Graft incompatibility syndrome in New Zealand Merlot vines involves another possible variant of GLRaV-2.

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Laboratory investigations into some recent cases of distressed or declining young vines in New Zealand vineyards have revealed the presence of a previously unknown closterovirus, which appears to be a distinct variant of the Grapevine LeafRoll associated Virus type 2 (GLRaV-2). We have called this virus the "Alfie virus" until we learn a bit more about it and develop a more descriptive name.

Many winemakers and viticulturists will be aware of the negative effects of Grapevine LeafRoll associated Viruses types 1 and 3 (GLRaV-1 and GLRaV-3) upon fruit quality, especially in the more sensitive red varieties. Few people are aware of the effects of GLRaV-2 and its associated variants. GLRaV-2 is known to be associated with graft incompatibility problems, and the combination of GLRaV-2 and some other viruses resulting in such incompatibility problems is a topic being extensively researched at the University of California at Davis (Golino et al., 1993, 2003). In New Zealand, the only reported findings of GLRaV-2 so far concern the Bordeaux clones of Sauvignon Blanc, 316 and 317, and we have observed incompatibility reactions with these clones when grafted onto some rootstock varieties. The Alfie virus appears to be a variant of GLRaV-2 and also produces similar incompatibility problems with some rootstocks. Another recently reported virus, the Grapevine Rootstock Stem Lesion associated Virus (GRSLaV) (Uyemoto et al., 2001; Rowhani et al., 2002) appears to be another variant of GLRaV-2.

Our molecular genetic studies on the Alfie virus so far have given us genetic sequence data on three important viral genes in the Alfie virus, and the closest relative we can find for this virus is GRSLaV. The next most closely related virus to the Alfie virus after GRSLaV is GLRaV-2. GRSLaV can cause severe incompatibility problems on susceptible rootstocks (Uyemoto et al., 2001), and while it was first found in the table grape variety "Red Globe" it has more recently also been found in some wine grape varieties.

Field observations in New Zealand
Merlot 481 is a relatively new arrival in New Zealand. The clone was only released in 1992, and the first wines were not made until 1996. Extensive plantings have since been made without a thorough working knowledge of the clone's performance and properties.

Field observations were made across a number of vineyards which had been supplied by a variety of sources. The young vines that we inspected that were either distressed or in decline were all Merlot clone 481, of about three to four years of age, grafted onto either 3306C or Riparia Gloire (RG).

The symptom that first alerted the viticulturists to the problem was that the vines went alarmingly red very early in either their first or second season (see figures 1 and 2). In some cases the vines showed very poor fruit set and an overall lack of performance, while in other cases, the vines seemed to be performing well despite the early reddening. Some growers were quite happy with the performance of the vines but were puzzled by the rather dramatic early reddening. Other growers, after three to four years of persevering, were ready to pull the vines out because of continued very poor fruit set and continuing decline.

We have seen the problem of a moderate to severe early reddening of Merlot 481on RG many times, and we have observed that after three to four years the vines usually seem to grow out of it and perform well. The worst-affected Merlot 481 vines we have seen with this problem have all been on 3306C.

Rootstock incompatibility
We have found examples of this Merlot clone grafted onto 3306C, 3309C, 101-14mgt and Riparia Gloire. On inspection, all the vines grafted onto 3309C and 101-14mgt were doing very well; they showed little or no early reddening and the growers were happy with their performance. The vines grafted onto RG had caused some concerns to the growers and showed a marked early reddening (see figure 3), and in some cases difficulty with fruit set, but for the most part they were performing well and after a few years the early reddening problem diminished. The Merlot 481 vines grafted onto 3306C showed a dramatic early reddening and some were also showing very poor fruit set and were causing the growers great concern.

On some low stress sites, the Merlot 481 vines grafted onto 3306C were still showing the dramatic early reddening but were making good fruit set and were certainly not described as "declining." This differential response when grafted to different rootstocks is consistent with what is reported for GRSLaV (Uyemoto et al., 2001), except that GLRSaV seems to react badly to a different group of rootstocks than the Alfie virus does. This leads us to speculate about the involvement of another rootstock-related factor that exacerbates the response of the rootstock when grafted with a scion variety carrying the Alfie virus or a similar GLRaV-2 variant.

Site-related response
We have observed that the negative response of the Merlot 481 vines when grafted to 3306C is lessened when the vines are planted on easier and more stress-free sites, such as flat ground with deeper soils and more sheltered aspects. On what we perceived to be the more stressful sites, comprised of heavy clays or exposed sites on thin-soil hillsides, the response of the vines was worst.

Lab findings
None of the standard PCR primers we commonly use for the detection of GLRaV-1, GLRaV-2, GLRaV-3 or any of the other known grapevine leafroll viruses, including GRSLaV, are able to detect the Alfie virus. However, in ELISA testing, the Alfie virus does show quite strong cross-reactivity with antisera specifically raised against GLRaV-2 (Agritest, Italy). An isolate of GRSLaV found in New Zealand – which is 99% identical in nucleotide sequence to the Californian isolate of GRSLaV (Zhang and Rowhani, GenBank accession AF314061) – also shows quite strong cross-reactivity to the same GLRaV-2 antisera.

The Alfie virus is quite difficult to find in the affected Merlot 481: only a relatively small number of positive vines can be detected by using PCR (approximately 10%), but a higher number of positive vines and a more consistent pattern are found when using ELISA testing based on the GLRaV-2 antisera (Agritest). We have tested a range of different sources of the Merlot 481on different rootstocks and all sources give us some level of positive vines, with the symptomatic vines giving us the highest percentage of positive results.

Initially, interesting indications of the presence of the Alfie virus were found by using degenerate closterovirus PCR primers made on the HSP70 gene (modified from Tian et al., 1996), but since then we have made a series of specific PCR primers designed to extend the sections of known sequence. Some of a series of PCR primers we designed to differentiate across a number of different genes between GLRaV-2 and GRSLaV have also been shown to be able to detect the Alfie virus. We have fully sequenced the HSP70 homologue gene, a gene which is common to all three viruses and is also an important gene for taxonomic comparisons of grapevine leafroll viruses. The similarities we have observed in the laboratory diagnostic results and the phylogenetic studies based on the genetic sequences of the HSP70 gene lead us to conclude that the Alfie virus, GRSLaV and GLRaV-2 are all closely related viruses and could all best be described as different members of the GLRaV-2 cluster.

Discussion
The Alfie virus is present in New Zealand's most popular and top-performing clone of Merlot, yet most viticulturists and winemakers are very happy with its performance. Our laboratory research has also revealed additional findings of GLRaV-2 in other cultivars, as well as another (possibly significant) variant of GLRaV-2 – this time, in a minor rootstock variety. It is also known that some clones of the most popular premium grapevine varieties currently in vogue around the world are infected with GLRaV-2 or one of its variants.

This raises several questions. What are the effects of this group of viruses on grapevine performance and fruit quality? Why are they present in some premium high quality clones and how did they get through the clonal selection process? The short answer is that when these clones were selected, the technology to detect and identify these viruses probably did not exist. However, the technology does exist now, and we have become aware of the presence of these viruses. This raises several more questions. Some people will say that these viruses may be responsible for the defining features of these clones, such as reduced vigour, yield and bunch-size. Others will say that trying to work with virus-infected clones is a dangerous business, fraught with potential loss of control over the normal viticultural processes.

The influence of viruses on viticultural performance is a poorly understood subject, and, as we are slowly finding out, there are far too many unknowns to be able to confidently predict the outcome. There are scion-rootstock incompatibility situations to deal with, which may not become apparent for two to four years. It also appears that the effects of the virus may also be moderated or exacerbated by the variations in sites. Each site has its own unique profile of stress factors. It is quite probable that vines

infected with these viruses will react more extremely to variations in site-related stress factors and to the use of different rootstocks than vines that are not infected with these GLRaV-2 type viruses.

Unfortunately, information on scion-rootstock incompatibility issues and site-related stress factors is not yet widely available or even well understood. In New Zealand, where many plantings are still under five years of age, some of these problems are just becoming evident. It is recommended that the possible implications for a vineyard based on a clone with a GLRaV-2 type virus in it be explored before planting begins.

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On the up-side, there is no evidence that these GLRaV-2 viruses are spread in the vineyards by insect vectors, and it has been shown that mealybugs will not transfer these viruses (Golino et al., 2003).

Obviously, the time has come for a full appraisal of the role of the GLRaV-2 group of viruses in high-performance viticulture.

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