short, medium and long term.

Program

Day 1 Wednesday November 2, 2005

Time	Item
9:35 am	Melbourne and Adelaide flights arrive in Hobart. Book into hotel room.
10:30-10:50 am	Registration and morning tea, load powerpoint files
10:50 am	Meeting commences & welcome by **Dr Kathy Evans** (TIAR, UTas) – statement of purpose, house keeping & introductions
11:10 am	Opening address by **Mr Tony Scherer**, organic grower, Frogmore Creek Vineyard, Coal River Valley.
11:20-11:40 am	**Dr Philip Elmer** (HortResearch, NZ) sets the scene. Flower & fruit infection pathways. Chairperson: Kathy Evans
11:40-11:50 am	Questions/Comments/Discussion
11:55-12:40 am	**Dr Bob Emmett** (DPI, Vic.) on research over the last 6 years. Key outcomes and recommendations for future R&D Chairperson: Jacky Edwards
12:40-1:00 pm	Questions/Comments/Discussion
1 pm	Lunch
1:30 pm	**Group Session 1 - The pathogen & disease epidemiology** 1. General instruction on how the group sessions will proceed – Kathy Evans 2. The pathogen –detection, quantification. Chairperson: Barbara Hall 3. Inoculum potential, dispersal & infection pathways. Chairperson: Jacky Edwards 4. Environmental factors, including host physiology. Chairperson: Chris Steel 5. Monitoring & modeling epidemics. Chairperson: Rob Beresford
3:30 pm	Afternoon tea
3:50 pm	**Group Session 2 – Integrated disease management** 6. Manipulation of inoculum sources & the host. Chairperson: Bob Emmett 7. Crop protection – chemical & biological. Chairperson: Philip Elmer 8. Evaluating integrated management strategies. Chairperson: Trevor Wicks
5:30 pm	Close meeting for the day
7 pm	Dinner at Meehan's Restaurant

Day 2 Thursday November 3, 2005

Time	Item
8:30-9:00 am	**Dr Ian Dry** (CSIRO) Potential new strategies for disease resistance through increased understanding of host:pathogen interactions.
9:00-9:30 am	**Dr Tony Reglinski** (HortResearch, NZ)– Induced resistance
9:30-10:30 am	**Group Session 3 – From molecules to adoption of technology** 9. Host-pathogen interactions. Chairperson: Ian Dry 10. Technology transfer. Chairperson: David Braybrook 11. Carryover issues from previous sessions and additions to the list of knowledge gaps. Chairperson: Kathy Evans
10:30 am	Morning Tea
10:50 am	**Final Group Session - Bringing it all together** **Facilitator: Duncan Farquhar, Department of Primary Industries, Water & Environment, Tas.** 1. Display knowledge gaps identified under each theme 2. Discussion about groupings, across-theme items followed by regrouping 3. Synthesis of topics for preliminary research proposals 4. Individuals vote on priority of proposals suggested (coloured dot method) 5. Delegation of leaders to develop proposals further, including contacting researchers not present at this workshop. 6. Leaders collate list of individuals present at meeting or identified elsewhere that can provide input on proposal development 7. Discussion about sources of funding or other 'enabling' mechanisms 8. Post workshop tasks and reporting dates determined 9. Information on field trip and dinner with Tas. growers/Meeting close.
1:00-1:30 pm	Lunch
	Optional post-workshop events
1:30-5 pm	Afternoon visit to Coal River Valley– Frogmore Creek via Richmond /Airport/ Meadowbank & Hood wines
3:40 pm	Three participants to airport for 4:40 pm flight
5 pm	Participants delivered back to Motel
5:30 pm	Bus to Moorilla winery from Motel
6:00 pm	Dinner with the Vineyards Association of Tasmania (VAT) at Moorilla winery. Convenor: Duncan Farquhar (DPIWE)

Report on proceedings

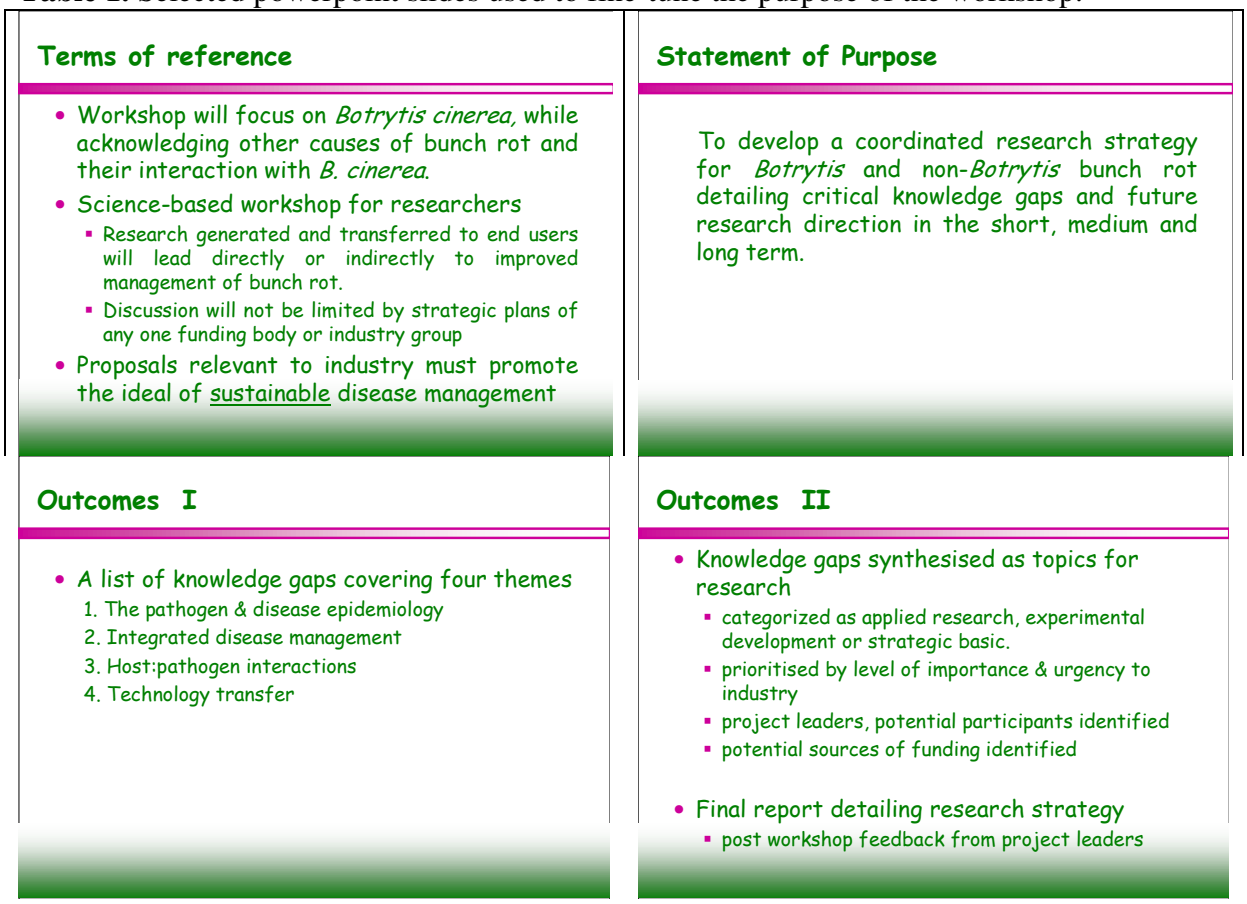
1. Opening session

During introductions, members of the group were asked to rate on a scale of 1-10 how well they thought growers in their region were managing bunch rot in terms of their knowledge and application of technology. While a number of participants gave a score of 5 out 10, a comment was made to the effect that growers' manage bunch rot well when conditions are unsuitable for pathogen activity or bunch rot development. Already, a knowledge gap was evident and it related to the timing of sprays according to pathogen activity.

During the opening address, Mr Tony Scherer, an organic grape grower from Frogmore Creek vineyard in the Coal River valley, emphasised that there were no 'silver bullets' available to manage bunch rot. He explained the multiple ways in which he managed the vineyard environment (organically) for preventing expression of disease. The importance of row orientation and managing humidity with open canopies were examples of his 'plant positive' approach. Mr Scherer also encouraged the group to consider biological approaches to managing bunch rot, a problem that is ranked as one of the top three production issues for growers in Tasmania.

Kathy Evans then outlined the task ahead and requested input and consensus from the group on this matter. Prior to the workshop there was a dichotomy in opinion as to whether the event should focus on *Botrytis cinerea* or whether other causes of bunch rot should be addressed. It was decided that the group would focus on *Botrytis cinerea* for discussion about host-pathogen interactions and disease epidemiology. However, discussion about other bunch rot pathogens would be discussed with regard to their identification/quantification, interaction with *B. cinerea* and/or research issues relevant to the management of bunch rot complexes. The presence of three researchers from New Zealand was highlighted with the aim of fostering and coordinating trans Tasman research for mutual benefit. Table 1 contains a selection of the slides used in this session.

Table 1. Selected powerpoint slides used to fine-tune the purpose of the workshop.



2. Formal presentations over the two-day workshop

Dr Philip Elmer, from HortResearch NZ, set the scene of the workshop by presenting a talk on 'Botrytis in the vineyard'. This presentation began with a summary of the disease, economic importance and impact during winemaking. It was noted that the majority of grape growers are still highly dependent on repeated applications of fungicides. This practice results in high yields and premium quality wines "That no one wants to buy!" This provocative statement was then framed in the context of drivers such as retailers in the UK who demand wines free of fungicide residue. Phil Elmer then posed the question: "Is this high level of botryticide dependency going to change?" Wineries in NZ have responded by reducing fungicide inputs but there have been consequences in the short term. These include loss of disease control, which Phil Elmer argues highlights the deficiencies in our approach to *Botrytis* management and/or the lack of late season products available for application. Clearly, alternative Botrytis management strategies will require a thorough understanding of the ecology and epidemiology of *B. cinerea* in the vineyard.

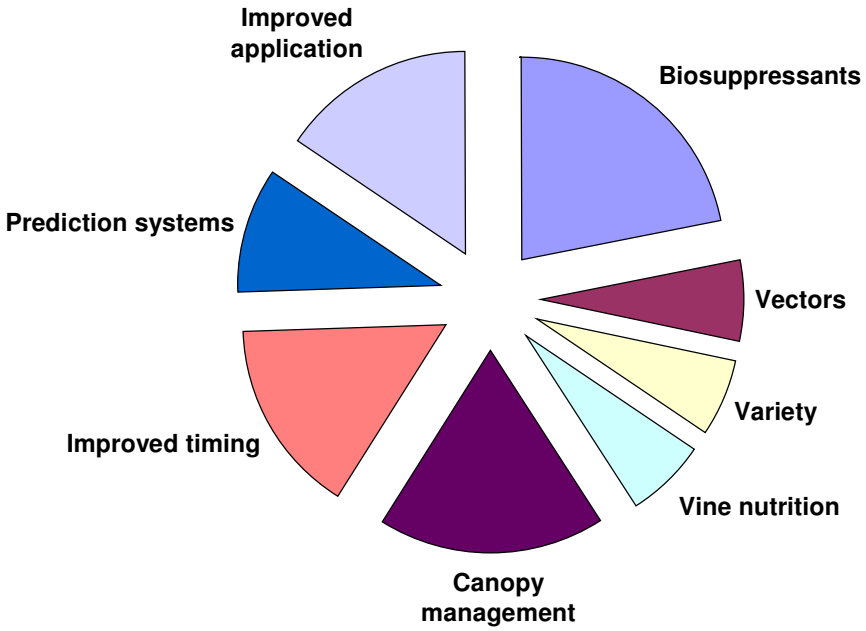
Dr Elmer then gave a pictorial tour of the multiple infection pathways that have been identified for flowers and fruit, noting that sources of inoculum and the infection pathway might vary from season to season year and from one region to the next. The phenomenon of latency was reviewed plus factors influencing disease progression. Most of the pathways described involve a latent phase yet our understanding of the infection criteria (host phenology and environmental factors) for latent infection is incomplete. There were also pros and cons for traditional methods of detecting latent infection.

Dr Elmer stated that the challenge posed for scientists and vineyard managers was to devise cost effective Botrytis management plans that break the various infection pathways. The issues, according to Phil Elmer, were

- How best to use the knowledge gained by many researchers world wide?
- What more can be done early, mid and late season to break infection pathways?
  - Reduce overwintering inoculum after machine harvesting?
  - Break pre-flowering infection pathways? Are *B. cinerea* infections latent in cap tissue prior to 5% flowering?
- How can we achieve greater integration of vineyard management practices to reduce fungicide inputs?
- Can we manage according to risk associated with each cultivar, instead of the one management program fits all approach?

Figure 1 is based on Dr Elmer's presentation to illustrate the multiple components required for Botrytis management in viticulture.

Figure 1. A hypothetical, fully integrated approach to management of bunch rot in viticulture, with the size of each wedge depending on local production constraints.



Questions directed to Phil Elmer highlighted the large task of technical transfer and ultimately the level of technology uptake. Barriers include an overemphasis on managing "from the back of the tractor".

Relevant reference:

Elmer P.A.G., Michailides T. J. (2004) Epidemiology of *Botrytis cinerea* in orchard and vine crops. In: Y. Elad *et al.* (eds) Botrytis: Biology, Pathology and Control. Pages 243-272, Kluwer Academic Publishers: The Netherlands.

Dr Bob Emmett, from DPI Victoria, gave the second formal presentation. His topic was 'Grape bunch rot research: some key outcomes and recommendations for future R & D' (Table 2). The presentation covered key aspects of two final GWRDC reports:

1. DAV 95/1 Strategic management of Botrytis bunch rot and lightbrown apple moth on grapevines.

2. DAV 92/1 Effects of grapevine canopy management on disease development and spray deposition and distribution.

Reference was also made to studies of the biology and management of black Aspergillus bunch moulds outlined in the GWRDC report on Project CRV 99/23 'Fungal contaminants and their impact on wine quality'.

Overall, the presentation highlighted the key elements for integrated management of Botrytis; namely, vine cultural practices and chemical/biological treatment programs. Furthermore, growing seasons can be divided into three categories, which determine which elements become important.

1. Environmental conditions unfavourable for disease development – no bunch rot
2. Conditions marginal and moderate with the impact of vectors on bunch rot potentially significant
3. Ideal conditions for disease development – significant bunch rot, regardless of vector activity.

While significant gains in knowledge have been made, more efficient and effective bunch rot management will depend on addressing issues for future R&D listed in Table 2.

Following Bob Emmett's presentation, the following questions (Q) and answers (A) were noted:

Q: Do you have a feel for the populations of the different pathogens in different situations and seasons?
A: We are monitoring populations of black Aspergillus now. We know that there are certain weather conditions, for example hot and dry leading up to veraison that select out the Aspergillus population. If there is rain toward harvest, then disease caused by Aspergillus is expressed.

Q: Bob got the job of holding together this last set of projects based on recommendations of the 1999 workshop. Was the model a good one?
A: Getting researchers together to share information is important. The previous workshop came along after much work had already been done. We have come a long way in the last 10-15 years and viticulture has changed in that time. The workshop environment is a good technique to focus on priorities. Management of large projects takes time, especially reporting. There are also the logistics managing projects when staff leave jobs and work is completed as components of PhD projects. However, there is great value in studying management strategies in different regions.

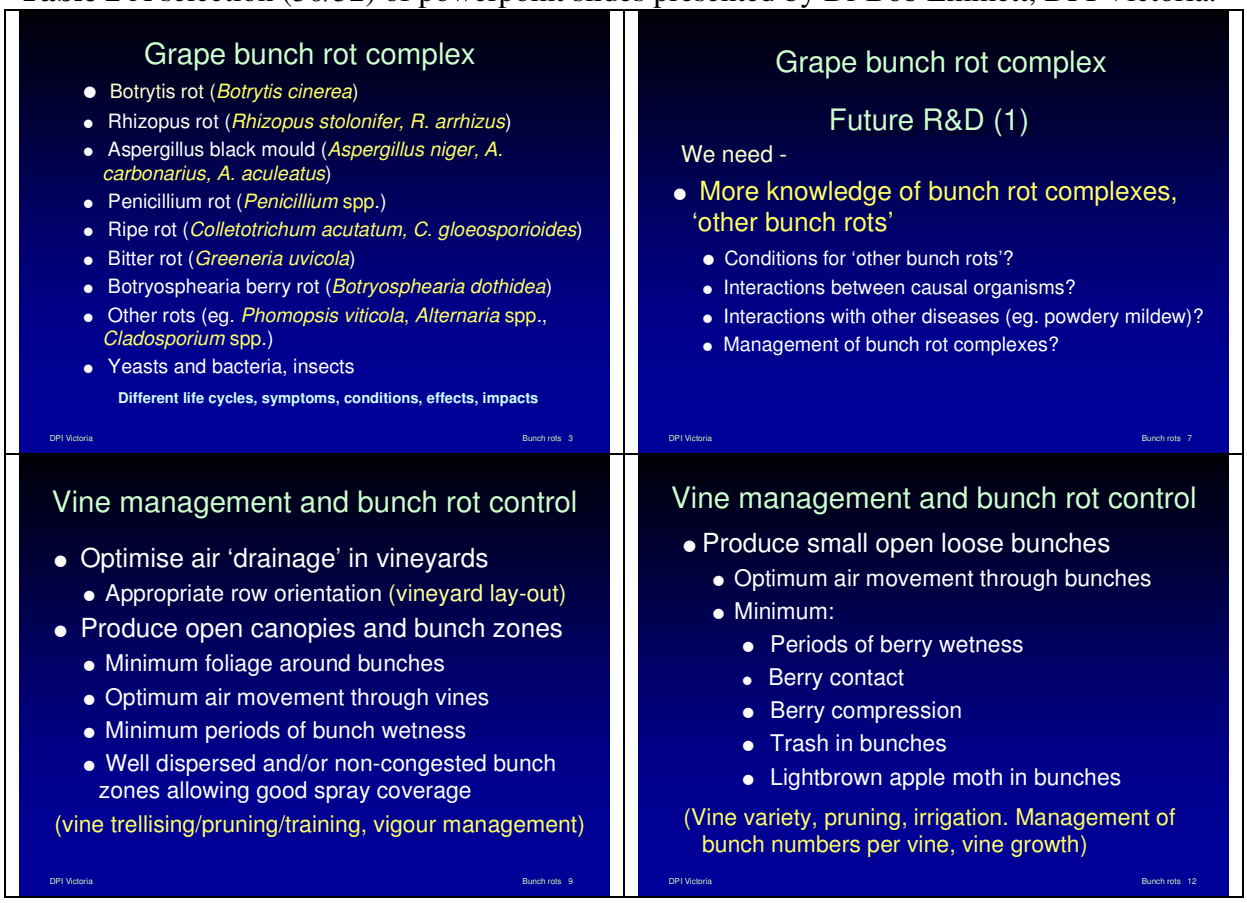
Q: Our knowledge is better as a result of recent work, but is the industry better off in terms of losses from bunch rots?
A: Measuring crop loss is difficult as it is hard to obtain the value of the grapes. As it is an extensive task, it would need to be combined with collection of other data. We can get fungicide expenses. Quantification at the winery?

Q: Will spray programs offered by chemical companies be useful as a model for Aspergillus?
A: No. In Europe, they can use Switch pre-harvest. We don't have that option. In Mildura, spray programs are applied for Botrytis, but the bunch rots are caused by other pathogens as well. Growers need to know when each type of bunch rot will or won't be a problem. (One participant commented that Botrytis control could be opening up a niche for the other bunch rot pathogens).

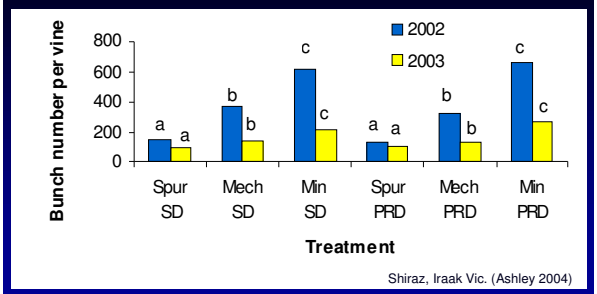
Following these questions there were a raft of comments from participants, including the issue that if we get better quantification at the winery, then the industry might keep lifting the bar on what grapes are acceptable. While Botrytis can be managed in the winery, the cost of this would cover additional trial work! There is also the issue of separating out which pathogens are causing the rots and in some regions like the Hunter, many of the bunch rots are not caused by Botrytis. With regard to seasonal and regional variability, it is not known whether people understand what is driving bunch rot in a particular season. We have the Checklist, CDs and workshops, but how do we get change in practice? What are the drivers at a particular site? It requires significant investment in a national extension effort (like the dairy industry extension effort) to get individuals to sort out their own situation. For example, a single grower may learn, through correct application of knowledge, that if he/she pulls out the trees in the gullies, then this will add significantly to the management of bunch rot.

Bob Emmett concluded the session by emphasising that the messages we deliver need to be clear and simple.

Table 2 A selection (30/52) of powerpoint slides presented by Dr Bob Emmett, DPI Victoria.



Effects of pruning and irrigation treatments on bunch numbers per vine



Shiraz, Iraak Vic. (Ashley 2004)

Effects of pruning and irrigation treatments on bunch architecture



Vine management and bunch rot control

- Minimise damage to berries and bunches
  - Grape varieties with resistance to rain damage
  - Careful irrigation between berry softening and harvest
  - Prevent sunburn of bunches
  - Control pests and diseases (lightbrown apple moth, powdery mildew)
  - Other strategies (vine covers, summer pruning)

(Vine variety, irrigation, nutrition, trellising, training, pruning, pest and disease management)

Vine management and bunch rot control

- Minimise pathogen inoculum in vineyards and in bunches
  - Removal of over-wintering bunch remnants from vines?
  - Minimum cultivation? Use of selected cover crops, mulches, irrigation practices?
  - Use of chemical/biological treatments to reduce bunch spore load and berry infection

(Vine pruning. Irrigation. Management of vineyard floor. Treatment of bunches)

Vine management and bunch rot control

Future R&D (2)

We need -

- More knowledge of the effects of vine and vineyard management on pathogen populations and bunch rot development
  - Combined effects of different practices (eg. vine pruning, training, irrigation, nutrition, vineyard floor management) on inoculum load, host susceptibility?
  - Regional differences?
  - Differences between vine varieties?

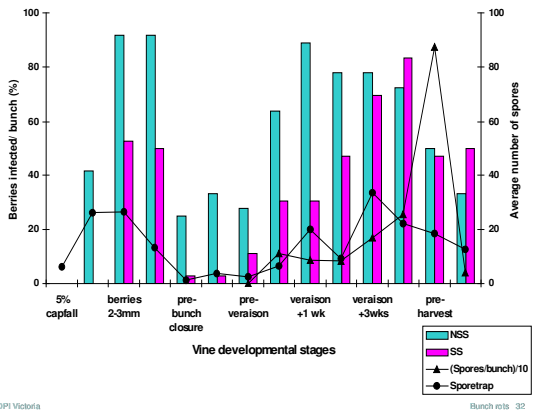
Integrated management of Botrytis rot

Chemical/biological treatment programs

- Routine (insurance-based) spray programs
  - Spraying regardless of seasonal *Botrytis* activity (high chemical use, costs)
- Strategic (risk-based) spray programs
  - Spraying based on assessments of seasonal *Botrytis* activity (eg. *Botrytis* incidence, weather events) (reduced chemical use, costs)

Monitoring incidence of *Botrytis* in grape bunches and vineyards

- Assessed
  - Number of air-borne spores (air spores)
  - Number of spores washed from bunches (bunch spores)
  - *Botrytis* incidence and severity in SS and NSS bunches



Monitoring incidence of *Botrytis* in grape bunches and vineyards

- Air spores, bunch spores, *Botrytis* incidence and severity: fluctuated through season, lower from pre-bunch closure to pre-veraison.
- Air spores were higher around flowering and towards harvest
- Air spores and bunch spores were related to *Botrytis* severity in bunches
- *Botrytis* severity in bunches provided better estimates of *Botrytis* activity than *Botrytis* incidence

Monitoring *Botrytis* severity in grape bunches and vineyards

Future R&D (3)

We need -

- More efficient and reliable methods for assessing *Botrytis* severity in bunches, tracking *Botrytis* activity in vineyards
  - Assessment of bunch spore load, berry latent infection and/or bunch infection potential?
  - Sampling protocols?

Bunch rot disease risk models

- Predictive model for bunch rot at harvest (Hunter Valley NSW) based on *Botrytis* severity (% berries infected) at flowering (Nair *et al.* 1995)
- Model tested in 5 other districts over 3 seasons
- Regression analyses on predicted and observed disease incidence and severity
- The model had low predictive value in the other districts

Bunch rot disease risk models

- Alternative 'weather and disease' models were developed by including other parameters (length of season, rain days, days over 35°C)
- Examined relationships between weather, disease incidence/severity at 5% or 80% cap-fall and disease incidence/severity at pre-harvest and harvest
- Higher confidence in 'weather and disease' models for predicting bunch rot severity. Further tests required

Botrytis bunch rot disease risk models

Future R&D (4)

We need -

- A model to reliably predict bunch rot severity at harvest as guide to the need for control measures
  - Suitable for regional application?
  - More efficient use of fungicides?

Variation in *Botrytis* isolates

- Isolates from cool and warm climate vineyards
- High genetic variation
- High variation in pathogenicity
- High incidence of benzimidazole resistance, despite absence of recent exposure

Monitoring fungicide resistant populations

- *Botrytis* isolates from samples of bunches (in season) and bunch remnants (dormancy)
- Tested isolates for benzimidazole, dicarboximide and anilinopyimidine fungicide resistance
- Minimum of 30 samples required to monitor incidence/ fungicide resistance
- Action on presence of resistance not severity. Response guidelines developed

Sources of *Botrytis* inoculum in vineyards

- *Botrytis* sporulation on vine canes, bunch remnants, tendrils, buds (from vines and vineyard floor)
- *Botrytis* on other plants in and around vineyards
- Cross inoculation of isolates?
- Field treatments (fluazinam and summer oil) reduced *Botrytis* on over-wintering bunch remnants and canes but did not improve bunch rot control at harvest

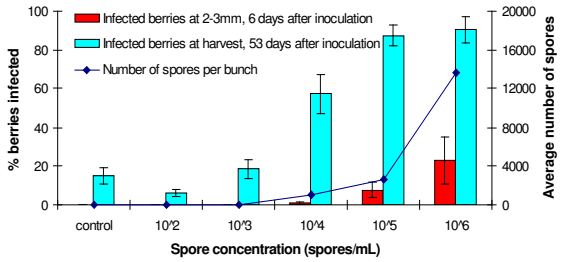
Botrytis variation
Future R&D (5)

We need -

- More knowledge of the effects of *Botrytis* variation on epidemic development and disease management
  - Strain population shifts? Effects on the efficacy of treatments? Climatic differences?
  - Size of resistant populations for loss of fungicide efficacy?

Botrytis spore levels and bunch rot development

- Bunches inoculated with different *Botrytis* spore suspensions at 80% cap-fall.
- Assessed washed spores/bunch, % infected berries in bunches just after berry set and at harvest
- > 1000 spores per bunch (from 10^4 spores/mL) at 80% cap-fall were required for berry infection at harvest



Time of inoculation and bunch rot development

- Bunches inoculated with *Botrytis* spore suspensions at 80% cap-fall, pre-bunch closure and 2 weeks after veraison
- *Botrytis* persisted until harvest in bunches inoculated at each growth stage
- Periods of moist incubation overnight 1-3 weeks before harvest promoted Botrytis rot in bunches on vines by harvest

Botrytis latent infection
Future R&D (6)

We need -

- More knowledge of *Botrytis* latent infection and its management
  - Conditions for latent infection? Role in epidemics?
  - Important for prediction of disease risk, development of control measures?

Fungicide applications at different stages of bunch growth

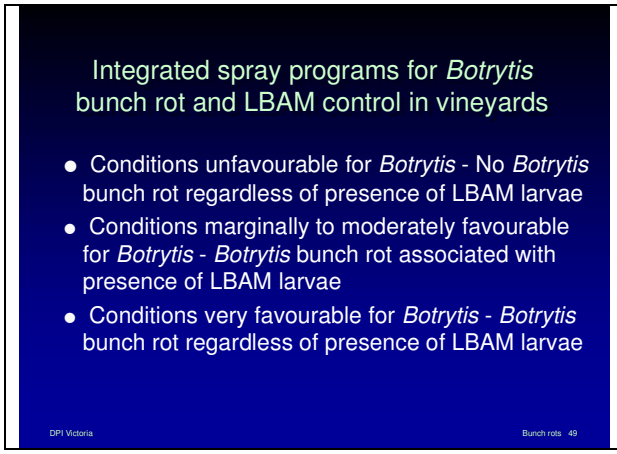
- Fungicide sprays (Scala) were applied to bunches at berry set, pre-bunch closure and 3 weeks after veraison
- Bunches were inoculated 3 days after treatment
- Fungicide treatments closer to harvest provided better disease control

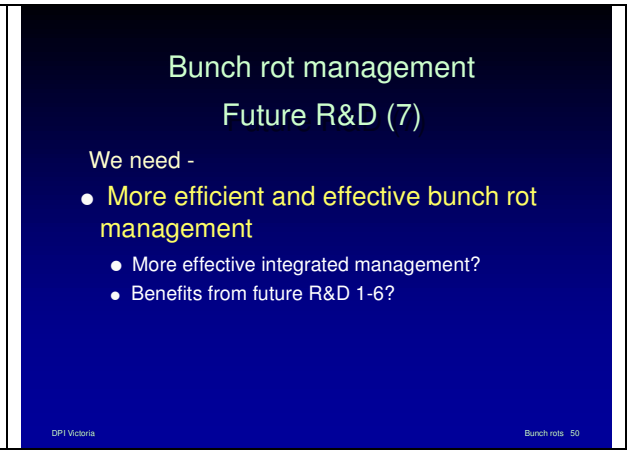
Transmission of *Botrytis* by lightbrown apple moth (LBAM) larvae

- Presence of *Botrytis* contaminated LBAM larvae in bunches at veraison increased bunch rot incidence at harvest
- Viable *Botrytis* propagules were also recovered from faecal pellets of LBAM larvae

Integrated spray programs for *Botrytis* bunch rot and LBAM control in vineyards

- Fungicide sprays were applied at 80-100% cap-fall, pre-bunch closure, veraison or up to 2 weeks after veraison, and at pre-harvest
- Insecticide sprays were applied at 80-100% cap-fall and pre-bunch closure
- Fungicide treatments and other factors affected *Botrytis* incidence in bunches





Dr Ian Dry, of CSIRO, gave a presentation on the potential for developing disease resistant varieties of grapevine, through an increased understanding of host-pathogen interactions. Two broad topics were reviewed: the regulation of stilbene production and cluster architecture. Resveratrol is a type of stilbene or grapevine phytoalexin that has antimicrobial properties. Ian Dry described how the biosynthesis of resveratrol can be induced in berry skins and how levels decrease during ripening. However, resveratrol is not being investigated further as a research strategy because of the effect of elevated levels on delaying ripening. Other research at CSIRO has demonstrated that percentage bunch rot is directly proportional to percentage berry set. A primary aim now is to identify grapevine gene(s) that control bunch architecture. It is known that rachis internode length at flowering is the main determinant of bunch openness. A major gene, the RIE or rachis internode elongation gene, has been identified and the question now is how to modify the gene, either by application of a chemical during early bunch development or by marker-assisted breeding to achieve modified expression of RIE in varieties with tight cluster architecture. Ian Dry then illustrated the wonders of modern genetics by showing a picture of a shoot of a mutant grapevine where all the tendrils had been converted to bunches as a result of a single nucleotide change in a single gene.

Ian Dry then described events happening at the molecular level during early infection by *B. cinerea*. The plant responds by producing reactive oxygen species (ROS) that lead to programmed cell death. The localised death of plant cells can restrict further colonisation of biotrophic pathogens, such as the powdery mildew fungus. However, the necrotrophic *B. cinerea* appears to utilise this biochemical pathway to create dead tissue for colonisation. This hypothesis might explain why ozone damage in plants results in more Botrytis. Ian Dry's group and researchers internationally are now trying to find genes and their products that block programmed cell death. BOS1, for example, is a transcription factor that mediates the response to ROS production. When BOS1 is switched on there are more scavenging enzymes for ROS, and when it is constitutively active in plants it can result in traits such as tolerance of drought and heat. Furthermore, proteins have been identified in animal cells that block programmed cell death and researchers are now trying to find plant homologues (Dickman *et al.* 2001). Ian Dry's group is looking at a class of these proteins, called cystatins. Overexpression of cystatin in tobacco has been reported to block programmed cell death (Belenghi *et al.* 2003). Phytocystatin also appears to be a potent inhibitor of *B. cinerea* (Martinez *et al.* 2003). In order to obtain transgene expression of these proteins from veraison until maturity, the grape GRIP4 promoter could be utilised. CSIRO have also developed a high throughput breeding system that uses microvines (dwarf mutant) capable of two flowering cycles per year. The group noted that these microvines could be useful for pathology research in general.

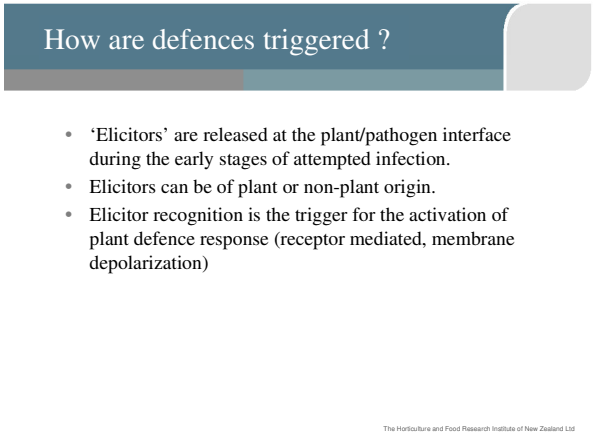
Following Ian Dry's presentation there was general discussion about the practical hurdles that would need to be overcome before application of this 'blue sky' technology. Aside from issues associated with genetic modification, there was the issue that this type of genetic resistance might interact negatively with any type of resistance developed for powdery mildew. There is also the wine industry "obsession" with existing varieties, which would be a significant marketing issue for any new technology. Trevor Wicks suggested placing the transgenes in rootstocks in order to overcome the issue of naming the GM variety. Ian Dry responded by saying that some gene silencing elements can move through the plant. All genetic work with phytocystatin to date has been carried out using leaves, and so the model for berries is yet to be developed.

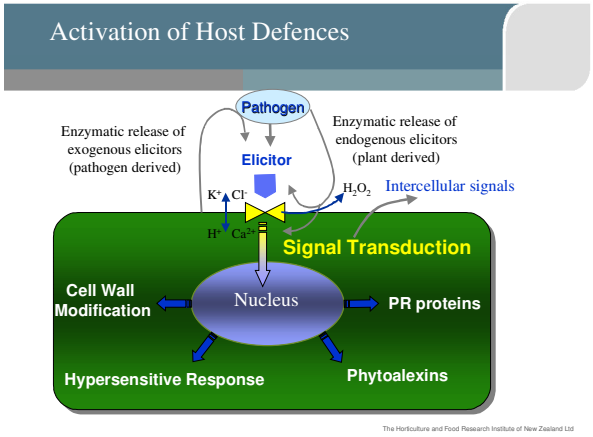
Reference of interest:
Mlikota Gabler F, Smilanick JL, Mansour M, Ramming DW, Mackey BE (2003) Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. *Phytopathology* **93**, 1293-1273.

The final formal presentation was given by **Dr Tony Reglinski,** HortResearch NZ, on the subject of induced resistance. Following a review of the different types of plant disease resistance, Tony Reglinski, defined the various types of inducible defences in plants, including those characterised in grapevine to date. A case study of the application of 5-chlorosalicylic acid highlighted the potential negative impacts associated with some elicitors of induced resistance (Table 3). Practical application of elicitors will depend on a greater understanding of the variation in response in relation to host physiology and environment. As elicitors are not a replacement for conventional chemicals, a strategy for integration with other management measures will be required.

During discussion on this subject, it was suggested that induced resistance probably had greater potential for management of powdery mildew, rather than bunch rot. It was also suggested that any fitness cost leading to reduced vigour or yield penalty might be useful at some vineyard sites. Tony Reglinski distributed a review of biosuppression by Elmer and Reglinski that will be available for citation from *Plant Pathology* in 2006.

Table 3 A selection (14/31) of powerpoint slides presented by Dr Tony Reglinski, HortResearch NZ.





Inducible Defences in Grapevines

Phytoalexins –fungitoxic compounds

- Resveratrol is the major phytoalexin in grapes
  - concentrations range from 40-400 µg per g in berry skins and leaf surfaces (Jeandet et al, 1991; Adrian et al, 2000)
- *B.cinerea* germination was inhibited by 50% in the presence of 90 µg per g in lab assays (Adrian et al, 1997).
- Resveratrol levels are lower in flowers than in leaves and berries.
  - Phenolic concentrations are lowest in the receptacle area, typically where disease is highest.
- Resveratrol levels decline during berry ripening.
- Pterostilbene and viniferin are more antifungal than resveratrol but are present in lower concentrations.

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Inducible Defences in Grapevines

Pathogenesis-related proteins

- Chitinases, glucanases and thaumatin-like proteins
  - accumulate in berries and leaves in response to pathogen attack.
  - degrade structural components in fungal cell walls
- PR-protein expression varies depending on the tissue type, developmental stage and also on the type of infecting pathogen.
- Some PR-proteins are produced constitutively during berry ripening
  - Chitinase and thaumatin-like protein can account for half of the soluble protein in ripe grapes.
  - PR-proteins in ripe grape berries are reported to be responsible for clouding or haze in wines

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Practical Application

Characteristics of Induced resistance

- Requires an active plant response
- Most effective in actively growing tissues
- There is a delay between treatment and the onset of resistance (max response after 4-8 days)
- Induced response can be local or systemic
- Induced resistance is not pathogen specific
- The duration of the induced resistance is variable

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Effects of Inducing Agents on Grapes

Salicylic acid

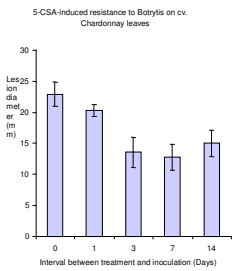
- Induced PR-proteins in grape leaves stems, roots and berries in cv. Pinot noir (Renault et al, 1996, Derckel et al, 1998).
- Ten applications of 0.2% SA (*ca.* 10 mM) reduced downy mildew (*Plasmopara viticola*) by 30% on leaves and by 40% on berries (Kast, 2000)
- Berry ripening of Shiraz grapes was retarded by 2-4 weeks after treatment with SA (7.2 mM) 2-3 weeks before veraison (Kraeva et al, 1998).
- SA treatment caused limited cell necrosis on the berry skin that may be indicative of phytotoxicity (Kraeva et al, 1998)
- Field application of SA may be impractical because of the narrow margin separating rates at which it is efficacious and the rate at which it is phytotoxic.

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Effects of Inducing Agents on Grapes

5-chlorosalicylic acid

- More potent than SA
- Induced resistance to botrytis infection on grape leaves
- Regular applications (every 14-28 days) of 1 mM 5CSA in reduced botrytis bunch rot by 60-90% in cv. Chardonnay and cv Merlot grapes.
- Reduced berry yield & leaf chlorosis
- Phenolics content reduced by 15% in Cabernet Sauvignon wines
- Residues on grapes and in wines



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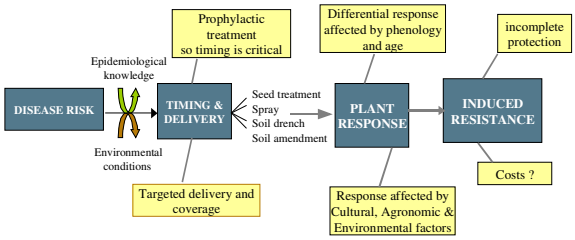
Practical Implementation

Possible Consequences

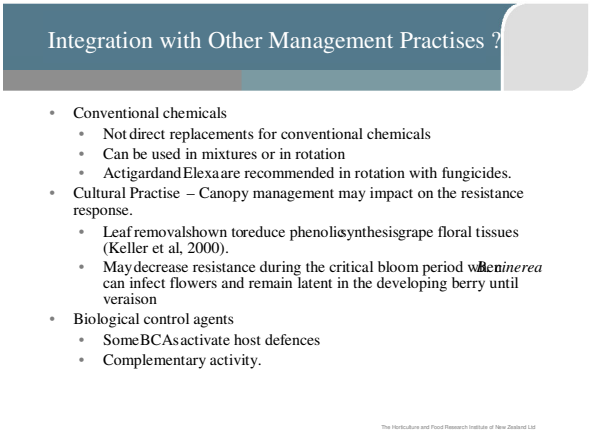
- Changes in phenolic profile in wine - taste
- Elevation of PR-proteins in wines - haze
- Metabolic costs
  - Growth inhibition
  - Yield penalties
- Phytotoxicity
  - Leaf yellowing
  - Vine vigour

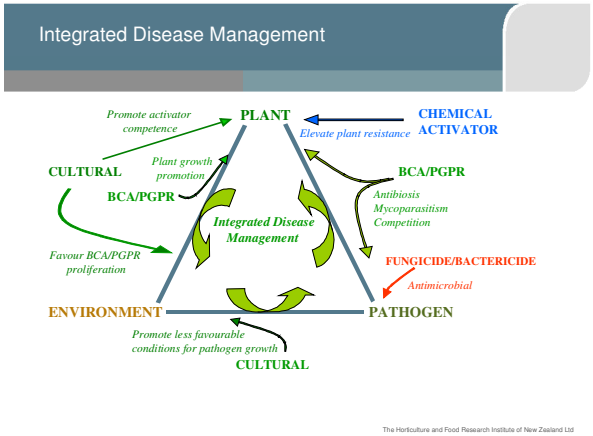
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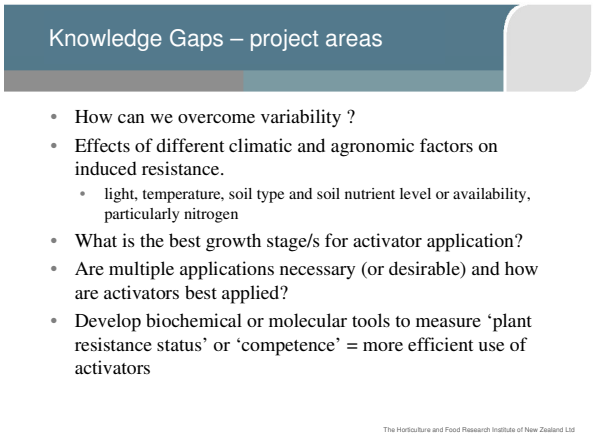
Practical Implementation

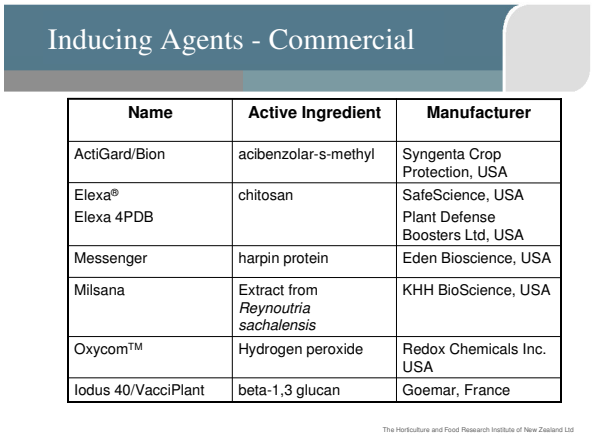


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Name	Active Ingredient	Manufacturer
ActiGard/Bion	acibenzolar-s-methyl	Syngenta Crop Protection, USA
Elexa® Elexa 4PDB	chitosan	SafeScience, USA Plant Defense Boosters Ltd, USA
Messenger	harpin protein	Eden Bioscience, USA
Milsana	Extract from *Reynoutria sachalensis*	KHH BioScience, USA
Oxycom™	Hydrogen peroxide	Redox Chemicals Inc. USA
Iodus 40/VacciPlant	beta-1,3 glucan	Goemar, France

3. Group discussion sessions

The Process

There were four group discussion sessions held over the two days as follows:

1. The pathogen & disease epidemiology
2. Integrated disease management
3. From molecules to adoption of technology
4. Bringing it all together

The first three group sessions were aimed primarily at identifying knowledge gaps under nine general themes. Prior to the workshop, Bob Emmett and Kathy Evans developed a list of elements under each theme, similar to headings that might be used in a review of the literature. Participants were given this list and selected literature before the workshop and asked to highlight, amend or add topics of relevance both to the aims of the workshop and to individual interests (Appendix A). Powerpoint slides of key elements identified by participants were then developed by Kathy Evans to guide discussion under each general theme (see below). A different researcher chaired each theme area, as indicated in the program. The notes page of each powerpoint slide contained the extended list of dot points, to guide the chairperson on the content of the discussion area. As each theme was reviewed, each knowledge gap was recorded on a single sheet of A4 paper and posted on the wall under the heading of the theme. Notes made by Duncan Farquhar during the discussion of each theme area can be found in Appendix B.

Theme 1 – The pathogen and disease

Theme 1 - The Pathogen & Disease I

- Detection of latent infection (*B. cinerea*)
  - crop stage-specific methods
  - molecular-based diagnostics?
- Quantification
  - fungal load at weighbridge, NIR based approach/chemical imaging
- Strain characterisation and variation (biotypes of *B. cinerea*)
  - correlation of biotypes with hosts or tissue types

N.B Standardised disease assessment is in Theme 4 & 7.

Theme 1 - The Pathogen & Disease II

- Economic importance of disease
  - Quantification of regional differences
- Crop loss assessment
  - Assessment of quality at the winery
  - Objective criteria for price penalty

Theme 2 – Inoculum potential, dispersal and infection pathways

Theme 2 - Inoculum potential, dispersal, infection pathways

- Sources of inoculum in relation to infection pathways
  - relative importance, dispersal to infection court
- Infection pathways and relative importance
  - leaves, flowers, fruit
- Epidemiology in conventional versus organic vineyards (also in Theme Area 5)
  - host, pathogen, environmental factors?

Theme 3 – Environmental factors, including host physiology

Theme 3 - Environmental factors, including host physiology

- Factors enabling & promoting infection
  - weather/microclimate/radiation etc
  - ecology of the leaf & berry surface
    - ecological niches of various bunch rot organisms
  - crop nutrition, water status
  - level of constitutive & induced host resistance*
    - chemical, soil-borne elicitors
- Factors enabling & promoting sporulation
  - as above plus resumption of growth after latency*

*Refer to Theme 8

Theme 4 – Monitoring and modeling epidemics

Theme 4 - Monitoring & modelling epidemics

- Standardised monitoring
  - detection of latent infection
  - within canopy microclimate, long range forecasts
- Standardised disease assessment
  - incidence & severity - what? how? sample size?
- Modeling
  - disease progress: temporal, spatial, crop phenology
  - dispersal - short & long range (eg. other crops)

Theme 5 – Manipulating inoculum and host

Theme 5 - Manipulating inoculum & host

- Manipulating inoculum levels
  - Sanitation - when is it necessary?
    - what? - (eg. rachides, aborted berries)
    - how? - (eg. biocontrol, vineyard floor/soil)
- Manipulating the host
  - Cluster tightness - breeding, chemical, clonal
  - Microbial ecology of the leaf & fruit surface
  - Organic versus conventional production systems

Theme 6 – Crop Protection

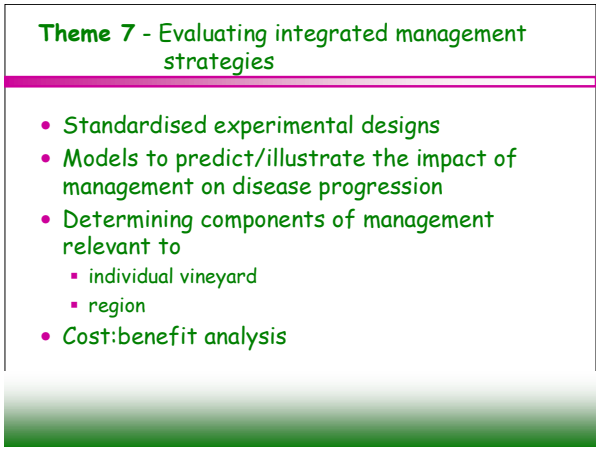
Theme 6 - Crop Protection I

- Status of biological control & 'alternatives'
  - single target and multiple target agents (multiple agents, compost extract)
  - restorative disease management
- Timing of chemical or inundative biocontrol based on
  - agent mode of action, chemical group, MRL
  - pathogen activity & weather-based disease forecasting
  - susceptibility of host tissue

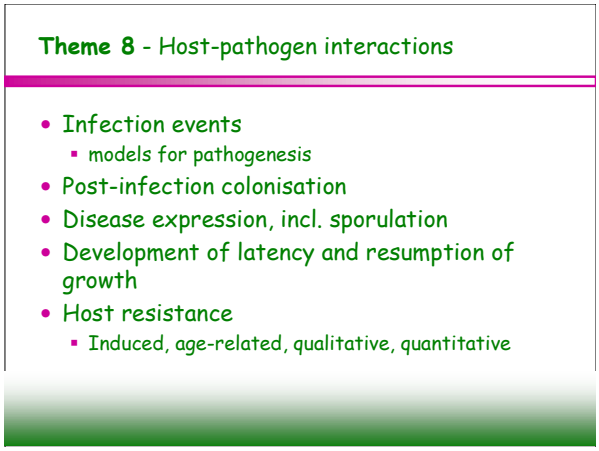
Theme 6 - Crop Protection II

- Minimising non-target, negative impacts
  - Ecological niches for other bunch rotters?
  - Spray coverage and effective dose
  - Impact of adjuvants on berry waxes
  - Detecting & managing fungicide resistance
  - Fungicide free wines in the POST MRL era

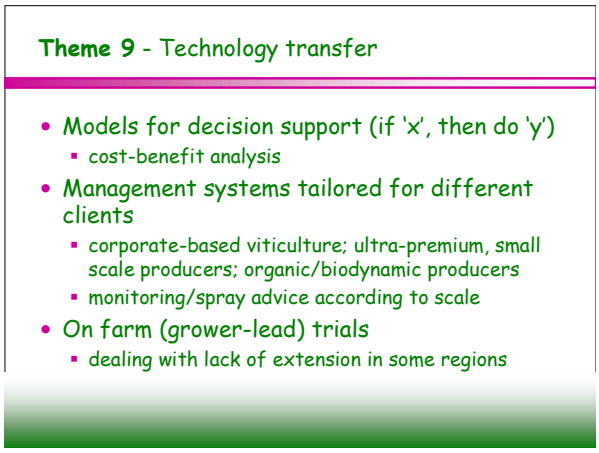
Theme 7 – Evaluating integrated management strategies



Theme 8 – Host-pathogen interactions



Theme 9 – Technology transfer



The final group session involved the group splitting into five working groups, each being allocated two theme areas from which to synthesise preliminary research proposals, using the knowledge gaps posted on the wall. The process included proposing a research topic and then listing the objectives of the research. There was also the option to categorise the research in one of three categories: applied research, experimental development or strategic basic. The group decided against categorising the importance and/or urgency of the proposed research to industry, given that there are other forums for such priority setting. Each working group was

advised to draw on items from other theme areas, if there was a relevant linkage and/or if some knowledge gaps were categorised inappropriately. The group was also advised to prepare their topics without a specific funding body in mind, so that each proposal would reflect the knowledge gap transparently and without skewing the proposed objectives towards the strategic plan of any one funding body. This would enable individuals to use the information in different ways, especially when developing full proposals for different sources of funding.

Once preliminary proposals were prepared, a representative from each working group presented their topics to the whole group and there was much discussion among the working groups when two groups identified the same proposal, and/or a different approach to the same issue. After this task, Kathy Evans led a discussion on options for obtaining research funding. The group was also reminded of the GWRDC RITA program for technology transfer and for engaging industry. Participants from New Zealand related their system and there was some discussion about mechanisms for coordinating trans-Tasman research. Finally, the group agreed that it would be useful to relate any application for funding back to this particular forum.

Outcomes

After the workshop, Kathy Evans collated the preliminary research proposals and sent them out to the group for corrections and/or additions.

Table 4 contains a list of topics for research developed at the workshop and is followed by the description of each preliminary research proposal.

Table 4 Topics of research grouped roughly under discipline area. Each topic or subset of research objective within a topic may be used as a module for developing a multidisciplinary project.

No.	Orig. No.	Title
		Host-pathogen interactions
1	3	Pathways of latent infection to symptom expression OR Impact of latency on bunch rot of grapes
2	4	Understanding the process of primary infection in mature berries and berry-to-berry spread of bunch rot
3	2	Impact of strains of *B. cinerea* on management of bunch rot
		Disease epidemiology
4	6	Predicting bunch rot severity at harvest
5	7	Identifying potential differences between organic and conventional viticultural systems in levels of pathogen infection and bunch rot expression
6	9	Understanding bunch rot complexes
		Biosuppression
7	8	Understanding the mechanism of disease suppression provided by BCAs and other beneficial organisms present either naturally, restored or augmented
8	17	Development of BCAs, elicitors of induced resistance & other biosuppressants.
		Disease management
9	11	Evaluating management strategies that reduce inoculum
10	12	To develop effective strategies for late-season management of bunch rot
11	10	Chemical or biological control strategies for sites with extended flowering periods and incidence of free moisture
		Technology transfer
12	14	Collating a *Botrytis* message framework
13	15	Making data available to researchers for continuous improvement of management strategies and benchmarking
14	18	Processes that support adoption of research at a regional level.
		Method development
15	1	Detecting latent infection consistently, rapidly and accurately
16	5	Standardised protocols for generating and managing data on microclimate and bunch rot incidence and severity
17	16	Tracking the fate of chemical residues in wine and options for removal at the winery
18	13	Standardised protocols for testing efficacy of developmental fungicides

Topic 1

Pathways of latent infection to symptom expression AND/OR
Impact of latency on bunch rot in grapes

Benefits of research

An ability to measure and track latent infection is likely to be more important than knowing the original source of inoculum. More specifically, the level of latent infection is likely to be a better indicator of bunch rot at harvest than knowing the source and amount of inoculum present at flowering. An understanding of this pathway and its relative importance may lead to novel measures for disease and risk management that account for differences among grape varieties and conditions necessary for establishing latent infections. The latter knowledge is essential for timing the delivery of BCAs or crop protectants in relation to pathogen activity. Variety-specific knowledge of the resumption of growth after latency and the mechanism involved may also provide clues for manipulating the host for increased resistance to disease.

End users of research

Other researchers
Growers, when improved management measures are developed.

Objectives

The first objective is to identify key variables (host, pathogen, environment) that determine the pathway from latent infection to symptom expression. Within this overall objective, the key aims are

1. To determine the role of host factors and the influence of germplasm (varieties) in establishing and maintaining latency
    - Are all varieties susceptible to latent infection?
    - Is there a genetic basis between clones of a grapevine variety?
    - Are *B. cinerea* infections latent in the cap tissue prior to the first fungicide application? (Are applications of protective fungicide at 5% flowering too late?)
    - Is the latent infection growing or quiescent during berry development? If quiescent, which structures are quiescent (hyphae, appressoria, germ tubes)? What is the biochemical nature of the quiescent phase?
    - What happens after the appressorium forms?
    - What is the relationship between berry development (ripening) on latency and fungal development?
2. To determine if the infection process is the same in berries and leaves.
3. To determine and quantify the effect of key environmental factors on establishment of latent infections and subsequent disease expression.
    - When do latent infections establish and what microclimate conditions promote latent infection and symptom expression? (refer to related proposal)
    - What are the infection criteria (Broom model?) for establishment of a successful latent infection?

The second objective is to quantify the relative importance of the latent infection pathway versus other infection pathways, especially the necrotic tissue pathway, as a function of bunch rot severity at harvest and in relation to weather events from flowering until harvest. This second objective could be incorporated in the proposed epidemiological study on ‘Predicting bunch rot severity at harvest’.

The third objective is to apply knowledge generated in this proposed project to evaluate efficacy and timing of applications of chemical or biological agents for the control of latent infections (refer to related proposal on extended flowering).

Preliminary ideas on approach to research

1. Material collected from the proposed epidemiological study on 'Predicting bunch rot severity at harvest' can be frozen for cytological/molecular-based studies.
2. Consider using 'dry' spores for inoculation in experiments on latency?
   Comment from Eileen Scott:
   "Recent experience with strawberry (Matchima Naradison's PhD project, with Amanda Able, Margaret Sedgley and myself) suggests that inoculating flowers to produce latent infection may be somewhat 'hit or miss'. Some humidity is needed to get infection established, but Matchima found that a considerable proportion of the flowers died as a result of inoculation. After much trial and error, she is applying a micro drop of spore suspension on individual flowers, then bagging each flower overnight to facilitate entry. This method arose from discussions with Dani Shtienberg and Yigal Elad. It would be good to liaise with them about what they have tried and, perhaps, to look at effects of inoculum concentration and relative humidity on incidence and success of latent infection."
   Comment from Bob Emmett:
   "Recent experience with *Aspergillus carbonarius* on grapes also indicated that inoculum concentration (bunch spore load) appears to influence the success of latent infection and/or the persistence of the pathogen in bunches from flowering until harvest. The pathogen persisted in bunches inoculated at 10^5 spores/ml but not in bunches inoculated at 10^2 spores/ml."

Topic 2

Understanding the process of primary infection in mature berries and berry-to-berry spread of bunch rot

Benefits of research

An understanding of this process may provide a novel means for breeding varieties of grapevine that are resistant to bunch rot. In the shorter term, this knowledge might give rise to development of non-toxic crop protectants that prevent adhesion of fungal appressoria or that block fungal penetration.

End users of research

Other researchers

Objectives

1. To determine the role of berry stomata (pores) in penetration and sporulation of *Botrytis cinerea*
   - What is the relationship between inoculum density and bunch rot severity?
   - Standardised technique for inoculation needs to be developed, either for wet inoculation (eg. 10^4 spores/ml) or (preferably?) for dry conidia applied in a spore setting tower.
2. To determine the genetic basis for stomate density
3. To investigate the impact of pore-plugging compounds on fungal penetration and sporulation
4. To determine the role of appressoria (biochemical events associated with appressorium formation and adhesion?) during infection of mature berries.
5. To identify events after formation of appressoria.
6. Why is moisture needed for sporulation on the berry surface?

Preliminary ideas on approach to research

Dose response experiments using dry conidia?
This research is potentially an 'across crop' issue, given work by others (Holz?) on cytology (c.f. Sclerotinia). Apply GFP (tagged) strains to microvines in a PC2 glasshouse?
Comment from Eileen Scott:
Confocal microscopy and Scanning EM could be useful here – last year I helped a student of Jenny Watling's (in environmental biology at UA) examine fungal penetration of stomata on leaves using these approaches, the combination worked well.

Topic 3

Impact of strains of *B. cinerea* on management of bunch rot

Benefits of research

Botrytis cinerea is a genetically diverse plant pathogen, yet current management measures are aimed at the species as a whole. Knowledge about pathogen strain variation may indicate the need to treat sub-specific causes of bunch rot differently. Understanding the evolution of *B. cinerea* (changes in genetic structure) may also indicate management practices that are selecting for higher disease pressure (eg. fungicide resistance, cultivars deployed, movement of deleterious strains to new areas).

End users of research

Researchers, growers

Objectives

1. To investigate variation in pathogenicity on different hosts (cultivars, species (other crops))
    - measures of pathogenicity may include symptom expression, parameters of fitness or dispersal
2. To investigate whether or not strains infecting grapevines have different infection pathways and/or contribute to variation in disease progression among sites
3. To develop diagnostic tools for understanding failures in disease management
    - What proportion of individuals in a population must be resistant before a reduction in fungicide efficacy is observed?
4. To understand the evolution of *B. cinerea* at different levels of hierarchy (within, among vineyards, among regions) using neutral (DNA) or selective markers (eg. fungicide resistance).

Preliminary ideas on approach to research

Follow up recent research conducted by Mary Cole's group.
Comment from Tony Reglinski:
Preliminary work at HortResearch indicated a difference in the response of different *B. cinerea* strains to induced resistance on grape leaves.

Topic 4

Predicting bunch rot severity at harvest.

Benefits of research

Once the relationship between bunch rot severity at harvest and key drivers (host, pathogen, environment) is quantified, then evaluation of specific management measures, such as leaf plucking at a particular time, can be assessed in terms of a readily quantifiable benefit:cost ratio. The ability to track pathogen activity in vineyards using standardised sampling protocols for key drivers will allow data to be used for decisions on disease management.

End users of research

Other researchers
Growers, if a simple tool/decision rule is packaged in a system of delivery.

Objectives

The following objectives were written for bunch rot caused by *B. cinerea*, but the experimental approach could apply to other causes of bunch rot.

1. To identify key variables (host, pathogen, environment) that predict bunch rot severity at harvest.
    - Is qualitative knowledge about canopy type sufficient for bunch rot management? Is it necessary to determine the relationship between key indicators of canopy density (e.g. leaf density in bunch zone? air flows? east or west exposure?) and final bunch rot severity? To quantify pathogen activity in the vineyard and how that translates to final bunch rot severity
        - What are the key environmental factors that allow establishment of latent infections and subsequent disease expression (refer to related proposal)?
        - What is the relative importance of the latent infection pathway versus other infection pathways, especially the necrotic tissue pathway, as a function of bunch rot severity at harvest and in relation to weather events from flowering until harvest?
2. To develop a model describing the relationship between key variables, late season disease increase and final bunch rot severity
    - Describe disease progress in each region and use the rate of late season disease increase to predict final bunch rot severity
3. To validate the model in different vineyards, regions and growing seasons.
4. To determine if the model can be applied universally and, if not, to modify and validate it to account for regional or site differences.

Preliminary ideas on approach to research

1. Identification of the sources of inoculum is a complex task, with sources likely to vary from vineyard to vineyard, from year to year and from region to region. Research efficiency will be improved if there is a focus on the level of inoculum in or on developing berries, for example, using indicators such as the level of latent infection.
2. Adopt standard protocols for microclimate and disease assessment across research sites as per the related research proposal on this subject.
3. Quantify latent infections and key drivers for their development accurately as per the related research proposal on this subject
4. Identify the relationship between Brix (maturity) and susceptibility of individual berries to infection. Brix at harvest can be predicted using microclimate data (R. Beresford). Therefore, harvest date can provide an indicator of the likely severity of bunch rot, if disease potential and/or rate of increase post veraison is known.

5. Develop an understanding of the ecology/ecological niches of the different bunch rotting organisms. Develop separate or integrated models that account for organisms that vary in ecology.
6. Refer to GWRDC soil and water initiatives on management of irrigation water in relation to bunch tightness and berry splitting.
7. Regarding nutrients like nitrogen and calcium, the direct and indirect effects of nutrients (eg. vigour) could be separated in a long-term study (more than one growing season).

8. Topic 5

Identifying potential differences between organic and conventional viticultural systems in incidence and severity of pathogen infection and bunch rot expression.

Benefits of research

- To determine the basis for anecdotal reports that lack of expression of bunch rot in organic vineyards is biologically based and not just the result of sub-optimal microclimate for infection and/or disease expression.
- If the difference is biologically based, to determine what factors limit bunch rot expression in organic vineyards. Management measures that promote these factors could then be trialed in conventional vineyards.

End users of research

Researchers, growers

Objectives

1. To quantify the difference in incidence of bunch rot infection and severity of symptom expression in organic and conventional vineyards.
   - conventional vineyards might be categorised further into 'type' of conventional vineyard or general production system.
2. To identify which host, pathogen and/or environmental factors are correlated with the difference, if it exists.
   - sources of inoculum, microclimate, host susceptibility (including nitrogen content and general vineyard nutrient inputs), leaf and berry microbiota, spray deposits, relative incidence of latency
3. To study the parameters associated with the difference for investigating 'cause and effect' and for explaining 'why' the difference exists.
4. To apply the knowledge generated to improve management in conventional vineyards.
   - How do rootstocks affect vigour and bunch/berry size?

Preliminary ideas on approach to research

1. This study will require collection of standardised epidemiological data from 30-50 vineyards, across regions and countries (Australia, New Zealand), in a longitudinal study conducted over a minimum of three growing seasons. The minimum data set should include standardised data for microclimate in relation to spray program and the level of latent infection and bunch rot severity at harvest.
2. If a difference between organic and conventional vineyards in bunch rot infection and/or expression is detected, then statistical analysis of indicators of crop or environmental status will need to be selected judiciously and analysed with sufficient statistical power. Examples of indicators for correlation analysis might include vigour at veraison, petiole analyses (nitrogen content), Brix at onset of visible symptoms and at harvest, above and below ground microbial activity, berry waxes and/or selected components of berry biochemistry.
3. Another approach is using 'matched pairs', where a vineyard of each type is inoculated at the same time to obtain controlled data.

Topic 6

Understanding bunch rot complexes.

Benefits of research

Bunch rot may be caused by pathogens other than *Botrytis cinerea*. Development of effective management `requires knowledge of the identity and ecology of species or species complexes causing bunch rot.

End users of research

Researchers, growers

Objectives

1. To develop rapid and accurate methods for identifying 'other bunch rot' (OBR) pathogens in the laboratory and the field.
   - To develop a key for growers to identify different causal organisms in the field
   - To quantify what and how much of each causal organism is present at the weighbridge
   - To contribute to development of economic thresholds for pathogen load at the weighbridge
2. To investigate the ecology and epidemiology of OBR pathogens
   - pathways of infection
   - host and microclimate conditions required for infection and symptom expression
   - dispersal gradients
   - interaction with other bunch rot pathogens, in the presence or absence of *B. cinerea*
3. To determine the impact of OBR pathogens or complexes on crop yield and quality
4. To quantify regional variability in incidence and severity

Preliminary ideas on approach to research

Comment from Eileen Scott:
"Effects of mixed inoculum and wounding, at various stages of berry development, on disease. As well as interactions of OBR with Bc, it would be valuable to determine what happens if *Aspergillus carbonarius* (all strains tested to date produce ochratoxin A) and *A. niger* (few strains produce OA) are co-inoculated? Also, should look at *Penicillium* spp. and *Colletotrichum acutatum."*
Comment from Bob Emmett
"Research centres such as DPI Mildura have Chardonnay vineyards (unsprayed for bunch rot control) that could be used to study the effects of different pathogens alone or in combination on the development of bunch rots."
Information from Chris Steel:
GWRDC project # CSU03/01 will commence in 2006
Title: Management of non-Botrytis bunch rots
This project will be largely based on sub-tropical rots, i.e. bitter rot and ripe rot. It will include

- An A3 poster for growers on bunch rot identification as part of the extension component of this project.
- Studies on the ecology and epidemiology of OBR pathogens, including pathways of infection, host and microclimate required for infection, symptom expression and interactions with berry microflora. Work includes how environmental factors affect the growth of different organisms in a bunch rot complex.
- Limited investigation of regional variability (NSW & Qld) on incidence and severity

Topic 7

Understanding the mechanism of disease suppression provided by BCAs and other beneficial organisms present either naturally, restored or augmented.

Benefits of research

Continued development of BCAs, especially those that are compatible with fungicides in integrated management, will expand control options and aid management of fungicide resistance and residues in wine. Understanding the mechanism of disease suppression by biological means adds to the theory of biological control with the likely outcome being more rapid and efficient development of effective BCAs. A greater understanding of microbial ecology in the vineyard will provide clues as to how the environment can be manipulated to promote beneficial organisms. The level of disease suppression obtained by augmenting the natural microflora, either with compost extracts and/or removal of certain pesticides, will depend on knowledge of the role that microbial abundance, activity and diversity play in limiting the development of bunch rot.

End users of research

Other researchers
Growers, if a simple tool/decision rule is packaged in a system of delivery.

Objectives

1. To characterise the interaction of single-target BCAs with their target pathogen
    - Does the pathogen have constitutive and induced defence reactions?
    - What are the mechanisms of disease suppression?
    - Can the BCA prevent latent infection?
2. Does manipulation of the microbial ecology of phyllosphere and/or fructosphere lead to disease suppression?
    - What is the mode of action of 'compost extracts'?
    - What is the impact of milk or whey on the natural microbiota in different regions?
    - Which fungicides reduce natural microbial diversity and, if so, what is the impact on bunch rot suppression when the fungicide residue decays below the lethal dose? (Restorative disease management is the subject of research by a group at HortResearch, NZ)

Preliminary ideas on approach to research

Comments from Eileen Scott:
Sampling of (leaves? and) bunches inoculated in the field (and glasshouse) with BCAs (single or combinations of BCAs) or treated with compost extracts, milk, whey or whatever for:

Population of BCAs over time
Population of pathogen over time
Population dynamics of natural/ indigenous microbiota (e.g. metapopulation studies using PCR-based identification of microorganisms – to look at total diversity, changes in individual species and/or groups of organisms). Liaise with GWRDC UA03/03, Sustainable control of powdery and downy mildews …Carol Walker is looking at microbial population and diversity on bunches.
Need to take into account environmental parameters, cv, canopy, presence or absence of wounds etc.

Comment from Dean Metcalf:
The key sites for these studies are flowers, latent infections in berries, spread within bunches. The key mechanisms to test for are competition, antibiosis, chitinases/glucanases, and plant host resistance mechanisms.

Topic 8

Development of BCAs, elicitors of induced resistance and other biosuppressants.
(refer to related proposals on late season disease management)

Benefits of research

Continued development of BCAs and other biosuppressants will expand control options and aid management of fungicide resistance and residues in wine. BCAs with different modes of action for concurrent or staggered application will improve integrated management, especially if they are also compatible with fungicides or other crop protectants.

Gene expression in plants can be activated for induction of disease resistance, either by natural or synthetic chemicals applied to the soil or foliage, or by microorganisms present in the soil or on plant surfaces. These biosuppressants are collectively referred to as 'elicitors'. To date, application of elicitors for suppression of bunch rot has produced variable results. This variability might be overcome with better knowledge of the effect of plant tissue 'competence' for induced resistance. Correct timing of elicitors could reduce variation in plant response and provide another tool for integrated management of bunch rot.

End users of research

Other researchers
Growers, if a simple tool/decision rule is packaged in a system of delivery.

Objectives

1. To identify and develop BCAs that suppress development of bunch rot early, mid and late season
   - Ideally, multiple BCAs (biological mixtures) with multiple modes of action
   - BCAs that are compatible with fungicides and integrated disease management
2. To survey Australia for the presence of *Ulocladium oudemansii*, so that BOTRY-Zen® can be developed/applied in Australia. (BOTRY-Zen® has been trialed in Italy)
3. To identify and develop natural or synthetic elicitors for suppression of bunch rot
   - Overcome variation in response to elicitors by understanding the effect of crop physiology (eg. nutrition) and environment (climate, soil ecology)on plant tissue 'competence'
   - Quantify potential impacts, such as changes in phenolic content, effects on berry yield, elevation of PR proteins in wine (haze), residues in wine and/or phytotoxicity
4. To develop biochemical or molecular tools to measure the status of plant resistance or 'competence' in relation to application of an elicitor.

Topic 9

Evaluating management measures that reduce inoculum.

Benefits of research

Identification of the sources of inoculum is a complex task, with sources likely to vary from vineyard to vineyard, from year to year and from region to region. However, once the relationship between bunch rot severity at harvest and key drivers (host, pathogen, environment) is quantified, then evaluation of specific management measures that reduce inoculum can be assessed in terms of benefit:cost of the management measure.

End users of research

Other researchers
Growers, if a simple tool/decision rule is packaged in a system of delivery.

Objectives

1. To demonstrate that reducing inoculum in the vineyard reduces final bunch rot severity at harvest.
2. To investigate the use of fungicides or BCAs to reduce overwintering inoculum, especially after machine harvesting
3. To identify and evaluate BCAs and other methods of reducing inoculum, for example, mycoparasitism of sclerotia.
4. To develop a management strategy for reducing inoculum in 'abandoned' vineyards, including neighbouring 'abandoned' crops and grapes left on the vine for economic reasons.

Preliminary ideas on approach to research

Comments from Dean Metcalf:
Inoculum reduction has been used with great success as a tool to manage *Botrytis allii* in onions. While *B. cinerea* is more ubiquitous, the principle of inoculum reduction cannot be ignored.

Fungicides are not likely to be effective against resting sclerotia.

Sclerotial parasites should be reasonably easy to find and screen.

Topic 10

To develop effective strategies for late-season management of bunch rot.

Benefits of research

While latent infection establishes *B. cinerea* in the grape cluster, it is the expression of disease in ripening berries plus direct infection and berry-to-berry spread that determines bunch rot severity at harvest. More effective management strategies applied post-veraison will ensure that the benefits of integrated management measures applied pre-veraison are realised.

End users of research

Other researchers
Growers, if a simple tool/decision rule is packaged in a system of delivery.

Objectives

The following objectives can be applied to *B. cinerea* and other causes of bunch rot.

1. To identify and develop BCAs that suppress development of bunch rot post veraison
   - Ideally, multiple BCAs (biological mixtures) with multiple modes of action
   - BCAs that are compatible with fungicides and integrated disease management
2. To investigate further the effect of spray adjuvants on development of bunch rot
   - Microscopy to characterise and quantify effect on waxes, cuticle, stomata
   - Potentially an 'across crop' issue
3. To identify and develop 'soft' chemicals that do not 'strip' waxes from maturing berries.
4. To time applications of fungicides in relation to accurate prediction of infection periods.
5. To improve/optimise spray coverage on berries.
6. To ensure replicated clonal trials or selection of rootstocks includes quantification in differences in cluster tightness, vigour and/or susceptibility to physical damage.

Preliminary ideas on approach to research

This proposal could be linked tightly with topic number 2 and other proposals involving BCAs.

Topic 11

Chemical or biological control strategies for sites with extended flowering periods and incidence of free moisture.

Benefits of research
Strategic application of fungicides during flowering will ensure materials are applied according to pathogen activity and microclimate conducive to establishment of latent infections. Improved protocols for evaluating fungicides during flowering will assist the regulatory process.

End users of research
Growers and researchers involved in evaluating control agents.

Objectives
1. To develop decision rules for timing fungicides during extended flowering periods, based on the characteristics of individual fungicides, the percentage capfall and the incidence of free moisture.
   - How many sprays should be applied and when?
   - What is 80% capfall when individual bunches can vary from 20 to 100% capfall?

2. To evaluate efficacy and timing of applications of chemical or biological agents for the control of latent infections using new knowledge of when latent infections occur (refer to related proposal on pathways for latent infection).

Preliminary ideas on approach to research
1. David Gadoury, Cornell University, has described percentage cap fall as a series of 'cohorts' for regions with warm winters, like Australia. These models could be applied for more accurate identification of stages of capfall.
2. With more accurate definition of the stages of capfall and simple methods for assessing this in the field, then field trials could focus on testing different application times (based on thermal time?).
3. Forecasts of rain could be incorporated in the decision rule for those sites where it does not take multiple days to spray the whole vineyard.

Topic 12

Collating a *Botrytis* message framework

Benefits of research
Improved disease management.

End users of research
Vineyard managers, consultants, company tech. reps, resellers.

Objectives
1. To develop a heirarchical web resource for information dissemination
   - photographs, other sources of information plus FAQ
     eg. How important is grapemarc mulch as a source of *Botrytis* inoculum? When should leaf plucking occur for optimising wine quality and bunch rot management?
   - accessible to reseller networks, company technical representatives, vineyard consultants and managers
2. To deliver weather-based, region-specific prediction of bunch rot across Australasia.
   - Identify marginal years for bunch rot development and how many marginal years there are for each region. Use this knowledge to predict when additional management measures are needed.
3. To deliver techniques for sprayer set up, calibration and operation for optimising spray coverage and for achieving an effective dose in the bunch zone, including advice on the use of adjuvants

Preliminary ideas on approach to research
Utilise Bureau of Meteorology in product development.

Topic 13

Making data available to researchers for continuous improvement of management strategies and benchmarking

Benefits of research

Individual growers and wine companies have a wealth of recorded data that demonstrates their performance in terms of bunch rot management. The existence of benchmarks for bunch rot management would allow producers to compare their performance with an industry standard.

End users of research

Researchers, growers, wine companies

Objectives

1. To develop the means for growers and wine companies to provide researchers with data on bunch rot.
   - development and delivery of standard procedures for monitoring.
2. To use grower data to develop an industry standard or benchmark for bunch rot management
3. To develop a process for researchers to return data received from growers that indicates individual performance in comparison to the industry benchmark.

Topic 14

Processes that support adoption of research at a regional level.

Benefits of research
Booklets and information pamphlets do not drive adoption. How do we support adoption on a regional level, especially for infrequent events?

End users of research
Vineyard managers, consultants, company tech. reps, resellers.

Objectives
To investigate processes that lead to adoption of research

- Delivering a simple message for a complex system such as bunch rot
- Resource intensive extension to support people and decision making, where regions set their own priorities and framework eg. standardisation of weather networks

Preliminary ideas on approach to research
Rural sociology project

Topic 15

Detecting latent infections consistently, rapidly and accurately

Benefits of research

Current techniques are mostly based on ONFIT – or overnight freeze and incubation technique, although some researchers use paraquat instead of freezing. Worldwide, researchers use different methods to measure latent infection, with variation in the type of sterilisation technique and/or treatment of whole bunches or dissection of individual berries that are treated with paraquat/frozen then plated. This procedure is conducted inconsistently and consumes large areas of freezer and bench space and the lengthy incubation period means that results may be unavailable in time to make appropriate management decisions. This method also assumes that surface sterilisation techniques are effective. While ELISA methods have been developed for symptomatic tissue, the time of appearance of the test band is related to the concentration of *Botrytis* antigens in the sample. One participant indicated that this method requires presence of conidia of *B. cinerea* and would not be effective for use with presymptomatic material.

The benefit of developing a standardised technique that is rapid and accurate is that conclusions about the potential impact of latent infection on disease progression will become consistent and meaningful.

End users of research

Researchers, growers

Objectives

To develop rapid and accurate methods for laboratory and field application for identifying the incidence of latent infection in grape clusters.

- Determine appropriate sampling strategy and test material, accounting for within-block variability and regional differences
- Identify threshold levels of latent infection that contribute significantly to final bunch rot severity (refer to related proposal)

Preliminary ideas on approach to research

Real time or quantitative PCR?
Refer to related proposal on latent pathways.

Topic 16

Standardised protocols for generating and managing data on microclimate and bunch rot incidence and severity.

Benefits of research

- To ensure data generated in Australia and New Zealand are sufficiently consistent in rigour for pooling of data to address common research or management goals efficiently.
- To facilitate rapid assessment of applications by regulators for new technology.

End users of research

Researchers, regulators, growers

Objectives

To reach consensus on how bunch rot incidence and severity should be measured for each research or production goal.

To develop standard protocols with consistent replication and statistical power suitable for the following purposes:

For growers and extension/technical staff:

1. Tracking/quantifying pathogen activity before or during the growing season for making decisions on management.
2. Self assessment of effectiveness of disease management in relation to industry benchmarks generated using standard protocols for monitoring.
   - Develop a key for growers that enables identification of different causal organisms in the field
   - Develop a standard protocol for sampling and assessing the level of bunch rot at harvest
3. Low precision methods for regional surveys of disease impact
4. Quantification of fungal load at the weighbridge.

For researchers, technical staff and regulators:

5. Identifying key variables (host, pathogen, environment) that enable prediction of bunch rot severity at harvest (see related proposal).
6. Evaluation of crop protectants applied early, mid and/or late season.
7. Evaluation of biosuppressants (eg. BCAs) to reduce inoculum.
8. Evaluating modes of action of fungicides or BCAs
9. Evaluation of overall or specific vineyard management practices at various levels of hierarchy, such as within a single block, among vineyard blocks and varieties, between vineyards and across regions.

   For example, identifying potential differences between organic and conventional vineyards in bunch rot incidence and severity would be an example of a ‘between vineyard’ and ‘across region’ study.

Preliminary ideas on approach to research

1. Use existing data as a basis for protocol development
   - Collate what researchers/practitioners do now in terms of methodology. Develop a spread sheet for data formatting (across regions, countries)
   - A one-year ‘desk top’ study?
   - Deal with a lack of standard methodology to date by applying new biometrical, computational approaches for mining historical data (metaanalysis?)

Topic 16 continued

2. If necessary, conduct field experiments or grower-led trials to validate sampling techniques.
3. Prepare a 'living document' for incorporation of new technology (eg. molecular diagnostics) as it becomes available.
4. Study/interpret standardised methods of other countries (France?).
5. Linkages with current work in New Zealand on microclimate research using GIS tools for interpolation of weather stations.
6. Eileen Scott: there might be a role for Near Infra Red reflectance spectroscopy (NIR) for quantification of fungal load at the weighbridge. We have done some preliminary experiments with NIR and Botrytis as part of the CRCV project on fungal contaminants and wine quality. NIR showed some promise for bunch rot and is included in a preliminary project application that Belinda Stummer, Bob Dambergs (AWRI) and I put to GWRDC. If that is funded, there could be a link here.

Topic 17

Tracking the fate of chemical residues in wine and options for removal at the winery**.**

Benefits of research

There are severe restrictions on the type and amount of botryticides that can be applied post flowering. An ability to remove fungicide residues at the winery would increase options for late-seasons disease control. The disadvantage of this approach is that it adds costs at the winery and it might encourage people to grow grapes in suboptimal sites.

End users of research

Growers, Winemakers

Topic 18

Standardised protocols for testing efficacy of developmental fungicides.

Benefits of research

The label recommendation for the weight or volume of fungicide to mix per 100 L (dilute spraying) is derived from trials that usually involve application of the material with a hydraulic spray gun fitted with a single nozzle. The actual spray coverage and dose achieved in various parts of the grapevine canopy is usually vastly different to that achieved by grower operated machinery. Furthermore, regulators have difficulty assessing the 'real world' efficacy of the test material and the quality of work conducted when R&D staff use different methods to generate data. Standardisation of methods for spray trials that reflect commercial production conditions would ensure that the correct amount of fungicide is delivered in the right place at the right time. (An important component of sustainable viticulture)

End users of research

Researchers, regulators, growers

Objectives

1. To develop and publish instructions for building small-scale spray equipment that mimics large-scale commercial machinery
   - suitable for spraying single panels in randomised complete block designs
   - prototypes required for different trellis systems (eg. VSP versus Aussie sprawl)
2. To develop 'whole-of-block' experimentation for comparing a developmental spray program with current grower 'best practice'.
3. To identify all the crop production and microclimate parameters that should be reported for efficacy trials
   - Refer to respective recommendations for each country (eg. Australia, NZ, EU)
4. To incorporate standardised protocols for monitoring and measuring disease incidence and severity (refer to related proposal).
5. To utilise improved knowledge of when latent infection occurs (see related proposal) to test appropriate timing of a developmental chemical in relation to a program of industry standard chemicals applied at the right time.
   - Which chemical is best applied at 10% capfall? Which applications require 'kick-back activity'?

Post-workshop tasks

The preliminary research proposals have been circulated among the group and participants have been asked to nominate how their institution might contribute to the research proposed. This process will be completed by the end of November, 2005, and Kathy Evans will facilitate this process in the short term, as needed.

There are several scenarios relating to the next phase of project development. One institution from the list of nominees associated with a particular preliminary proposal may take leadership and coordinate the group of nominees to develop the research proposal further. Alternatively, several proposals may be grouped under arbitrary headings such as 'cool climate' or 'warm climate' issues, with an overall 'coordinator'. Another option is that subsets of objectives within a particular proposal may be selected for further development by individual researchers. In short, this set of preliminary research proposals can be used as 'modules' to help researchers build collaborative research and grant applications to suit relevant selection criteria. Workshop participants will be sent an email three months after the workshop to request that they inform the group of progress made on any aspect of project development. Other forums will be sought for maintaining the momentum.

Appendix A

Below is a list of elements provided to participants and subsequently modified by them before the meeting. The colour coding indicates feedback provided by a subset of participants prior to the workshop.

Red indicates 'hot topics' needing adequate discussion time.

Green indicates topics that are already understood well by the industry.

Blue indicates usefulness of knowledge might be restricted to the vineyard and season under study. Discussion potentially unproductive.

Violet indicates a research issue of low benefit:cost (much work/questionable usefulness) in terms of improving disease management in the short term.

Black indicates no comment (yet) or participants undecided at present

[Theme Area 1 The pathogen and importance of disease]

THE PATHOGEN (focus on *Botrytis cinerea*?)

- Identification of different causal organisms
- *Detection (latent infection)*
  - Establishing a standard for research purposes (see also Theme Area 4)
  - Role of molecular-based diagnostics
- *Quantification*
  - Fungal load at the weighbridge (see also 'crop loss assessment' below)
  - NIR based approach/chemical imaging
  - Establishing a standard for research purposes (see also Theme Area 4)
- *Strain characterisation and variation (biotypes)*
  - Correlation of biotypes with hosts or tissue types?
- Physiological specialisation

THE DISEASE

Economic importance

- Impact on grape yield and quality
  - Quantification of regional differences
- Crop loss assessment
- Economic and threshold considerations
  - Assessment of quality at the winery

[Theme Area 2 Inoculum potential, dispersal, infection pathways]

Epidemiology

- Inoculum potential
- *Inoculum potential*
  - *Sources of inoculum at flowering and post flowering, in relation to infection pathways*
    - necrotic tissue within bunches
    - within vine
    - vineyard floor
    - neighbouring crops or other hosts
  - Quantifying inoculum
  - Factors driving inoculum sources
  - Relative importance of sources of inoculum
- *Dispersal of inoculum* eg. grape trash to flowers and fruit

- *Pathways of infection and relative importance*
  - Leaves, petioles – necrotic tissues
  - Flowers – petioles, stamens, receptacle, rachis, cap scar, aborted florets, floral debris
  - Fruit – fruit pedicel, rachis, berry cheeks

[Theme Area 3 Environmental factors, including host physiology]

- Factors necessary for resumption of growth after latency
- Factors enhancing critical events such as infection and sporulation
  - Influence of weather/microclimate/radiation
  - *Microbial ecology of the phyllo- and fructoplane* (leaf and berry surface)
  - Interaction of bunch rot organisms
  - Constitutive and induced resistance of grape tissue to disease
    - Grape variety
    - Microbial ecology of the soil (induced resistance?)
    - Chemical elicitors
  - Canopy and cluster architecture
  - Water status (eg. berry splitting), nutrition (eg. Ca, N)

[Theme Area 4 Monitoring & modeling epidemics]

- Monitoring weather
  - Within canopy microclimate
  - Long range forecasts
- *Standardised disease monitoring and assessment*
  - Disease incidence and severity
  - Disease standard diagrams
  - Designing experiments and sampling
- Modelling epidemics
- Modelling epidemics
  - Temporal analysis and disease progress curves
  - Spatial analysis
  - Disease development in relation to crop phenology, age of host tissues
  - Dispersal – short and long range
  - Population genetics (e.g. fungicide resistant strains, evolutionary potential, host-mediated selection?)

[Theme Area 5 Manipulation of inoculum sources & the host]

- Manipulation of inoculum sources
- Sanitation practices – cultural, biological, vineyard floor, cover crops, surrounding vegetation.
- Disease prediction – chemical, biological
- Manipulation of the host
  - Site selection
  - Canopy type/trellis system
  - Crop load and ripening
  - *Cluster tightness – breeding, chemical, clone susceptibility*
  - Leaf plucking
  - Bunch thinning

- Berry size
- Nutrition
- Microbial ecology of the phylloplane, fructoplane and soil
- Organic versus conventional
  - nutrition, cuticular wax, leaf architecture?
- Minimising pest or mechanical damage

[Theme Area 6 Crop Protection – chemical and biological]

- Preventing infection
  - Choice of chemical and regulation (MRLs)
  - *Spray timing*
  - *Spray timing*
    - Strategies based on pathogen activity, host susceptibility
    - Accounting for characteristics of individual chemicals
    - Disease forecasting using empirical models
    - For bunch rotters other than *B. cinerea*?
  - Spray coverage and dose
  - Non-target impacts
    - Impact of adjuvants on berry wax
  - *Fungicide resistance*
    - More sensitive methods for detection?
  - Biological control – single target agents and multiple target techniques (eg. multiple agent, compost 'teas')
  - *Alternative control strategies*
- Crop protection – 'curing' visible sporulation
  - Choice of 'late season' chemicals and regulation (MRLs)

[Theme Area 7 Evaluating integrated management strategies]

- Models to predict/illustrate impact of management on disease progression
  - Identifying key factors driving epidemics eg. weather versus inoculum (when weather favourable)
  - Relative importance of each component of integrated disease management
    - Regional level
    - Within vineyard level
- Cost:benefit analysis

[Theme Area 8 Host-pathogen interactions]

Host-pathogen interactions

- *Infection events*
  - Role of enzymes, metabolites in pathogenesis
- Post-infection colonisation
- *Disease expression*
  - Sporulation
- *Development of latency and resumption of growth*
- *Host resistance*

- Induced resistance
- Age-related resistance
- Qualitative or quantitative resistance

[Theme Area 9 Technology Transfer]

Technology transfer

- Models for decision support (If 'x', then do 'y')
- Cost-benefit analysis
- Field monitoring
    - LBAM still an issue for some growers
- On-farm (grower-lead) trials
- Lack of extension in some regions
- Management systems tailored for different clients
    - Corporate-based viticulture – large scale production
    - Premium/Ultra-premium producers - small scale production
    - Organic/biodynamic producers

Appendix B

Note made by Duncan Farquhar during the workshop. These notes illustrate the nature of the discussion and may not provide an accurate record of comments made by individuals.

Detection of latent infection.

Jacky – Project in table grapes – in storage latent infection. How valuable is the information in the end. Elisa kits have to have sporulation. Tool that can tell you how much latent infection.

Chris – Latest *Vitis* detection of Botrytis using PCR

Phil – Threshold to pick it up

Contamination from infected tissue.

Standardise surface sterilisation for latent infection detection.

Trevor - Latent infection well documented.

Phil – Overnight Freezing and Incubation method

Jacky – Found method didn't know about all of the tricks that make it reliable.

Phil found ONFIT reliable. Can take a few days.

Quantification

Of both Botrytis and other bunch rots.

4% Is rejected – arbitrary figure
1% Ripe rots

Tossing sample in a magic machine at weighbridge and getting a number on all fungi. What causal organisms and how much of each.

Bob - Winery inspection in the field prior to going into the winery. Standardised keys for ranking bunch rots. Keys for different bunchrots. Simple quick process that can be done in the field.

Phil – Botrytis – Laccase test at winery

Sandra – Identification of each of the bunch rots – Colletotrichum – pink salmon coloured spores in the lab.

Regional differences – is latent infection more important in one region /season than another.

Aflatoxin from Aspergillus and Penicillium will be important for marketing wine.

Bob - Industry needs measure just prior to harvest. Researchers and growers need measures before this.

Inoculum potential, dispersal and infection pathways.

Sources of inoculum at flowering
Necrotic tissue in bunches
Quantifying
Dispersal from trash to flowers and fruit.
Do leaves and petioles play a role
Flowers and flower parts and fruit.
Conventional vs Organic growers – why does severity appear to decline over time in some organic systems?

Barbara - Different regions and climates. Rachi in one vineyard not important in another down the road. Leaf petioles in Hawkes Bay were important. Vintage variability also.

Kathy - Does it really matter where it is coming from.

Trevor – It's everywhere

Why worry about the hugely complex set of sources when bunches are important things.

Bob - Do we do things to reduce these sources of inoculum.

Trevor - Cost benefit

Bob – After harvest, minimally pruned vines have up to 500 rachi remnants per vine, not worth doing anything about this.

Dead material

Can learn a lot from organic growers as they go after sources of inoculum.

Research Questions vs management practices.

Ian – Mary's probes for detection in vineyard? Talked about.

Dispersal – Alison Seyb's PhD most (sources) within 1m

Variable population. Isolates that produce many sclerotia and some that don't. Not so sure how much this knowledge influences management.

Organic vineyards get less botrytis

Jacky - Adjuvant effects from Suzy Rogiers work?

Plant vigour lower in organic vineyards

Is the organic difference a gap

Kathy – a gap for every area

Phil – Important as the many little things additive is part of the answer.

Dean – Adjuvants – Peas and Beans, Onions and *Botrytis allii*. 2-3 times as many conidia will germinate and 2-3 times longer germ tubes. Water droplets are flatter.

Jacky – Downy Mildew in Spring onions – adjuvants.

Chris – Suzy's microscope work of affecting waxy layers
Kim Plummer – Natural antagonists. Long term trial of Organic systems.

Interactions on leaf and berry surface and microbial ecology.

Jacky- Fits in with Organic Trial

Rob – Study looking at different yeasts and filamentous fungi.

Role of open canopies on Botrytis and other bunch rots.

Chris slide showing non Botrytis bunch rots and E vs W exposure.

Inorganic N Sources

YAN – for stuck ferments

N and Canopies more important than direct N Effect – this was fuzzy.

Ca?

Bob – Water status affects berry compression and berry splitting.

Barbara - Soil and Water initiative.

Host resistance

Varietal Bunch Architecture

Chemical elicitors - stilbene conversation and data pre and post veraison

Tony - Dramatic decline during berry ripening. Inducible resistance in younger tissue but not old

Vineyard Floor

Modeling and monitoring

Standardised monitoring.

Rainfall and post veraison wetness and ONFIT data.

21 site years of data 5 sites but standardised monitor was not good enough. Final disease severity at vintage. Sudden late rush, Slow development or early development and stop. Robs protocol for standardised monitoring with different levels of intensity of monitoring for each different purpose.

Some standardised storage of –20degrees C – storage of plant material to validate new test methods like PCR

French standardised methods in Bordeaux check these before starting our own new methods.

Potential for Meta-analyses if we have good datasets.

What would models be used for?

Bob – Important for the management of Botrytis in different situations. Comparing regions and situations. For use by industry and researchers.

Rob – If you could predict pre-harvest severity at flowering it would be great. Poor correlation between harvest severity and pre-veraison measures.

First standardise methods and potential predictive variables.

Harvest date important to final disease severity.

Bob – Need understanding of variability between regions.

Covariates – Brix should be measured with any measure of Botrytis. Only record sporulating Botrytis. Slipskin definition of botrytis is risky – if in doubt leave it out.

Bob - Short and long range dispersal. With black Aspergillus moulds, a dust storm in Mildura dramatically increased bunch spore load.

Research on spore suspensions needs to be standardised. Spore settling towers.

Knowledge of population variation of infections shows that infection and rot often comes from a single spore.

Bob -standardised disease assessments in vineyards. Reference keys. Recommended thresholds?

How many bunches should be looked at?

Manipulating inoculum levels

Bob – What do you take out of the vineyard and is it cost prohibitive. Different approaches for different canopy management practices.

Ian Dry – Dispersal question is related to this.

Fungicides for inoculum management
Phil - Shirlan after machine harvesting – and had a result.

Standard practice in France

Trevor – Standard organic trash blow

Bob – A trial showed no effect of blowing trash out of bunches

Kathy – More data on these methods

Bob – Can be negated by seasonal favourability for bunch rot at the end of the season.

David B – Mowing inter row

Tarak don't process grape marc and if not composted how important.

How many marginal years vs how many bad and non years.

Timing of inoculum buildup.

Do we have to accept bad years?

Early leaf infection before flowering more important than previously thought in S Africa.

Hail damage in McLarenVale if no bad weather then OK.

Need to standardise airborne inoculum levels to watch spore loads change after through seasons.

Manipulating the host.

Crop load effects reasonably well understood.

Cluster tightness, breeding, chemical applied extensions, clones.

Trevor- Different varieties have different GA effects. Afects next years crop? Too difficult to manage.

Trevor – 10500 litre limit in Europe. Removing half cluster

David and Duncan - Mechanical Thinning in Coonawarra and Tas Trial

Clonal selection – need good data about clones.

Rootstocks and cluster tightness.

Philip - Leaf Plucking – well understood by industry as a botrytis management strategy. Well researched but people leave it till after flowering.

Microbial Ecology of leaf and fruit surface.

Organic vineyard spread within bunch reduced?

Biological control

Alternative Tools – Tony's review in *Plant Pathology* where successful biological suppression has been published. Successful at low to moderate loads. Reliance on using a single mode of action.

Botryzen based on Ulocladium oum…. Not isolated in Australia yet. Has been trialed in Italy. Aggressive competitor for space.

Future direction, combination of complimentary BCA agents occupying different niches, integrated with good conventional practices. Compatible with sulfur and copper can tank mix. Not compatible with Rovral. Differences between DMI's. Need to get better integration and timing.

Trevor – Persistance in dry climates.

Phil – Dark conidia drought tolerant

Dean – It's probably out there (in Australia) not on plant disease database list as it is not causing a problem.

Some *Ulocladium spp*. are pathogens

Registration in Australia question. – show that it does not produce any antimicrobial – saprophytic. Well adapted to a grape niche.

Biological and chemical control relies on good spray application. This is still important.

Q about drivers of biocontrol and where we are going with Chemical vs Biological controls/organic – Marks and Spencers are pushing toward reduction of pesticides.

Barbara Hall - Pesticide resistance testing of limited use. Growers problems are usually spray coverage.

Bob - Size of population that is needed to maintain efficacy – Chemical companies have this information.

Trevor – Modern Botryticides are V effective chemicals. All good fungicides are to be used at the wrong time of the year. Most efficient use of these chemicals, maybe to knock out latent infections.

Bob - Tan Nair showed kickback effect on latent infections of some fungicides.

Phil – Teldor Kickback

Barbara – Better as protectant or to clean up latents. Based on effective latent tool. Appropriate chemical timing is still a huge knowledge gap.

NZ winegrowers have funded this type of work.

Measure effect at latent and harvest.

Company programs knock back the chemical timings as residue issues come up.

Evaluation of integrated management.

Bob – Standardising what should be documented about spray trials. Especially for registration trials.

Trevor – Organic versus conventional still important.

David Braybrook - Targeted trial impact of cane removal on inoculum levels. Integrated approaches

Bob - Cost benefit analysis approaches, establishing real economic impact of diseases is difficult.

Barbara - Chemicals – what about the other rotters.

Chris – Useful to use chemicals that are available later

Barbara – Residue management in the winery – binding agents, bioremediation, Ion exchange Reverse osmosis,

Appendix C

Planning research toward sustainable management of bunch rot in grapes



Back row: Bob Emmett, Dean Metcalf, Philip Elmer, Andrew Taylor, Rob Beresford, Ian Dry, Stephen Guilbaud-Oulton, David Braybrook, Duncan Farquhar
Middle row: Angela Smith, Tony Reglinski, Kim Plummer, Barbara Hall, Eileen Scott, Chris Steel, Trevor Wicks, Alice Palmer
Front row (seated): Jacky Edwards, Kathy Evans, Michelle Warren, Sandra Savocchia





















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