'Shiraz disease'

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Background

Shiraz is a critical workhorse variety to the Australian wine industry and reports of problems with 'shiraz disease' from overseas countries cannot be ignored. At minimum, the Australian industry needs to be aware of the current thinking and understanding of these 'diseases' and have in place contingencies, in case we find similar problems arising in our plantings.

This paper addresses current issues noted in;

California	Red Vine
France	Syrah decline
South Africa	Shiraz disease and Shiraz (syn. Syrah) decline
Australia	'Shiraz disease'

Leafroll and other virus

Virus symptoms, particularly with single infection of leafroll 1 and 3 (see Figure 1) can be very distinct in their symptomatology. Intriguingly, many Australian viticulturists are not fully aware of the causes of the wide range of reddening symptoms that may occur where Shiraz vines are mechanically damaged (e.g. girdling, 'string' disease; see Figure 2), physiologically challenged (e.g. cold damage, water stress; see Figure 3) and diseased (e.g. leafroll).

In almost all cases, the reddening is caused by loss of chlorophyll (potassium reddening being a much darker colour ranging into a blackening effect) resulting in anthocyanin pigments being visible. Generally a reddening that is evenly spread throughout the leaf is a signal of mechanical or physiological damage where all leaf blade chlorophyll breaks down. Reddening in sectors of the leaves is often associated with insect, mechanical or water stress impacts. Distinctive veinal patterns are most often viral in origin, where there is blockage in the phloem tissue.

To ascertain the causes of the reddening, careful review of the incidence and severity is required, together with verification of the areas of the leaf affected in terms of 'pattern' and possible causative factors. Where leaves are speckled or patterned and symptoms are most prevalent on basal leaves, then virus may be suspected. Visual identification of virus infection requires high levels of infection and identification in a limited part of the season (cooler autumn periods after summer heat).

Where symptoms are not obviously related to physical factors, then virus testing is a tool to aid with investigation for causes. There are a wide range of known virus (see Figure 4), as well as many syndromes that do not give consistent results and yet behave similarly to virus infections (as this paper will demonstrate).

Figure 1. Variation in Leafroll symptoms showing Leafroll 1 on the right and Leafroll 3 on the left.

Virus testing

There are a number of laboratories established to provide these services. Two main techniques are used:

ELISA	Enzyme Linked Immunosorbent Assay
PCR	Polymerase Chain Reaction

Figure 2. Leaf reddening induced by tendrils restricting normal cane development.

Figure 3. Autumn colours induced by physiological damage and cold autumnal conditions.

PCR is a higher resolution method that relies on amplification of specific portions of the virus genome. This method requires a higher level of expertise to provide reliable results. In general, multiple samples of ELISA are cheaper than PCR as the 'kits' for testing allow for multiple tests, whereas PCR costs are related to the number of lanes on a test gel.

For reliable virus detection, there are at least three critical conditions that should be satisfied. The first is that the test method is working reliably which means that the operator is skilled and no contamination of samples is occurring. The best way to ensure this is to include both positive and negative controls as this demonstrates that the procedure is not giving false positives and negatives.

The second requirement is that a standard sampling method is in place ensuring that conditions such as the time of season (early season samples may have low levels of virus) and the tissue sampled are constant. Ideally, samples would be taken from both a clean 'control' and a 'suspect' to be sure of final results. This is however not standard practice due to the perceived high cost of testing.

The final requirement is that the titre (concentration) of the virus is at a level that may be readily detected by the method. ELISA samples that test negative for example, may test positive in PCR as the PCR method is considerably more sensitive. The other factor that makes sampling method important is that the titre of virus in any vine may vary according to where in the vine the sample is taken as well as at what time of year.

With all of these factors in mind, the only result we can be certain of with ELISA and PCR testing is a positive (where a positive control is used), as a negative result may mean the sample is clean or the virus was not detected as the sensitivity, sampling and method were not capable of detecting the virus.

Red Vine in California

Foster's experience

Foster's first noticed issues relating to Red Vine at its Camatta Hills Vineyard which is at Creston near Paso Robles in the Central Coast region. Development commenced in 1998 with the first plantings of green potted vines in 1999. The season was very wet and planting was late, with the growing season being shortened by early frosts. The vineyard showed a widespread scattering of reddened vines which was attributed to the short season of growth (see Figures 5 and 6).

Similar symptoms were seen in 2000 and were traced largely to material sourced from one nursery. This led Foster's to believe that the issues were related to graft unions, with problems relating to their physical strength (poor wrapping of grafts) and a tendency

Virus	Severity	Comments
Routinely tested virus		
Leafroll 1 (LR1)	Severe	Red veinal with yellow highlights, >30% yield loss
Leafroll 2 (LR2)	Medium	Variable symptoms, severe impact on topworking
Leafroll 3 (LR3)	Severe	Red veinal with green/purple, >30% yield loss
Leafroll 4 (LR4)	Medium	CAS 125 carries this virus
Leafroll 5 (LR5)	Medium	Red Emperor associated strain
Leafroll 9 (LR9)	Medium	Red Globe associated strain
RSPaV-1	Mild	Rupestris Stem Pitting variant 1
RSPaV-2	Mild	Rupestris Stem Pitting variant 2
GVA	Medium	Rugose wood variant A, a vitivirus type
GVB	Medium	Corky bark/Rugose wood variant B, a vitivirus type
GVD	Medium	Rugose wood variant D, a vitivirus type
GFkV-A	Medium	Grapevine Fleck A
GFkV-B	Medium	Grapevine Fleck B
GFLV	Medium	Grapevine Fan Leaf, a nepovirus
RG	Medium	Red Globe Virus, a LR2 strain in tablegrapes
Other virus		
Yellow speckle	Mild	Ubiquitous virus, speckle symptoms in Chardonnay, Cabernet Sauvignon and Merlot in warm seasons

Figure 4. List of virus currently routinely tested for by PCR

Figure 5. Red Vine symptoms in one-year-old planting at Camatta Hills in 1999 (image courtesy of Greg Pearce).

Figure 6. Red Vine symptoms in two-year-old planting at Camatta Hills in 1999 (image courtesy of Greg Pearce).

Figure 7. Red Vine affected bunch and leaves (left) as compared to unaffected bunches (photo courtesy of Simon Graves).

to showing symptoms of overgrowing of the scion on the stock. It was apparent that this was not the complete explanation as material from the other key nursery subsequently showed similar symptoms.

In the 2001 vintage, the manager noted significant grape quality issues with vines showing rapid onset of reddening and crisping of leaves. All vines with these symptoms also had bunches with delayed development, resulting in low Baumé, low colour, high pH and high potassium (see Figures 7 and 8). The vineyard manager determined that selective harvesting was required to resolve these issues and dropped all fruit from vines with symptoms. The site found that this had to be done the day before harvest as new symptoms manifested and the fruit impacted on wine quality.

In subsequent seasons, these symptoms persisted. The symptoms at Camatta Hills include reddening of leaves ranging from flecking, through to full inter-vein colouring in sections covering up to 50% of the leaf, with a predominance of colour in the upper sectors of the leaf (see Figure 9). At times, these symptoms resemble leafroll, but all testing at Camatta Hills returned negative for known virus. Crisping of leaf edges also occurred on affected vines (see Figure 10). This occurred as large necrotic regions on the leaf margins with symptoms developing rapidly as if an instant event crisped the leaf. At worst, almost the whole leaf became affected and later on in time, the vines defoliated when leaves were badly affected. In most vines, both crisping and reddening occurred, although it was possible to find either symptom in isolation (see Figure 11). Observations over several seasons showed that these symptoms were transient suggesting that the symptoms were not spreading and depended on seasonal conditions as to their expression and severity. We also noted that similar symptoms could be found in Durif and Zinfandel.

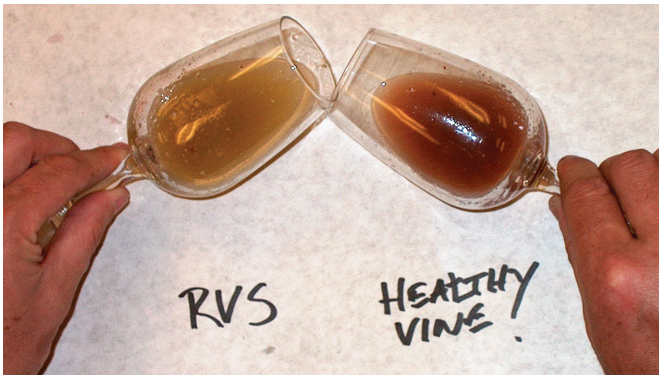
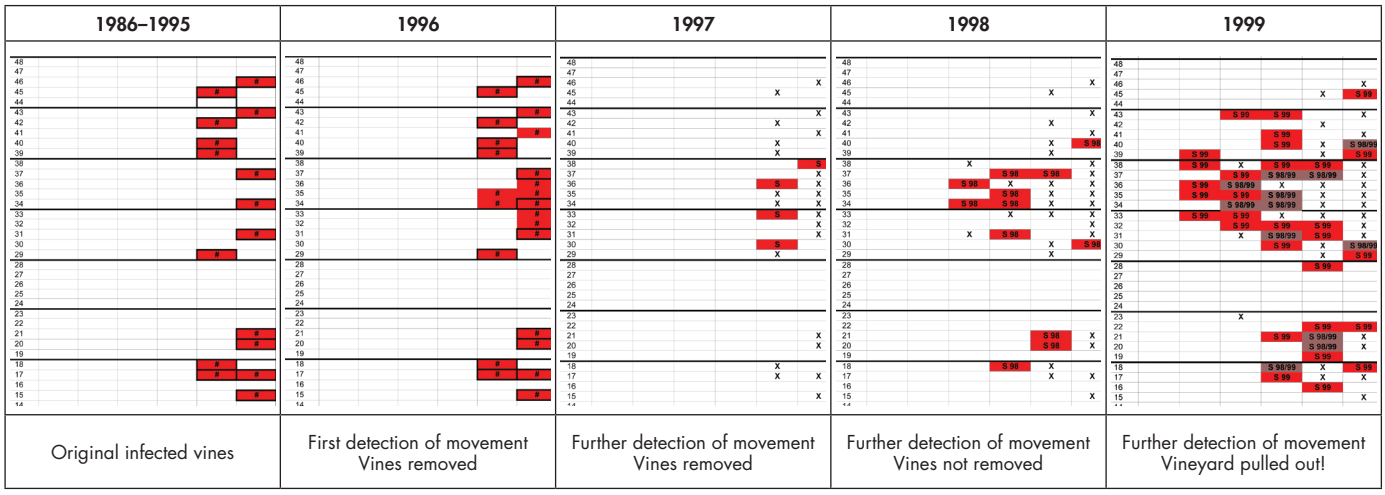


Figure 8. Comparison of juice extracts from Red Vine-affected Shiraz versus control material (photo courtesy of Simon Graves).

Figure 9. Leaf reddening symptoms associated with Red Vine in Shiraz.

Figure 10. Leaf crisping symptoms associated with Red Vine in Shiraz.

Figure 11. A badly affected vineyard in the Paso Robles region of California.



Legend: Original infected vines #, Vines removed X, Suspect vines S

Figure 12. Use of an Excel spreadsheet to map leafroll symptoms from season to season.

The use of an Excel or GIS-based mapping system can be a useful tool to determine changes occurring from season to season and as an aid to decision making. Figure 12 shows an example from a vineyard in South Australia where yearly mapping of leafroll symptoms in a private commercial vineyard helped the vineyard manager to make a final decision regarding the risk of leafroll Type 3 in the vineyard. This project was managed by the South Australian Department of Agriculture or Primary Industries and Resources South Australia as it is now known. This involved establishment of two vineyard clonal comparison trials in 1986, with newly imported clones of Pinot Noir compared against the then industry standards. The trial consisted of 13 clones randomised in 10 replicates with single vine plot comparisons. One trial was planted at the then Nuriootpa Research and Advisory Centre and the other in a commercial vineyard in another region. At the time of planting the presence of leafroll 3 had not been noted and little was known of the issues that would evolve. The trial on the Research Centre quickly showed signs of movement of the virus between vines. This culminated in the removal of the trial on the station in 1990 as the future risk of spread was unacceptable. The vineyard manager of the second trial was advised of this decision at Nuriootpa, however with no sign of movement in the commercial site, chose to leave the vines in place.

The block was monitored from this time onwards and in 1996, the first signs of movement were detected. This first detection convinced the vineyard manager to remove all of the infected vines, including the original leafroll 3 sources. More infections were noted in 1997 and again the vines were removed. Worrying that the vineyard was becoming non-commercial, the detections in 1998 were left in place with mapping in 1999 confirming that the vineyard was becoming non-commercial and it was removed entirely. Follow up at the Nuriootpa site suggested that mealy bug was the cause of viral movement in that trial. The commercial site was newly planted to vines and we believe this is why movement did not occur until the mealybug population built up enough to spread the leafroll 3.

Returning to the Red Vine issue in California, a similar assessment of performance of the vineyard was undertaken in 2004. The manager found that 25% of all plantings had up to 70% of vines affected; 50% of all plantings had between 30–70% of vines infected and the remaining 25% of plantings had less than 30% of vines infected.. Amongst the plantings at Camatta at that time, was one block established with material imported into California from Foster's proprietary selections and this block showed no sign of vine symptoms.

As part of our review of the impact of Red Vine on Camatta Hills we followed a monitoring process to help us determine likely cause and effect issues. In monitoring disease we are trying to find answers to key questions. These include:

- How much of the vineyard is affected?
- How badly is it affected?
- Are symptoms seasonal / increasing?

For the Shiraz at Camatta we documented disease expression using an Excel spreadsheet. The key is to categorise symptoms on a scale that reflects both the range and incidence of symptoms. To aid data collection in subsequent seasons it is important to mark any misses within the vineyard. Comparison and manipulation of the data between seasons enable determination of spread/transience occurrences.

Currently Shiraz from Australian sources continues to show no symptoms with two proprietary sources from our Magill vineyard in Adelaide and Kalimna in the Barossa. This includes the multiplication increase blocks on own roots as well as material grafted to certified virus tested Californian rootstocks. This has led Foster's to conclude that the Red Vine 'agent' is endemic in many of the Californian Shiraz selections and that it expresses with transient symptoms

Red Vine in California

Red Vine was first reported in 2000. The symptoms described included:

- Leafroll like symptoms
- Poor fruit quality (sugar, pH, juice and wine colour)
- Leaf burn symptoms
- A small number of Central Coast vineyards show 'Syrah decline' symptoms

Many factors have been implicated as causing the Californian symptoms. With a predominance of plantings from green potted material, discussions have included the impact of graft unions and planting practices include establishment late in the season with subsequent impacts of late season frosts.

Vine stress has also been considered as an issue with focus on water defecit isssues e.g. irrigation practices. Salinity has been implicated although the predominance of high quality water in California perhaps limits this impact to small pockets of vineyards with adverse soil/water relations. Nutrition has been implicated with extensive use of gypsum influencing salt levels in soil. The application of potassium has been discussed, in light of Australian experience, together with phosphorous deficiencies which manifest in foothills country with older soils.

Many attempts have been made to determine if an infectious agent is involved with nothing definitive to date coming to light.

Syrah decline in France

Syrah decline was first recorded in 1993. The symptoms described include:

- Early season yellowing, late season whole vine reddening
- Swelling and cracking at graft union

As for Red Vine in California, there are a number of studies focused on determining the cause of the decline. The impact of stock selection and Shiraz clone is variable, with hints that some clones are more susceptible than others. Use of a genetic marker correlated with decline symptoms has been used to allow a range of recommendations for clones rating from few symptoms through moderately sensitive to removal of clonal recommendations. This list currently includes clones 73, 99 (Clone implicated in South Africa), 301, 381, 382 and 383.

Research into pathogens and physiological causes have not as yet returned any strong correlations. The current view of the disease is that it appears complex and thought to have a number of factors involved.

From an Australian perspective, this relates to our current thinking on Restricted Spring Growth where Australian Grapevine Yellows is a pathogen associated with the syndrome, yet other factors appear to be involved albeit at differing levels (winter chilling, trunk physical damage, carbohydrate reserves, vine age, rootzone drying etc.).

Shiraz decline in South Africa

In contrast to the French situation, the symptoms are found only in Shiraz 99 (ex France in 1982). Vines with symptoms are reported to decline once affected over a period of 5–10 years. Many factors have been investigated, with the main common link being an association with a Rupestris Stem Pitting associated virus (RSPaV-SY).

Symptoms are similar to French descriptors:

- Swelling at graft unions

- Deep cracks in the bark
- Leaves redden
- Canes mature each year
- Vines weaken each year

Shiraz disease in South Africa

This disease was first noted in 1984. Alarmingly it affects approximately 2% of South African Shiraz vineyards and vines recorded with symptoms are documented as dying within 3–5 years.

In contrast with Shiraz decline, the symptoms are associated with Grapevine Virus A (GVA). With follow up on the disease, researchers note that many infected blocks that have been top-grafted with Shiraz show symptoms. Further it is believed that mealybug is spreading the disease

Symptoms include:

- Delayed budburst
- Reduced crops
- Reddening only

Shiraz disease in Australia

There have been a couple of reports suggesting that symptoms similar to French and South African reports have been seen in Australia. The reports suggested that PCR testing of these vines with reddening symptoms have shown positives in Australia which relate to a Rupestris Stem Pitting associated virus (RSPaV-SY) and another with Grapevine Virus A (GVA). Great care is needed in both making and assessing these reports, as with limited information, it is possible to assume we are seeing the first signs of a 'new' disease in Australia. We need to remember that reddening in vines can be caused by numerous factors and in addition, that PCR is a powerful, complex tool and that both positive and negative indicators must be included with testing for it to be reliable.

Fortunately, there have been limited appearances of these symptoms in Australia to date and we need to be alert to future detections and diligent in making assertions as to their associations. This is not to say that we should dismiss these views, but rather apply rigorous investigations to determine cause and effect.

The future

There are a number of syndromes in Shiraz around the world that appear to have 'virus-like' symptoms with transient expression that are of concern in terms of Shiraz productivity. Particularly as there are reports of Shiraz vineyards dying, Australia must keep a watching brief over these reports to ensure that we minimise the likelihood of exposing our considerable Shiraz resources to any future risk. At minimum, we need a focused watching brief to ensure awareness of developments and understanding of cause and effect.

This situation reinforces the need to monitor developments in all vineyards whenever 'disease' pressures occur. As described in this paper, a directed monitoring protocol documenting incidence and severity and following seasonal variation is an essential tool in determining cause and effect. Finally, the reports of 'Shiraz disease' in other countries is a strong reminder of the importance of planting material and certification and adherence to our current quarantine regulations.