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Producers' Own Trials

Assessment of botrytis or powdery mildew

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These slides and notes provide information on how to assess botrytis bunch rot or powdery mildew when doing a strip trial.

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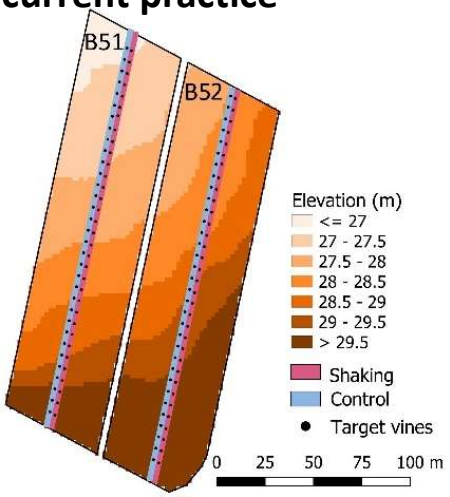
Assessing disease in a strip trial

to compare a **'test treatment'** versus **'current practice'**

When, How and What?

For:

1. Botrytis bunch rot caused by *Botrytis cinerea*
2. Powdery mildew caused by *Erysiphe necator*



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A 'strip trial' is one where we compare a 'test treatment' versus current practice. The 'test treatment' is something that we want to try for whatever reason.

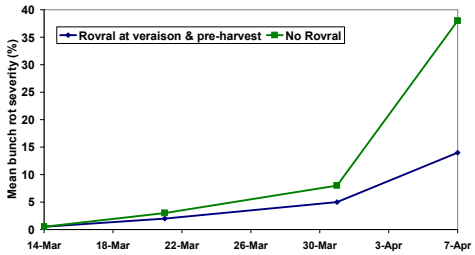
These slides provide information on when, how and what to assess in the designated assessment row of a trial strip. The methods are very similar for both botrytis and powdery mildew, apart from the keys we use to assess disease severity and the timing of the disease assessment.

Botrytis bunch rot: When?

As close to harvest as possible

- Magnitude of the difference between trial strips can vary according to the assessment date
- Differences may narrow or become greater as 'hang time' increases

Trial result from 2007



Rovral® = iprodione

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Starting with botrytis, we try and assess bunch rot as close as possible to harvest. This is because differences in disease severity between the 'test treatment' and 'current practice' can change over time.

For example, pictured are the results for a trial conducted decades ago in a block of Riesling vines. These vines were treated or not with Rovral fungicide late in the season which is something that we wouldn't do now. Nonetheless, the graph shows how botrytis developed differently if treated with Rovral…which is the purple or lower line on the graph.

You can see that the difference in botrytis severity - between treated or not treated – increased over time. In other botrytis trials, the reverse is true in that the difference might narrow as the 'hang time' increases.

The bottom line is that if we only have time to assess botrytis once, then do it as close to harvest as possible.

What if mouldy bunches are dropped?

Mouldy bunches may be removed when first seen or prior to machine harvesting. If so,

1. When walking down a trial row being used for disease assessment, drop the mouldy bunches into a bucket
2. At the end of the row, count and record the total number of mouldy bunches in the bucket
3. Repeat steps 1 and 2, each day mouldy bunches are removed (if this is done more than once)
4. Add up all the mouldy bunches removed per row (over time) and compare this result with your disease assessment near harvest

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In some vineyards, a vineyard scout might walk the rows and drop any bunches with visible bunch rot. There might be 2 or 3 passes to drop bunches before harvest. Alternatively, mouldy bunches might be removed by hand a day or two before machine harvesting.

If this occurs, then obviously it would impact any final assessment of botrytis severity.

One solution is to count the numbers of mouldy bunches removed from the vines for each assessment row.

Carry a bucket as you go down the assessment row and drop the mouldy bunches into it. Then when you get to the end of the row, count the total number of mouldy bunches in the bucket.

Let's say there were 3 passes of this row at different times, then you would add up the total number of bunches across the 3 passes. You can then use this information to aid interpretation of final botrytis severities at harvest.

Powdery mildew: When?

Bunch closure to veraison

- Symptoms are easier to see and score when mildew colonies have stopped expanding and are still producing spores (white/grey, powdery)

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The timing for a powdery mildew assessment is different to that for botrytis because most of the symptoms on fruit develop between flowering and veraison. If disease assessment is done at harvest, then it can be hard to score mildew colonies that have turned black and/or dried up.

The best time to assess powdery mildews is close to veraison because the disease symptoms on berries will not get worse from this time onwards..

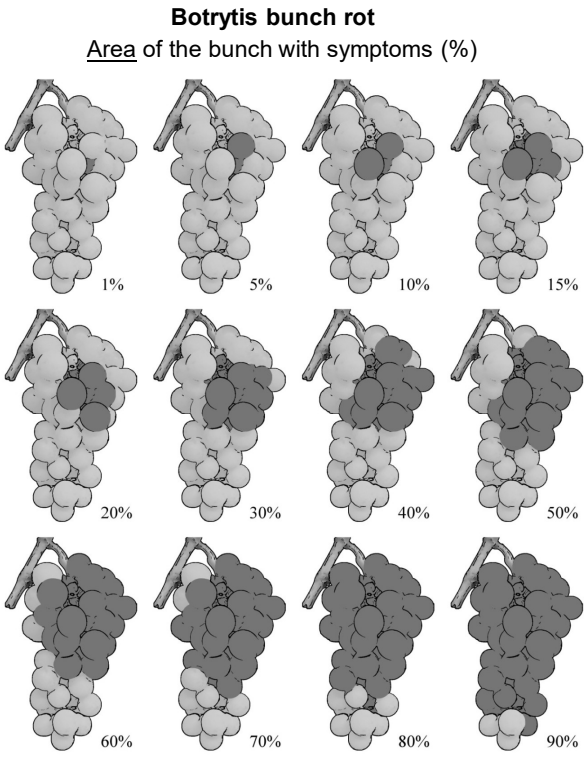
HOW?

1. Scoring individual grape bunches

How do we score individual grape bunches for either botrytis or powdery mildew severity?

Key to assess botrytis severity per bunch

Source:
Hill GN, Beresford RM, Evans KJ (2010) Tools for accurate assessment of botrytis bunch rot (*Botrytis cinerea*) on wine grapes. New Zealand Plant Protection 63:174–181. Available from: http://www.nzpps.org/journal/contents.php?vol=63



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To assess botrytis on a single grape bunch, estimate the percentage area of the bunch displaying symptoms. We can generally get a better estimate if we train our eye with the aid of a standard area diagram like the one shown here.

Let's say a bunch is fully rotten then we would score it as 100% severity. If about half the area of the bunch is rotten, then we give it a score of 50% and so on. Let's say less than half the bunch is rotten, then mentally divide the bunch into thirds or quarters to help locate what part of the diagram to focus on.

Once we have trained our eyes and have been doing some disease assessments, it can be useful to come back to this standard diagram to check the consistency of your assessments.

Use the key to estimate botrytis severity on this bunch

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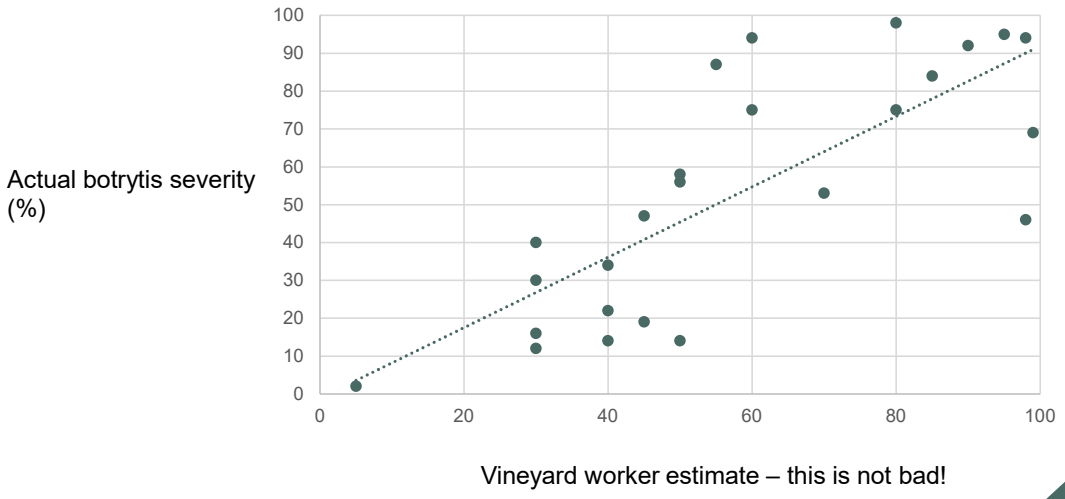
Here is a bunch with typical symptoms of botrytis bunch rot. What percentage severity would you give this bunch?

The first thing to notice is that there are more healthy berries than discoloured ones. This means that the botrytis severity must be less than 50%. Mentally divide the bunch into quarters to see if the severity 25%, or less. Decide the percentage severity and do not dwell on your assessment as the first number that comes into your head is as good as any.

Self evaluation of scoring ability

Sadly, Bunch Rot Assessment Trainer (BRAT, NZ) is no longer accessible.



Before you start the disease assessment it can be a good idea to evaluate your scoring ability.

There used to be a website call BRAT were you could enter scores for a number of ‘virtual’ bunches and then compare your own estimates with the actual percentages. Ideally you would see a scatter of dots around a trendline that has a slope of 1. Sadly, it appears we can’t access this tool anymore.

Pictured are some actual results for a vineyard worker who had never assessed botrytis before. You can see that they have scored high on some bunches and low on others – and that’s OK.

The main thing is that we sample and score enough bunches from which we calculate an average that is a reasonable estimate of botrytis severity.

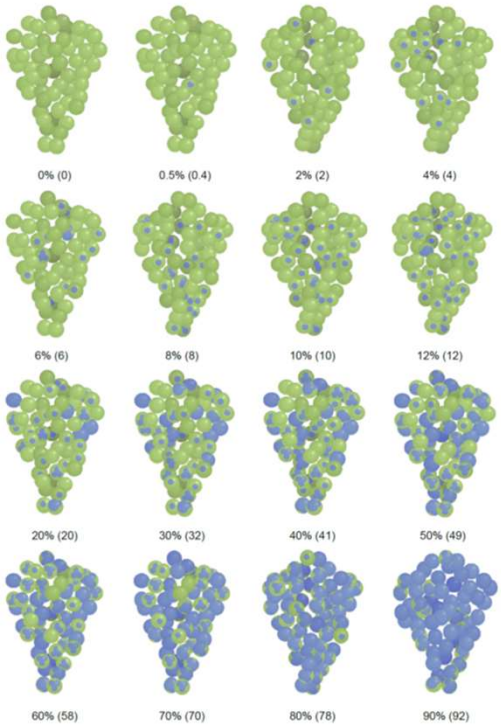
Key to assess powdery mildew severity per bunch

Source:
Scott, E. et al. (2015) A diagrammatic key to assist assessment of powdery mildew severity on grape bunches. *Australian & New Zealand Grapegrower & Winemaker*. 623: 46-49.

The number in parenthesis is the percentage blue measured by ImageJ©

Powdery mildew

Area of the bunch with symptoms (%)



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Here is the disease assessment key for powdery mildew. You can see that they key is different to the one used for botrytis and that's because the mildew fungus grows across the surface of the berries and the symptoms can appear more diffuse than what we see for bunch rot.

Try scoring a few bunches first

- Walk along a row with co-worker and look at some rotting or mildewed bunches with the scoring key in hand
  - Discuss with each other what score you would give and why
- Important to be consistent in how you score each bunch
  - One person does all the scoring
  - It's OK to routinely score 'high' or 'low' versus an unknown 'actual'

Whatever disease you are scoring it is a good idea to practice your scoring before starting the job.

We suggest walking along a row with a co-worker and, together, look at some rotting or mildewed bunches with a laminated scoring key in your hand. Score a few bunches each and discuss with each other what score you gave, and why.

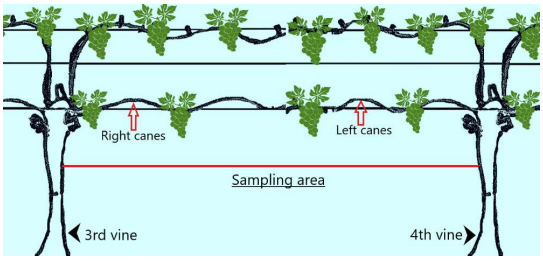
The main thing is the same person does all the scoring for consistency.

HOW?
2. Selecting grape bunches per vine

Now that you know how to score an individual bunch, the next step is to know what grape bunches to select for each vine being assessed.

Estimating disease severity per vine

- One vine is the distance between the trunks of two adjacent vines
- Choose one side of canopy to assess e.g. west or east side
- Stop at the first vine to be sampled
- Sample 8 bunches arbitrarily between the two trunks, score and record the disease severity for each bunch
  - Avoid bunches that are atypical or unusally small



In any given trial row, we define a single vine to be the distance between the trunks of two adjacent vines.
Depending on the time of day, choose one side or the other to do your disease assessment. Just stick to that side of the canopy for the entire assessment.

When you stop at the vine to be sampled, then select 8 bunches arbitrarily between the two trunks. Look at the first bunch selected and give it a severity score. Look at the second bunch and give it a severity score and so on. There is no need to remove the bunches from the vine.

Trying to sample arbitrarily can be tricky, so just do your best.

Importantly, avoid any bunches that appear atypical or unusually small.

Record sheet

Assessor _____ Date _____ Time start _____

Recorder _____ Assess side West Time end _____

No	K511. Row 9	botrytis severity (%)							
	No.of vine	Bunch1	2	3	4	5	6	7	8
1	3								
2	7								
3	11								
4	15								
5	19								
6	23								

Before you go to the field print a set of recording sheets and place them in clipboard with a pen (not a pencil).

We use a pen because it is too easy for pencil marks to rub off.

Each time you start a trial row to be assessed, then record the details at the top of the sheet. The ideal scenario is having one person assess the grape bunches and calling out numbers and the other writing down the numbers onto the sheet.

Don't forget to add the date and the side of the row being assessed as well as the start and end time. That will let you know roughly how long the assessment task takes, which can be handy next time you schedule a disease assessment. The more of these assessments you do, the faster it becomes because your eyes have become well trained and calibrated against the assessment key.

Example of completed scoring sheet

No	Vine No.	botrytis severity (%)							
		Bunch1	2	3	4	5	6	7	8
14	21	–	–	–	–	–	–	–	–
15	23	–	–	–	–	–	–	–	–
16	25	15	–	25	–	–	–	–	10
17	27	–	–	–	–	1	–	–	–
18	29	–	–	–	–	–	–	30	–
19	31	–	–	–	–	–	–	–	–
20	33	–	–	–	–	–	–	–	–
21	35	–	–	–	–	–	–	–	–
22	37	–	–	–	–	–	–	–	–
23	39	–	–	–	–	–	–	–	35
24	41	–	–	–	–	25	–	–	–
25	43	–	–	–	–	–	–	–	–
26	45	20	25	–	30	45	35	20	15

A dash means that bunch rot symptoms were absent

Here is an example of a scoring sheet from a strip trial. The second column shows the vine number along the row. We happened to assess every second vine based on the total number of vines in the row.

Across the top of the sheet you can see the numbers 1 to 8, representing the eight individual bunches per vine.

When scoring, we use a dash when no botrytis is observed. This example illustrates the variability in botrytis severity from vine to vine and bunch to bunch.

Calculate average severity per vine

No	Vine No.	botrytis severity (%)							
		Bunch1	2	3	4	5	6	7	8
14	21	–	–	–	–	–	–	–	–
15	23	–	–	–	–	–	–	–	–
16	25	15	–	25	–	–	–	–	10

A dash means that bunch rot symptoms were absent

Average severity for vine #25
= (15 + 0 + 25 + 0 + 0 + 0 + 0 + 10) / 8 = 6.25%

When you get back to the office, then the data are entered to an excel spreadsheet.

Here we give an example of how to calculate the average severity for vine number 25 in that row. Simply add the values for the 8 bunches assessed and divide by 8. In this example, even though individual bunches had severities of 10, 15 and 25%, when you include the bunches with no botrytis, then the average severity for 8 bunches to 6.25%

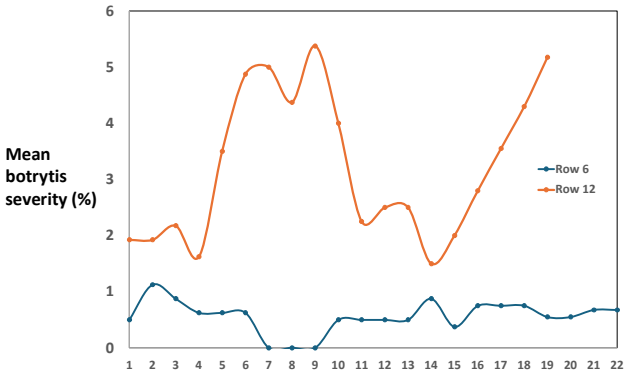
HOW?

3. Selecting vines to assess per row

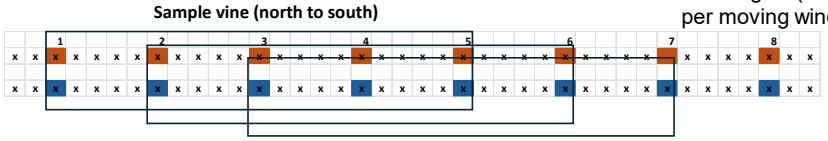
The next step is to decide how many vines per row to assess.

Sampling frequency per row

Aim for 25-30 sample vines per row to achieve at least 20 moving window averages per row.



Sample vine (north to south)



Rectangles (black border) show 5 samples per moving window average

etc

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The number of vines to sample per row comes back to how the trial results are presented.

This is a graph of moving window averages for two rows – one being the current practice and the other being the test treatment. We need at least 20 moving window averages to produce a useful graph of results. Given that one point on this graph is the average for 5 sample vines, then we would need at least 25-30 sample vines to produce a graph of at least 20 moving window averages.

Count the number of vines per row to estimate how frequently we need to sample vines going down the row.

Identifying sample vines based on row length

Assumptions:

- 5 sample vines are used to calculate each moving window average
- The two end vines per row are avoided (edge effect).

Example 1:

- 135 vines per row
- Every 4th vine will provide 33 sample vines and 29 moving window averages

Example 2:

- 28 vines per (short) row
- Every vine is sampled resulting in 23 moving window averages

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Here are some examples.

We have assumed that 5 sample vines are used to calculate each moving window average while noting the window can be any length you want it to be. The moving window simply smooths the graph line to make it easier to read.

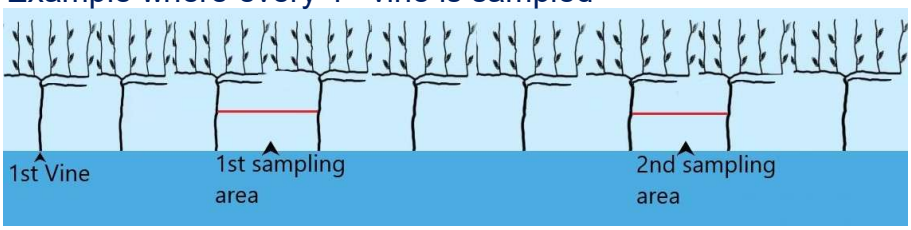
In the first example, there are 135 vines per row. Assessing every 4th vine would give us 33 sample vines and for the graph and 29 moving window averages.

In the second example, there are 28 vines in relatively short rows.
In this case it would be necessary to sample every vine. If so, there would be 23 moving window averages for the graph.

Sampling vines

- Avoid the two vines at the end of each row
- Start the assessment at vine 3
- Whether you sample every vine, or every 2nd, 3rd, 4th or 5th vine (etc) will depend on the length of the row
- When starting a new row, start at the same end as the previous row e.g. the northern end
- Take some photos as a record of the disease symptoms observed

Example where every 4th vine is sampled



Whatever the sampling regime, always avoid the two vines at the end of each row. That means the disease assessment will start at vine 3.

Again, whether you sample every vine, or every 2nd, 3rd, 4th of 5th vine etc will depend on the length of the row.

Once you have finished one row, then walk back to the start of the next row and start the assessment from the same end of the row as the previous row. Assess a row by moving in the same direction e.g. moving north to south. That's because we want to directly compare results for the test treatment and current practice for vines that are roughly in the same position in the row.

Don't forget to take some photos that illustrate typical disease symptoms or anything else of interest.

WHAT?

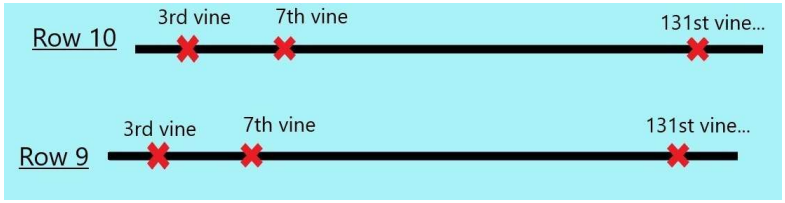
What to consider as you move down the row.

What else do we need to consider as we move down an assessment row?

Aligning sample vines among trial rows

Ideally, the sampled vines in each row will align so that the graph of moving window averages along each row can be compared at each 'location'.

- The red circles/pink tags show the actual position of the 7th vine in rows being compared. The alignment is often imperfect but it's good enough for the purpose of the 'moving window' graph.
- If a vine is missing, then assess an adjacent vine(trunk to trunk) - then make sure the next sample resumes the intended sequence.



This slide illustrates how we are trying to get alignment of sampled vines from row to row.

You can see that the 3rd and 7th vines in rows 9 and 10 are not aligned perfectly, and that's OK.

However, if a vine is missing, say the 7th vine, then we could sample vine 6 or 8 instead; however, we need to get back to the intended sequence of sampling after that. If every 4th vine is being sampled, the next vine in the sequence after the missing vine would be vine 11.

This is the last set of instructions for this presentation. Keep in mind that your disease assessment is likely to be faster than the time it has taken you to read these notes!!

Acknowledgments

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- Wine businesses who helped develop and test in-vineyard trial methods

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Thanks for your attention!