Proceedings of the Syrah Vine Health Symposium

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Syrah Vine Health Symposium

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The Syrah Vine Health Symposium brings together researchers from the University of California and abroad to present the current state of knowledge about the unique growth problems specific to the Syrah winegrape variety. The purpose of the symposium is to provide growers and other industry members an opportunity to hear from several of the researchers who have addressed this issue; what they have learned and what investigations are underway.

Nearly 19,000 acres of Syrah (Shiraz) are currently grown in California and from 1996 through 2001, bearing acres increased more than 10 fold; from 931 to 9,573 acres (California Grape Acreage, http://www.nass.usda.gov/ca). Not long after the planting boom began, some growers noticed unique foliar symptoms and trunk growth in their Syrah vineyards. Affected vines could be scattered within a vineyard block or comprise the entire planting. There is a gradual decline in canopy growth which results in poor wood selection during winter pruning. In some sites, vine loss has occurred in as few as two years after the onset of these unique growth issues.

Vine symptoms may include leaf blades with red color which occasionally appears similar to symptoms caused by grapevine leaf roll associated viruses; vertical crevasses in the wood very near the graft union; swollen unions; and necrotic areas along the length of the trunk. When the bark is removed on such vines, it is common to see necrotic pits in the wood just above the union as well as necrotic regions that sometimes involve half or more of the diameter of the trunk. Not all symptoms are present on affected vines.

These symptoms are more prevalent in the North Coast and the Sierra foothills, yet they also occur in the Central Coast. In that growing region, the term “Syrah Disorder” has been used to characterize numerous irregularities in vine performance that include delayed fruit maturity, poor fruit quality, and brown leaf color.

These proceedings include extended abstracts of presentations given at the symposium as well as selected articles found in the literature that address Syrah vine health issues. These articles appear in the Appendix and have been reproduced in their entirety and without modification from journals which have granted copyright permission. The Selected Bibliography includes citations of articles that appear in the Appendix and others found in the literature.

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A brief summary of Syrah Disorder/Decline on the California Central Coast

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Introduction

In the early 2000's, growers on the Central Coast began observing widespread poor performance of Syrah, while other varieties under similar conditions did not show similar behavior. Poor performance ultimately included either very low quality fruit (low sugar, high pH, low color), or poor vine survivability, with either situation eventually leading to non-economic production in many cases. Similar behavior of the variety may have existed locally prior to this period, but it may not have been as obvious when the bearing acreage was smaller and market was less demanding of fruit quality.

At the time this predominantly local performance problem was labeled "Syrah Disorder", to distinguish it from what appeared to be the different "Syrah Decline" observed in France. Over the past several seasons, observations of local Syrah plantings have allowed for the separation of the initial 'disorder' into multiple 'disorders' which affect predominantly (but not always exclusively) this variety. These 'disorders' can be summarized as: 1) identifiable leafroll virus, 2) symptoms identical to leafroll virus, but in which virus testing does not identify leafroll, 3) leaf burn consistent with drought/salinity effects, and 4) symptoms similar to the "Syrah Decline" observed in France. To confound things, the above symptoms may occur together or singly.

Leafroll virus like symptoms

Symptoms resembling leafroll virus are prevalent in numerous area Syrah vineyards, and are not uncommon in other varieties. Typically in late July or early August the leaves begin to show red or reddish-brown splotches in the interveinal areas, with the veins remaining green. In dry years the symptoms typically show up sooner and more dramatically, while in wetter years generally later and less notably. Regardless of the severity and timing of the symptoms, they appear to some degree year after year in affected plantings. The percentage of symptomatic vines within a planting can vary widely, from a few percent to virtually 100%; in the latter situations the condition is actually not as obvious because no contrasting healthy foliage exists for comparison.

The major complaint with Syrah plantings with leafroll symptoms is the poor quality of the fruit; ripening is delayed, and the juice has consistently lower sugar, higher pH, poor color, and sometimes much higher potassium as compared to fruit from healthy vines. Wine quality is reduced accordingly, thus making the fruit uncompetitive in the market.

However, the vegetative growth and survivability of affected vines appears to be little affected by the presence of the leafroll virus; infected vineyards typically make robust and healthy-appearing spring growth and often set normally sized crops. Affected vines generally do not show any signs of trunk deformations or graft union incompatibilities.

It should be noted that the above effects of leafroll virus are not unique to Syrah; foliar symptoms, and similar effects on fruit quality, can be very obvious and widespread in other varieties such as Zinfandel.

Some Syrah vineyards show all the symptoms of the leafroll virus condition described above, but even repeated comprehensive virus tests have returned negative results. This suggests that there may exist a strain of leafroll virus which our current laboratory tests cannot identify. However, this condition is likely also not limited to Syrah, as very similar leafroll-like foliage and fruit symptoms have been noted in other varieties locally as well, for which comprehensive virus testing has also not identified any causal agent to date.

Leaf burn symptoms

Leaf burn symptoms, in which much or all of the leaf area develops the dry, crumbly consistency of potato-chips in late summer, appear to occur mostly in the hotter and drier inland areas of the Central Coast. The symptoms are consistent to what is typically described for severe water and/or salt stress. In some cases only the outer leaf margins show burn symptoms, while in more severe cases it is virtually the entire leaf blade. These symptoms are often ephemeral, appearing very severely in some years but not at all in other years. These symptoms appeared to be more widespread in the years 2003 and 2004, which followed very dry winters, but less severe in 2005 and 2006, which followed wetter winters, and again made a resurgence at some sites following the very dry winter prior to the 2007 season. It seems likely that irrigation and salinity management practices play an important role in the appearance of these symptoms; in particular, periods of significant and perhaps abrupt water stress late in the season, when little reserve soil moisture is available, may help trigger the rapid onset of the condition. Whether this behavior is related to the unusual drought response physiology of Syrah is unclear. Effects may be related to the characteristically vigorous vegetative growth of the variety, which may lead many growers to impose relatively severe water stress in efforts to keep the vegetative growth under control.

Early descriptions of leafroll virus also included references to similar leaf burn conditions; as such this behavior may also be related to the virus. However, locally leaf burn symptoms can occur in vineyards which show no obvious red leaf coloration like that which is typically associated with leafroll virus.

While Syrah appears to be the variety most prone to these severe leaf burn symptoms, other varieties, in particular the genetically related Durif or Petite Sirah, can also show similar, if perhaps not quite as extreme, behavior.

Leaf burn symptoms which occur well before harvest will likely have significant effects on delaying fruit ripening due to loss of photosynthesizing leaf area, and will also greatly increase fruit exposure to excessive amounts of sunlight. Recreating leaf burn symptoms on a small scale by irrigation reduction in field trials has not been very successful, whereas increasing soil salinity in small plots has led to similar symptoms.

The fact that these burn symptoms are not reported widely elsewhere may indicate that some of the more unique characteristics of the inland regions of the Central Coast, such as the relatively dry winters, high evaporative demand in the summer, and sometimes marginal quality irrigation water, may play significant roles in the symptom expression.

Decline symptoms

A relatively small proportion of the Syrah vineyards on the Central Coast have shown symptoms similar to the ‘Syrah Decline’ described in France and reported in other parts of California. These symptoms include notably deformed graft unions with very poor connectivity between the rootstock and scion, the presence of deep wounds on the permanent wood, and ultimately the death of the vine, usually over the winter following a previous season of very weak and fruitless growth. Fall leaf symptoms may or may not show early reddening. Symptoms generally do not affect all vines at any given time, and symptoms may appear over the span of many years, eventually affecting even those vines which had made robust and productive growth for numerous seasons. Fruit quality does not appear to be affected in any negative way; in fact a number of affected plantings have been producing exceptionally high quality wines, which makes their gradual decline even more frustrating. In this area, the only common factor amongst affected plantings is that clone 877, on various rootstocks and from various sources, seems to be the most commonly affected clone. Comprehensive and repeated virus testing of several affected plantings to date have not indicated any obvious viral agents.

Syrah Decline in France: Historical background and first approaches

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Introduction

Syrah is one of the most important grapevine varieties cultivated in southern France. Syrah is the 5th most important variety in France (67500Ha, i.e. 160650 acres) and the 2nd largest grafted variety with more than 18 million plants grafted by nursery industries in 2006. Its origin area is the north Rhône Valley where Syrah is the only red variety cultivated in the famous "appellations" Tain l'Hermitage and Côte Rotie. Because of its great potential to produce high quality wine with intense color, good balance between polyphenolic structure and aromatic compounds, Syrah was rapidly propagated in Mediterranean vineyards in Languedoc and Provence, beginning in the 1970s, In 1993 growers and viticulture farm advisors observed the first symptoms of Syrah decline in Languedoc. Then the symptoms appeared progressively in different areas in Languedoc and Provence and more recently in the north Rhône Valley, followed by all Syrah vineyards exhibiting symptoms of the syndrome. In 1997 a National Working Group was established with various technical partners and advisors to collect observations and data and to propose and conduct experiments.
ENTAV manages this group and in 2001 a permanent researcher was appointed.

Symptom description

Syrah decline is characterized by symptoms at two levels: in the canopy and at the graft union.
-canopy level:
The first symptom to appear during the growing season is an early leaf yellowing. Then at the end of summer an early leaf reddening occurs, confirmed during fall by a characteristic intense reddening on all blades. All the leaves on a vine are involved.
-graft union level:
The reddening is associated with a swelling and cracking at the graft union. On this localized area (6 to 10 cm) the abnormal enlargement is associated with deep and parallel grooves easily observed after removing the bark. The wood becomes hard and necrotic zones develop in varying degrees.
Sometimes the scion declines and then dies but the rootstock may stay alive.
Finally, the most important characteristic symptom of Syrah decline is the development of huge cracked graft unions, followed or not by a leaf reddening and more often by death.
-Spread of the decline:
Syrah decline can be observed on different types of soil (clay, schist,…), clone sources of Syrah, rootstock varieties, management techniques and environmental conditions. Usually symptoms appear mainly on 6-10 year old vines, but recently symptoms could be observed earlier on 4-5 year old vines.

Biometrical analysis

The expression of the declining is more or less severe and depends on different uncontrolled factors. It is not possible to predict the evolution (slow or rapid) of the syndrome in a specific vineyard.
Several surveys and plant by plant mapping of symptoms performed over 4 years (1997-2001) across 38 vineyards in various situations, allowed researchers to conduct biometrical analysis. The aim of this study is to describe a temporal and spatial evolution of the symptoms.
In a global approach the evolution is:
First, swelling and cracking vines with green leaves (noted B0), then cracking and leaf reddening vines (noted B1) and more or less rapidly dying vines (noted M). The symptoms seem to extend from one plant to another along the same row. Groups of reddening vines (B1) are observed among the cracking

vines (B0).
The probability to die is more likely in a reddening vine (B1) than in a cracking vine (B0).
The probability to die is more likely in a cracking vine (B0) than in a normal vine.
The probability to redden is more likely in a cracking vine (B0) than in a non-symptomatic vine.
The probability is relatively stable within the same plot, but is very variable from plot to plot.
The vintage seems a non-determinant factor, but environmental conditions seem more efficient.
Between two successive years the probability to die for a normal vine is about 1%, for a cracking vine is 2-4% and for a reddening vine is 19-26%.

Histological studies

Various histological studies were carried out to understand the cracking formation at the graft union.
-First, serial transversal sections at the graft level were cut in small pieces to observe the evolution of grooves and necrotic zones from the rootstock area to the scion area.
The comparison between cracking vines (B0) and reddening vines (B1) showed that the live tissue became smaller and smaller and could explain, in part, the declining behavior.
-Second, thin sections at the cracking area were observed under the microscope, and showed a clear dysfunction of the cambial zone.
This dysfunction involves a final stop of cambial activity with a lack of formation of new vessels. The phenomenon is associated with characteristic production of phenolic compounds.
Finally, disrupted flow between rootstock and scion lead to death.
-Third, a comparison study of the graft region of Syrah and control varieties Grenache and Cabernet Sauvignon shows that Syrah presents a specific behavior. Serial thin slices taken from one month-old to fifteen month-old vines confirm the incorrect production of vessel connections and the early formation of necrotic zones in the Syrah vines, in contrast with the control varieties.
New histological studies are on going.

Grafting factors

At the beginning the growers thought that the problem was the result of new grafting techniques. In particular, the use of a new type of graft associated with the application of hormonal substances.
To check this hypothesis, bench grafting trials were conducted.
Comparative trials were done on Syrah with two types of graft systems (omega and long wipe), two types of wax and three concentrations of hormone at the base of the grafted cutting.
These eight grafting modalities were planted in two vineyards on the basis of ten replicates of ten vines per modality.
After ten years of symptom observations we do find any differences between the two types of grafting.
But an important increase of cracking symptoms is observed in both graft types. Less than 50% of vines do not have any symptoms and 4-7% are dead.
These trials demonstrate clearly that the type of grafting is not involved in the Syrah decline.
Moreover green grafting and field grafting were tested as well. Only a few vines are beginning to express cracking symptoms. Results from these tests may indicate a technique that could delay the decline.

Syrah Decline in French vineyards:
Rootstocks and Syrah Clone Impact, Pathological and Genetic Studies

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Rootstocks and Syrah clone impact

The understanding of the impact of rootstocks and clones on symptom expression in Syrah decline is essential for future plantings. Numerous trials were followed in all the French Syrah vineyards from 2001 to 2004.

Five trials were monitored from 2001 to 2003 to study grapevine rootstock behavior. All of these rootstocks were associated with Syrah decline. At least 30% of vines displayed cracks while 2-10% of vines were already dead (global death, all different reasons included). Nevertheless, two rootstocks (110R and 99R) presented increased sensitivity with about 70% of plants showing cracking symptoms (often associated with reddening). The use of both 110R and 99R is now not advised. The difference in symptom expression was not significant in other rootstocks (1, 3).

Observations on the clone aspect were carried out during 2003 and 2004 in 40 vineyards consisting of increase blocks and experimental vineyards. The 16 certified ENTAV-INRA® clones have been checked. Numerous and reproducible results were obtained which gave important information on the behavior of clones in the Syrah decline. Even if no clone seemed totally free of symptoms, great differences of behaviors were noticed (1, 4). Three groups of different behaviors were thus identified:
- 470, 524, 747 and - in a less manner 471 - show very few symptoms
- 100, 174, 300, 525, 585, 877 appear moderately sensitive
- 73, 99, 301, 381, 382 and 383 seem to be more sensitive and are currently not recommended

Following these results, we began a new Syrah clonal study where the behavior of clones with respect to syrah decline is a major criterion. Data were collected in 2005/2006 in two sites where candidate clones had been grown since 1995. We selected 14 clones without any symptoms and which had a large diversity of agronomic potential. Two new trials will be planted in 2008 in a larger scale to verify their "resistance" towards Syrah decline and their qualitative potential.

Research on pathogens

Studies were conducted to look for any associated transmissible agent(s). Some transmission tests were carried out and gave interesting results. Canes taken from declining Syrah (so called "Syrah +") were grafted onto different varieties as scions or rootstocks to observe potential symptom expression on other *Vitis vinifera* that would prove a pathogen transmission. A fastening expression of cracking was observed on "Syrah +" grafted onto 110R, indicating a possible increase of the symptom expression in the offspring of declining plants. Cracks could also be observed when "Syrah +" was grafted onto different *vinifera*. Finally, the observations of some double-grafted plants indicated that cracks were only present on the Syrah area of the plants. The cracking formation, when Syrah is grafted onto *vinifera* and even onto Syrah, seems to exclude the hypothesis of a genetic incompatibility. For the moment, transmission cannot be proven by these trials; if a pathogen is involved in Syrah decline, it may be unable to induce the same symptoms on other varieties.

Beside these tests, analyses were carried out to determine if the main classes of pathogens (bacteria, fungi, phytoplasma and viruses) were present in symptomatic plants.

- No phytopathogenic bacteria (Crown Gall, Bacteria Blight, Pierce's disease) were found in the samples analyzed.
- Concerning fungi, some of them involved in wood diseases were found in symptomatic plants but also in control vines. Their presence could not be correlated with the specific Syrah decline. Nevertheless, fungi involved in wood diseases might play a secondary role in increasing or quickening the decline of already weakened plants as they could induce necrosis leading to plant death. Involvement of these fungi in Syrah decline will be studied in vineyard experiments.
- As some of the symptoms (leaf-reddening and problems with wood maturation) appear similar to some of the physiological disorders classically observed in grapevine yellows, implication of phytoplasma in Syrah decline was investigated. The possible association of phytoplasmas with this disorder has been tested by molecular method (nested-PCR) in samples taken from symptomatic and non-symptomatic plants. Numerous samples of petioles/veins and flowers/clusters were collected from May to October 2004, from plants showing different degrees of Syrah decline. Sampled vines included those without any symptoms; vines showing only swelling and cracking at the graft union without leaf reddening; and vines expressing both cracking at the graft union and foliar reddening. Positive samples were found in both symptomatic and symptomless plants (Table 1). The sequencing of PCR fragments revealed the presence of two distinct phytoplasmas. One phytoplasma belongs to 16SrXII group (Stolbur) and the other phytoplasma belongs to 16SrI group (Aster yellow). No correlation could be established between the type of sample (tissue, sampling date and degree of symptoms) and the group. It was the first time the 16SrI group phytoplasma was reported in grapevine in France (2). However, there is still no obvious correlation between phytoplasma infection and Syrah decline; further investigations are in progress.
- Virus studies were conducted in collaboration with the INRA ViVe team (Lemaire O, Beuve M and Ignatovic L.). The goal was to compare the sanitary status of 22 clones presenting different behavior with respect to syrah decline: 16 certified and 6 non-certified, the latter used as "model clones". Among these 6 "model clones", two appeared totally free of symptoms, two were very sensitive and two came from sanitized plants produced by shoot tip propagation from sensitive clones. RT-PCR assays were performed using primers for 14 grapevine viruses (GLRaV-1, 2, 3, 4, 5, 6, 7, 9; GVA; GVB; GFkV; GFLV; ArMV and RSPaV). Nine to ten plants were analyzed per certified clone and five to ten per non-certified clone. The 200 vines tested were free of 12 viruses whereas 17 were infected with GFKV and 194 gave positive results with RSPaV primers. All of the six samples that tested negative for RSP came from shoot tip-propagated plants. Phylogenetic analyses were performed to study RSPaV variability in Syrah clones. More than 50 sequences obtained from 20 clones were analyzed. Sequences showed up to 14% variability indicating important molecular variability between isolates. No correlation could be established between these types of isolates and Syrah clone sensitivity. Furthermore, two plants of the same clone can be infected by two different isolates. Even if all these results seem to exclude this virus as responsible for Syrah decline, complementary studies have to be conducted before a conclusion can be made.

Genetic studies

The different behaviours of the 16 certified clones led us to work on the possibility of identifying them with specific markers. Among the microsatellite markers identified as giving different profiles for these clones, one named VMC5g7 gave results of specific interest. Tested on 100 different clones (certified and not), a high level of correlation (86%) was established between its profile and clone sensitivity (Table 2). This result will have practical and fundamental applications. It is already used as a help for current clonal selection in eliminating clones with the profile linked to the presence of more frequent symptoms (198-216-218). Moreover it opens new perspectives and especially a genetic approach of the Syrah decline issue. The very recent communication on grapevine genome sequencing has led us to localize this marker on chromosome 2, in a gene promoter known in other species (such

as *Arabidopsis)* to be a heat stress transcription factor (HSFA1D). This opens a new exciting field of investigation to understand Syrah decline.

Conclusions and perspectives

Syrah decline appears to be very complex and probably involves numerous factors. Even if the causes have not been identified yet, many important results have already been obtained concerning the different behaviours of Syrah clones and the identification of a genetic marker.

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Type of vines	Positive samples
No symptom (control vines)	**8/42 (19%)**
Cracking, green leaves	**9/42 (21%)**
Cracking, red leaves	**15/46 (33%)**

Table 1: Results of PCR detection of phytoplasmas in 130 samples of Syrah plants. x/y corresponds to number of positive samples onto total samples tested. Nested PCR with P1/P7 followed by U5/U3 primers were utilized.

Number of clones / VMC5g7 profile	"not sensitive" clones	"sensitive" clones	
198-216	**56**	**14**	**80% "not sensitive" 20% "sensitive"**
198-216-218	**0**	**30**	**100% "sensitive"**

Table 2: Correlation between clone sensitivity and VMC5g7 profile. Clones presenting less than 5% of cracking are considered to be "not sensitive". By contrast the sensitive clones present at least 20% of cracking.

Investigation of association of viruses of *Viti-* and *Foveavirus* genera with Shiraz disease and Shiraz (Syn. Syrah) decline in South Africa

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Shiraz disease (SD) and Shiraz decline (Sd) are two emerging diseases that threaten grapevine cultivar Shiraz in South Africa. Results suggest that viruses of of the family *Flexiviridae* are associated with these diseases.

Symptoms

Shiraz disease
Canes of SD-affected plants never mature fully (Fig. 1A). Cross-sections show weak wood and excessive phloem development. Affected grapevines look clearly different from disease-free plants in vineyards at the end of the growing season, canes are “droopy” and leaves with leafroll-like symptoms are shed much later. The disease affects growth, delays or prevents budburst and severely affects the production of fruit. Once a grapevine shows definite SD symptoms it never recovers, and dies within 3-5 years. Grapevine cvs. Merlot, Malbec, Gamay and Viognier are also susceptible to the disease. Other cultivars, although infected (SD-positive), do not exhibit symptoms. The disease is transmitted from them to disease-free plants by grafting of infected tissue and/or by mealybug *Planococcus ficus* (Goszczynski and Jooste, 2003a). Shiraz disease spreads in vineyards, frequently along rows, but there is no clear leafroll-like pattern of its spreading. Although, according to rough estimation, only about 2 % of South African Shiraz is affected by Shiraz disease, whole blocks are sometimes affected after top grafting of Shiraz to SD-infected rootstocks. A similar disease, called Australian Shiraz disease, has been reported from Australia (Habili and Randles, 2004).

Shiraz decline
Shiraz decline is observed in South Africa only in clone Shiraz99, which was imported from France in 1982. The clone was subjected to heat therapy (101 days at 36-38 ^{o}C) in 1986 and multiplied from in vitro meristem tip cultures. The buds from established plants (clone 99B) were grafted to Richter 99 rootstock (SA clone RY30) and planted in 1997. Locally selected Shiraz clones 1, 21 and 22, as well as clones 5 and 9, imported from Argentina, which are growing in blocks next to clone 99B, are not affected by Shiraz decline (Spreeth, 2005). Symptoms of the disease in clone 99B are similar to those reported by French researchers (Renault-Spilmont and Boursiquot 2002). Graft unions of infected plants are abnormally developed, swollen, with deep cracks in the bark of Shiraz scion that reach xylem tissues (Fig. 1C). Cross-sections reveal heavy modified wood. In severe cases, after the removal of bark, grooved wood is visible in the trunk above the graft union. Later in the growing season, leaves become intensively red and usually do not show the rolling symptoms characteristic of leafroll disease. Unlike with Shiraz disease, canes of Shiraz decline-affected plants mature fully but new growth becomes weaker every year. Affected plants die in 5-10 years. The disease does not affect rootstocks.

Suspected viral pathogens associated with the diseases

Shiraz disease
A vitivirus, Grapevine virus A (GVA) has been found to be associated with Shiraz disease in South Africa (Goszczynski and Jooste, 2003a). Results showed that of three divergent molecular groups of the virus, which were identified (Goszczynski and Jooste, 2003b), variants of molecular group II, are closely associated with the disease (Goszczynski, 2007). This conclusion was based on results of comparative analyses of GVA variants from grapevines with differing SD status. The grapevines, that were used in the study, were field-collected or propagated in the laboratory from plants that originally

were symptomless or exhibited only very mild symptoms of the disease. Analysis was possible after the development of a method of rapid identification of GVA variants of molecular group II, which is based on the single-strand conformation polymorphism (SSCP) technique (Orita et al., 1989). A short, 234 nt, fragment of GVA genome was RT-PCR amplified and then single strands of the DNA product were electrophoresed in polyacrylamide gel. DNA strands with different nucleotide sequences migrate in gels differently and produce different patterns of bands. The SSCP bands of amplified 234 nt sequence of GVA variants of molecular group II are unique, as they migrate very slowly and/or are diffuse in appearance (Goszczynski, 2007). SSCP analysis of randomly collected SD-free and SD-affected grapevines revealed bands that slowly migrate and are diffuse in appearance, indicative of variants of molecular group II, and were observed in majority of SD-affected grapevines but were sporadic in disease-free plants (Fig. 1B). The variants of molecular group II were also dominant in SD-affected plants that were multiplied in the laboratory from mother field-collected plants that originally did not show symptoms of the disease. The analysis revealed that GVA-infected but SD-negative plants, both field-collected and those multiplied in the laboratory, were commonly infected with variants of molecular group III. It suggests that variants of this molecular group may protect grapevines from infection with GVA variants of molecular group II associated with Shiraz disease. This would explain the lack of clear pattern of spread of the disease in SA vineyards, despite the common presence of the insect vector of GVA, mealybug *P.ficus*.

Shiraz decline

The report of detection of a highly divergent molecular variant of *Rupestris stem pitting-associated virus*, called Syrah strain (RSPaV-SY), in Syrah decline-affected plants (Lima et al. 2006) and the similarity of symptoms of Shiraz decline to those of rugose wood diseases of grapevines, were reasons for launching the investigation of the presence of viruses of the genera *Fovea-* and *Vitivirus* in affected and healthy Shiraz in South Africa. A total 101 plants of different clones, including 57 and 19 Sd-affected and symptomless plants of clone 99B, and 15, 5 and 5 plants of the disease-free clones 9C, 22B and 22F, respectively, were collected. Plants that were potted in the laboratory from rooted cane cuttings of 2 Sd-affected and 1 symptomless plant of clone 99B, and nucleus plants of Shiraz clones 99B, 9C, 22F and Richter 101-14 clone AA219, kindly supplied by Vititec (KWV) were also used in the study. Potted plants were kept in growth chambers at about 25 ^{o}C and with a 12-h photoperiod.

Preliminary RT-PCR amplification of dsRNA extracted from cane shavings of 4 Sd-affected plants of clone 99B, 2 field-collected and 2 potted 8 month-old plants, using primers specific to RSPaV-SY (Lima et al., 2006), was consistently negative. On the other hand, strong amplification of these dsRNAs was obtained using degenerate primers for simultaneous amplification of viruses of the *Fovea-* and *Vitivirus* genera designed by Dovas and Katis (2003). Cloning of 199bp DNA products of amplifications, SSCP analysis of clones and sequencing of clones with differing SSCP patterns, revealed that plants were infected with populations of virus variants related to RSPaV-1, -SG1 and -BS (79.8-93.5 % nt similarity), and RSPaV-SY, GVAIs151 and GVBItaly (85.4-89.9, 74.4-76.9, 74.4-81.9 % of nt similarity, respectively). The same techniques were used for the analysis of 52 Shiraz plants of clones 99B with differing Sd status, and disease-free plants of clones 9C, 22B and 22F, which were collected at the beginning of the growing season in SA, in January 2007. Leaves of Sd-affected plants were pale red in color. Results of RT-PCR based on degenerate primers showed that all grapevines, independently of Shiraz decline status, were virus-infected. The plants were also tested using RT- PCR based on GVB-specific primers. GVB was detected in many plants of Shiraz but there was no association of the virus with the disease. GVB-negative plants of various clones with differing Sd status were selected for further analysis. Results revealed that virus variants related to RSPaV-SY (86.2-90.4% nt similarity) are commonly present in Sd-affected and symptomless plants of clone 99B as well as disease-free plants of clones 9C, 22B and 22F, and rootstock clone AA219 (Fig. 1D). Population of these variants was relatively uniform, sharing 92.8-98.0% nt similarity. Variants of RSPaV and GVA were also detected in these plants. There was no clear association between any detected virus and the disease. Similar results were obtained after RT-PCR testing of 31 plants of Shiraz clone 99 with differing Sd status and 5 plants of disease-free clone 9C, which were collected in May 2007. Leaves of Sd-affected plants were very red in color. All plants except one were virus-infected. Study of 6 plants selected for detailed analysis revealed that variants

related to RSPaV-SY were common in the plants of clones 99B and 9C. Unexpectedly, the sequence data showed that one plant of Shiraz 99B was infected also with a virus variants sharing 72.7-73.4% nt similarity with a *Foveavirus*, Apple stem pitting virus (ASPV). Further study revealed that 18 month-old potted plants of clone Shiraz 99B were infected with a dominant and extensively heterogenic population of variants related to ASPV. The variants shared 73.7- 82.8% nt similarity with ASPV reported by Jelkmann (1994) and 76.8-82.3 % of nt similarity between variants (Fig. 1D). The suggestion that ASPV may be associated with Shiraz decline was supported by results of a study of the Fovea- and Vitivirus status of nucleus plants of Shiraz clones 99B, 9C, 22F and rootstock Richter 101-14 clone AA219G. The virus was detected only in Shiraz clone 99B. Other clones were infected with various molecular variants of RSPaV (clone 9C), RSPaV-SY and GVB (clone 22F). A nucleus plant of Richter 101-14 (clone AA219G) was infected with variants of RSPaV-SY, GVB and a *Trichovirus*, Apple chlorotic leaf spot virus (ACLSV). The possibility that ASPV is associated with Shiraz decline is presently under investigation. Detection of ASPV in grapevines was reported earlier by Hu et al. (1990). The virus is commonly present in commercial cultivars of apple. It mainly occurs as a latent virus but it is also reported to be associated with a number of diseases of apples and pear trees, including symptoms of abnormal wood development of some rootstocks and decline of apple varieties if grafted to these rootstocks (ICTVdb Management, 2006). ASPV is mechanically transmitted to herbaceous hosts, but it is not known whether or not it can be transmitted by insects.

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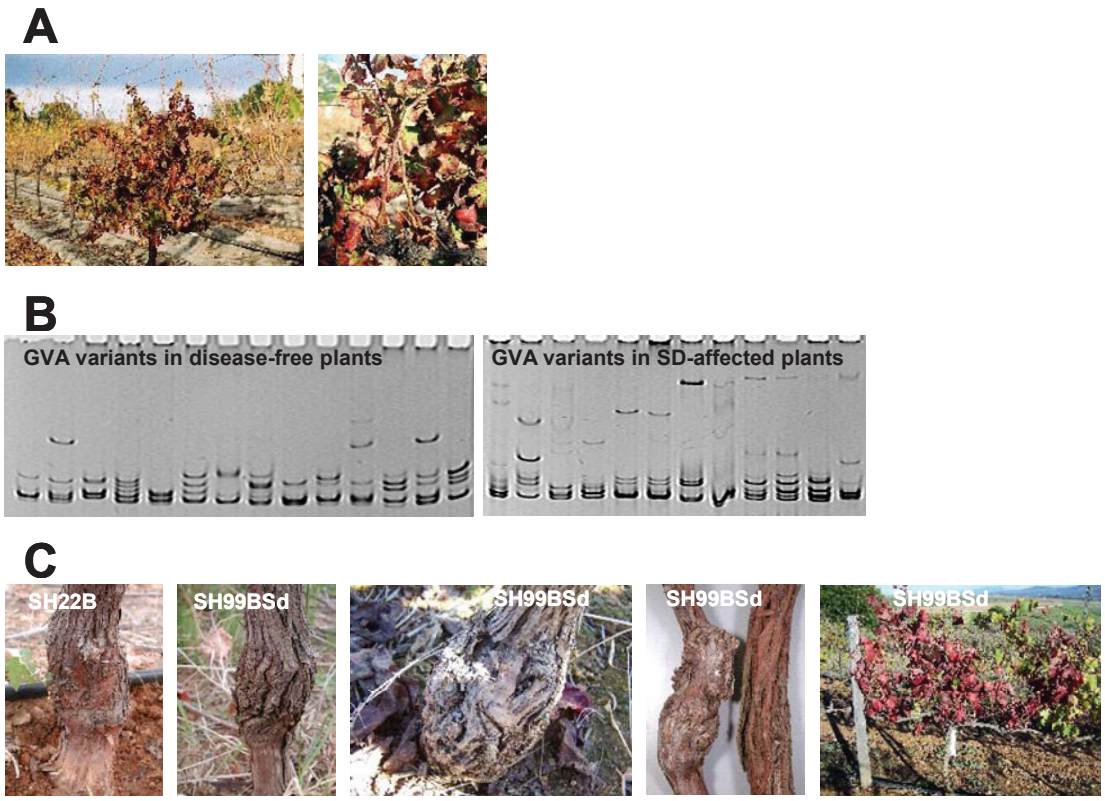
Fig. 1 Symptoms of Shiraz disease (SD) (**A**) and Shiraz decline (Sd) (**C**) and selected results of virus status analysis (**B**, **D**)



Syrah Decline: Viral or non-viral?

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Declining young grapevine plants of *V. vinifera* variety Syrah suggesting a unique problem for this popular variety. Generally the affected vines show red canopy symptoms starting from early to late summer due to the stress caused by affecting factors. In many cases, symptoms in problem vineyards could be attributed to specific factors, including frost damage of young vines, water stress, poor planting and training techniques and other physical damages on the trunk. However, in our survey of number of different Syrah vineyards in California we found many cases that the symptoms on the trunk were suggestive of the involvement of a virus or viruses. These symptoms were similar to those reported in France characterized by swelling and cracking of the graft union, pitting and cracking of the woody cylinder which sometimes extended to the tip of the cordon (Fig. 1) and finally vine declines. When these plants were tested by RT-PCR for known vitiviruses, closteroviruses, and nepoviruses, all tests were negative, but many were tested positive for *Rupestris stem pitting associated virus* (RSPaV).

The possibility of the involvement of an uncharacterized virus or viruses in the decline syndrome was examined in a Syrah plant on 101-14 Mgt rootstock. Dormant cuttings were collected from the plant and cambial tissue was scraped and used for dsRNA extraction. DsRNAs were used as templates for producing a cDNA library for sequencing the genome of the viruses present in the plant. The sequence information revealed the presence of a virus closely related to RSPaV. The nucleotide sequences of different genes of the new virus showed similarities ranged from 72 to 89% (data not shown) and the overall sequence identity of 77% to other published RSPaV sequences (Zhang *et al.,* 1998; Meng *et al.,* 2005). This virus was considered to be a new strain of RSPaV and named RSPaV-SY.

The phylogenetic analysis of RSPaV-SY compared to 27 isolates from different geographical regions and different grapevine varieties showed that this strain clustered together with few other isolates (Figs. 2A and B). In comparing the replicase gene, the RSPaV-SY isolate was grouped with the PN53 and CHS4G isolates, constituting one of the main branches of the phylogenetic tree (Fig. 2A). Considering the phylogenetic pile up of coat protein, RSPaV-SY clustered together with seven other isolates (Fig. 2B), including PN53 and CHS4G, the same isolates that clustered for the replicase fragment.

To investigate incidence of RSPaV-SY isolate in commercial vineyards, by RT-PCR, two pairs of primers targeting two different genes were used. One set of primers, RSP 48V/49C, was designed from a more conserved region (coat protein) and used as RSPaV-universal primers, which amplified a 331-bp fragment. The second pair, SY9F/SY8R, was designed from a more variable region (replicase gene) of theRSPaV-SY, which specifically detects this strain, and amplified a fragment of 628 bp. Among 399 plants tested, including 86 Syrah plants, 259 (65%) tested positive using RSPaV-universal primers (48V/49C); 73 of the positives were Syrah plants. Using the RSPaV-SY-specific primers (SY-9F/SY-8R), 48 (12%) of the RSPaV-positive samples were also positive for RSPaV-SY, including 41 of the positive specimens from Syrah plants. Thus, roughly half of the diseased Syrah plants, but very few of the other infected grape varieties (7/399), were infected with the SY strain. To confirm the specificity of the RSPaV-SY primers, twenty of the amplicons generated by these primers were sequenced and found to share about 90% nucleotide identity to RSPaV-SY and about 73% to RSPaV sequences in the database. Among the Syrah specimens in the collection tested, 16 asymptomatic plants (without any canopy symptoms) were included in these assays and 15 plants tested positive for the 48V/49C universal and 12 for RSPaV-SY (SY-9F/SY-8R) specific primers. However, in our survey work we were not able to establish correlation between the virus (RSPaV-SY) and the disease in Syrah (red canopy).

We are pursuing our work to check for the possibility of involvement of different virus (es) in the disease syndrome using more modern technologies which were became available very recently.

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Zhang Y-P, Uyemoto JK, Golino DA and Rowhani A (1998) Nucleotide sequence and RT-PCR detection

TABLE 1 – List of oligonucleotide PCR primers used in this study.

	Sequence	Location in the genome	Length (nt)	Product size (nt)
48V	5'AGCTGGGATTATAAGGGAGGT3'	8,177-8,198	21	331
49C	5'CCAGCCGTTCCACCACTAAT3'	8,506-8,526	20	
P-35F	5'ATGGTTGCATGATCACAGCCA3'	3,545-3566	21	776
P-43R	5'AGTGGCCAGCCTTCAATCC3'	4,300-4,319	19	
PN-1F	5'GATGGATACAAGTTACGGGC3'	3,442-3,462	20	866
RR-43	5'ACATCCCACCCTTCCTTCTT3'	4,289-4,308	20	
CP-1F	5'GGTTTGAAGGCTTTAGGGGT3'	7,709-7,728	20	803
49C	5'CCAGCCGTTCCACCACTAAT3'	8,506-8,526	20	
SY-9F	5'AGGATTCCAAACTGTAGAGCAA3'	2,083-2104	23	627
SY-8R	5'TTGGTCGTCATCTTCCAGTT3'	2,689-2,710	20	
CP-3F	5'TGAAGAAATTGATTATC3'	7,741-7,757	17	-

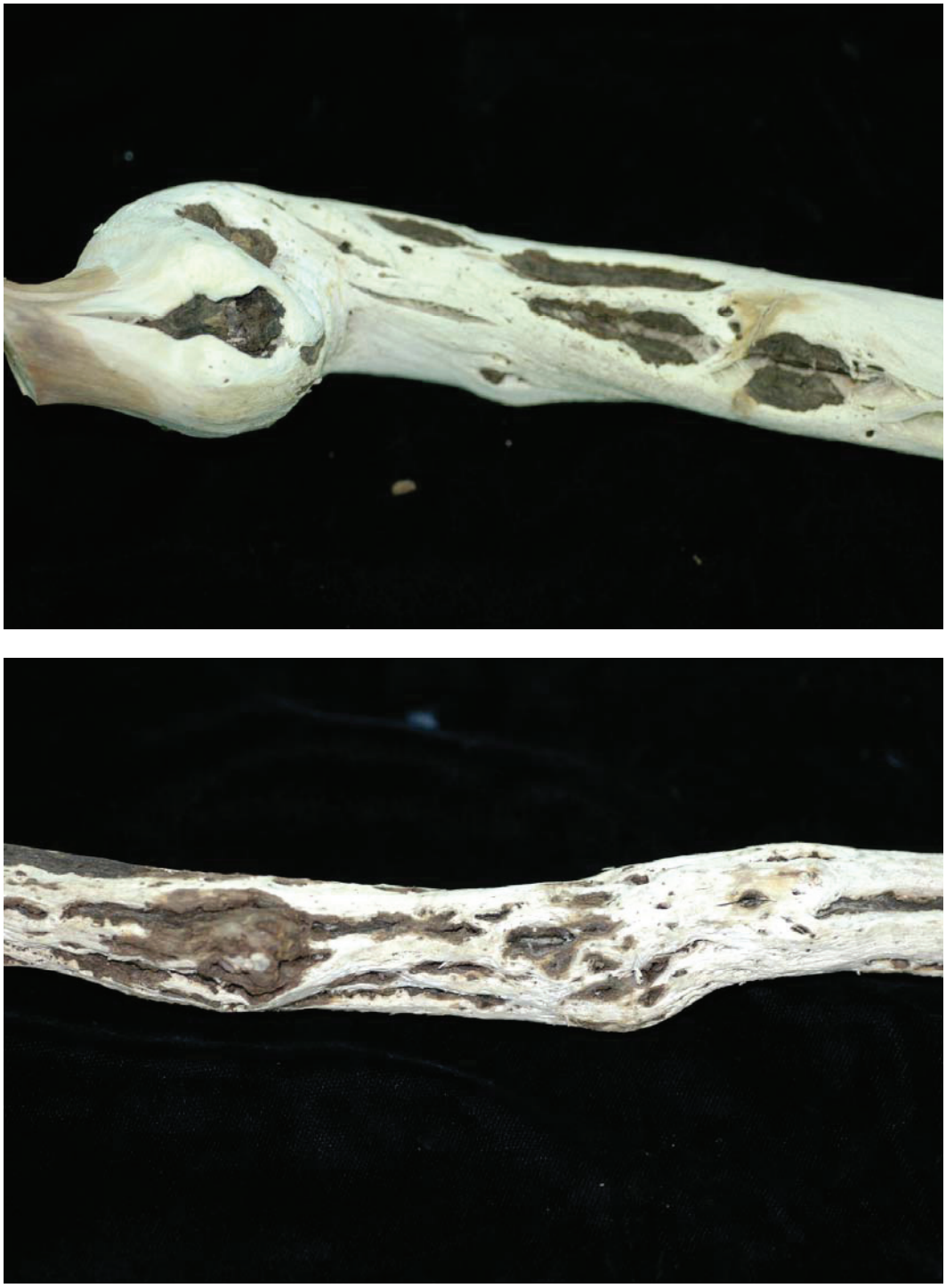

Figure 1. Woody cylinder of a Syrah plant affected with the disease. Pitting, grooving and dead tissue showing on the wood are similar to those that cause by some of the viruses in the rugose wood complex.

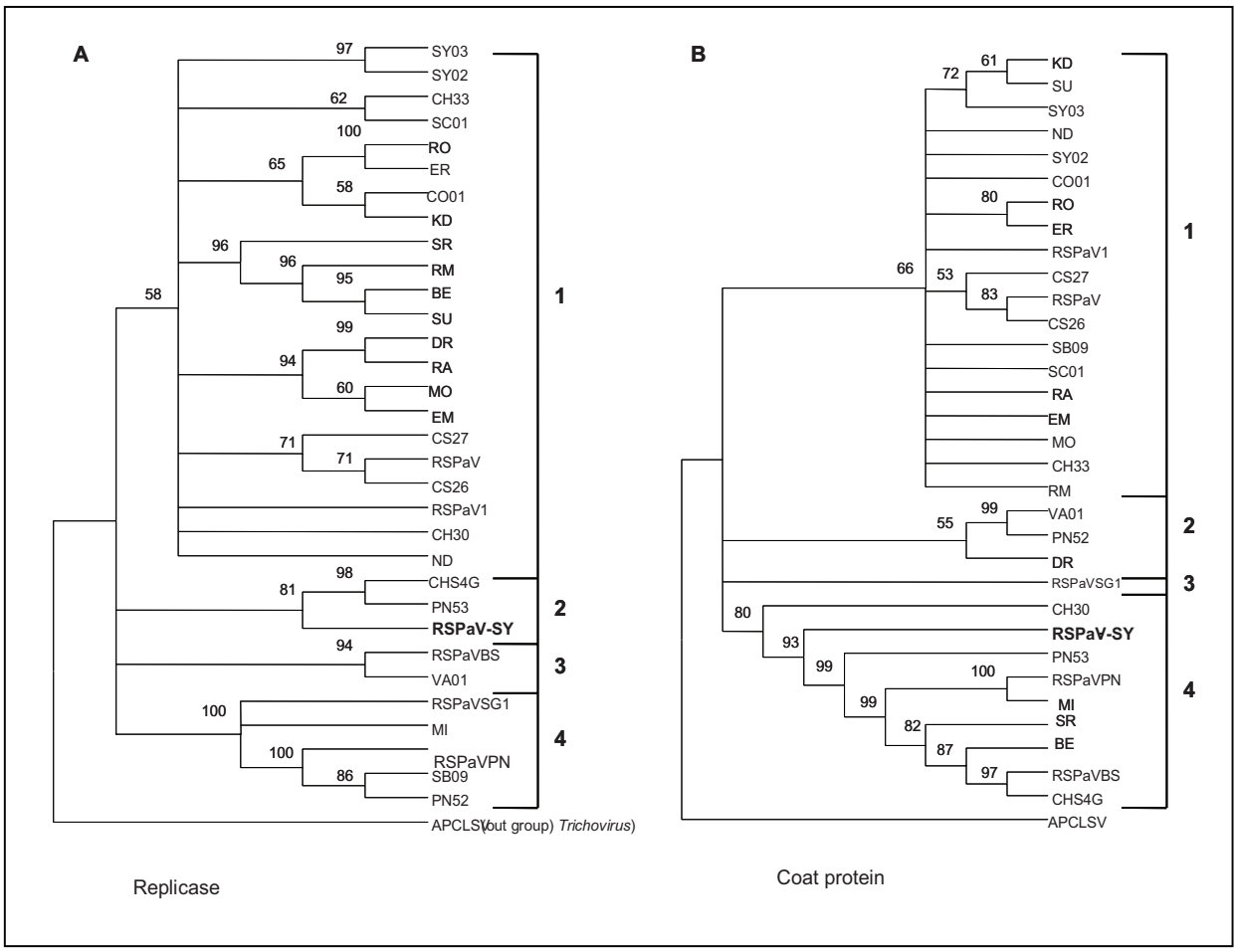

Figure 2. – Phylogenetic analysis showing the relationships among RSPaV-SY isolate and other 31 RSPaV isolates including the four isolates which their full length sequences are available in the database [RSPaV (AF026278); RSPaV-1 (AF057136); RSPaV-SG1 (AY881626) and RSPaV-BS (AY881627)]. For the comparison, fragments of the replicase gene were amplified by RT-PCR using primers P-35F/P-43R or PN-1F/RR-43 and the overlapped 664nt fragments were used in the phylogenetic analysis of ORF 1(A). For the coat protein analysis, fragments were obtained with primers RSP-49C and CP-1F (Table 1). However, due to difficulties in sequencing this product in some isolates, an internal forward primer, CP-3F, was designed based on sequences of several isolates and used on the remaining isolates. Complete sequences of the resulting products (670 nt in length) were utilized in the phylogenetic analysis of the coat protein (B). *Apricot pseudo-chlorotic leaf spot virus* [APCLSV (AY713379)] in the genus *Trichovirus* was used as an out group.

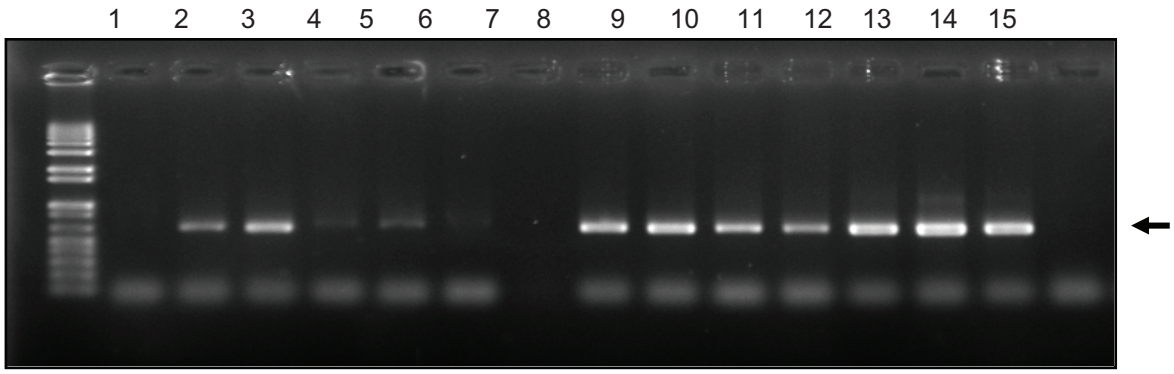

Figure 3. The RT-PCR analysis of number of Syrah samples showing the red leaf symptoms. Lane 1 is healthy control. Lanes 2-15 are samples from different Syrah plants. The primers SY-9F/8R were used for the test. The arrow shows the specific PCR products.

Red leaf grapevines: Diagnosis of putative causes based on type of stem markings (includes comments on syrah red leaf - necrotic stem)

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Premature onset of red leaf symptoms is a function of girdling caused by either biotic or abiotic factors. Some unique stem markings have been associated with the presence of specific biotic agents while others occur in response to defined abiotic conditions.

Biotic disorders

Among viral and putative viral like agents, stem markings develop on trunks of hypersensitive rootstocks as tissue necrosis.

Rootstock stem lesion (appearing as discrete pits or extensive blemish-like necrosis requires more than one year to develop) and is incited by *Grapevine leafroll associated virus-2 Redglobe* (GLRaV-2RG) on rootstocks 3309C, 5BB, 5C, 1616C, and 1103P. Canopy symptom consisted of solid red leaves on red fruit varieties or yellow leaf curl on white fruit varieties. After initial onset of off-colored leaves, affected grapevines decline rapidly and frequently die following one or two dormancy periods. Besides GLRaV-2RG, up to five sources with different lethal graft-transmissible agents (lethal GTAs) have been identified. These lethal GTAs were differentiated based on responses in a range of grapevine rootstock hosts. Work is in progress to characterize them.

Rootstock necrosis-distortion and **Rootstock necrotic union** were recently recognized and have been observed to only impact rootstocks 3309C and 110R, respectively. Symptoms of necrosis-distortion appear as extensive necrotic tissues formed in between raised longitudinal ridges. With necrotic union, a black line develops at the scion-rootstock junction. Both diseases cause red leaf, decline and death of grapevines.

Abiotic disorders

Plastic wrap embedded in proliferated scion tissues have been observed on young developing grapevines. The wraps served as protective covers over freshly constructed green bench-grafts. However, if left beyond one growing season, wraps become imbedded in the proliferating scion tissues; thereby, girdling the vine.

Insect grub injury was diagnosed in a vineyard established on a virgin hill site with a natural dense growth of native grasses. During the spring, St.George rootstocks were planted then fall-budded with Zinfandel and buried with soil. Early the following spring, the soil was removed and the rootstocks pruned back to the bud insert to promote Zinfandel bud push and shoot growth. Nearly all scion buds produced shoots. Later in the growing season however, scattered vines developed premature reddening of leaves and some eventually died. Close examination of the bud union revealed at the interface, a tunnel and remnants of insect frass, an indicator of past insect grub activity.

Heat canker. In 2003, several second-leaf vineyards contained unthrifty grapevines with red canopies. Close inspection of several impacted grapevines revealed a narrow channel of exposed woody cylinders a meter or so in length up scion trunks. Presence of callus tissues on the exposed edges of bark suggested an abiotic etiology. The suspected cause was grow tubes used to promote early growth of newly planted grafted vines. To test this hypothesis, in 2003 a grow tube experiment

was established in a vineyard block planted that year. Grow tubes were modified to create three treatments: “sealed” with soil at the bottom and not painted; sealed and painted white; and not sealed and not painted. Ambient temperatures were taken from the nearest automatic weather station and air temperatures were monitored inside the tubes. Ambient temperatures were lower than those found in all three tube treatments. Sealed, unpainted tubes had average maximum temperatures of 45.5C (114°F) at a corresponding maximum ambient temperature of 32.5C (90°F). Over the same period, average maximum temperature inside tubes that were sealed and painted white as well as tubes that were unsealed and not painted was 40.5C (105°F). In a different recording period, maximum ambient temperature was 39C (101°F) while corresponding temperatures inside sealed, non-painted tubes was 46.5C (116°F) and both sealed-painted and unsealed, non-painted tubes were 41C (106°F). During the 2003 growing season, the maximum ambient temperature was 40.5C (105°F) and trunk heat canker did not develop. This contrasts with a maximum ambient temperature of 43.5C (110°F) recorded on July 10 in 2002, a year in which vines were putatively damaged by heat.

Chemical toxicity. Paraquat injury was diagnosed on second-leaf Cabernet Sauvignon where a section of trunk was devoid of bark tissues. Concomitantly, the edges of remaining bark developed callus tissues, an indicator of wound “healing” which does not occur in response to biotic agents.

Herbicide toxicity was also assumed to be affecting the rootstocks in three commercial vineyards. One grower with two vineyards of Sauvignon blanc/110R planted in 1999 had approximately 450 vines fail. In mid-summer, potted green-bench grafts were supplied as replacement plants, which were held in the grower’s yard and planted spring 2000. In late fall 2000, a herbicide application was made. During summer 2001, an inspection of both blocks revealed scattered grapevines that had either collapsed or exhibited solid yellow canopies. These grapevines had rootstocks with trunks that were partially or completely devoid of bark tissues. Affected grapevines numbered 450, and by inspection, were determined to be the replacement vines. Symptomatic vines were smaller in trunk size than unaffected green canopy vines. Another grower with a vineyard of young Cabernet Sauvignon on SO4 also had vines that showed similar rootstock damage.

Syrah red leaf-necrotic stem. We initiated a study of declining red leaf Syrah grapevines in the fall of 2004. Chronic symptoms of affected grapevines include red leaves, stunted shoots, and scions with deep bark cracks-swollen trunks (rough bark) near graft unions. Grapevines decline and become unproductive. Also, stems of scions, but not rootstocks, exhibited necrotic cankers and fissures (necrotic stem) formed above the scion-rootstock junction. Inspections of several specimens suggested that onset of necrotic stems was initiated at the scion shoot-rootstock juncture.

Based on research experiences on stem marking disorders, growers’ vineyard practices, and combined with anecdotal observations, our working hypothesis is that Syrah red leaf-stem necrosis is due to chemical sensitivity, specifically herbicides. Vineyard trials have been established recently to investigate the role of herbicides and red leaf-necrotic stem in Syrah grapevines (see Steenwerth *et al*., this Proceedings).

Is Syrah grapevine sensitive to herbicides?

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Introduction. In California, Syrah grapevines have developed symptoms consisting of leaf reddening, weak canes, scion trunks swollen-rough bark above the union, and necrotic stems (woody cylinders), herein referred to as 'Syrah red leaf-necrotic stem'. Similar symptoms were reported in France and named 'Syrah decline'. In California, several Syrah vineyards statewide were surveyed for grapevine viruses, but a positive correlation between symptoms and viruses detected could not be established (Battany *et al*., 2004). Early investigations linking symptomatic grapevines and nutrient status also failed to show any correlations. In the Central Coast, there were indications that water stress may be involved (Battany *et al*., 2004). However, in the North Coast (e.g., Sonoma County) symptomatic grapevines occurred in irrigated vineyards (R. Smith, unpublished data).

Surveys of Syrah vineyards and collections of plant materials were made in Sonoma, San Joaquin, Yolo, Madera, San Luis Obispo, Sacramento, and Calaveras counties. Syrah vineyards with symptomatic vines have also been documented in El Dorado County. Information on management practices, Syrah clones, and rootstocks were obtained from growers. Overall, Syrah clones 877 and 174, irrespective of rootstocks, developed common symptoms. Furthermore, the position of rough bark-necrotic tissues in scion bark and woody cylinders above the graft unions were suggestive of non-viral etiology and onset of red-leaf was attributed to a girdled host response (see Uyemoto and Steenwerth, 2007, Syrah Vine Health Symposium Proceedings). We therefore initiated an investigation on the sensitivity of Syrah clones to herbicides commonly used in vineyards.

In France with Syrah decline, pathogens were posed as incitant factors of early red-leaf and necrotic stem symptoms (Renault-Spilmont and Boursiquot, 2002). Hence, we are concomitantly investigating the presence and potential roles of biotic agents, i.e. bacterial, fungal or graft-transmissible agents (GTAs), detected and identified in assays of symptomatic Syrah grapevines. For bacteria and fungi, margins of necrotic tissues were plated on standard media for bacterial and fungal isolation (assays performed in Dr. Doug Gubler laboratory). In assays for GTAs, graft inoculations onto asymptomatic test plants of bench grafted Syrah grapevines were made and planted in nursery rows for observations. Also, symptomatic and asymptomatic canes of Syrah were bench-grafted onto 110R rootstocks, callused, and rooted and also prepared as own-rooted plants. These propagations were transplanted in nursery rows. In addition, RT-PCR virus assays of these collections were performed in Dr. Adib Rowhani laboratory.

Importance of Research. In 2005, Syrah was the fifth most common red variety grown in California in terms of total tons crushed and purchased (CDFA, 2006). It also comprised 6% of standing acreage in California, ranking fifth highest among all red wine grapes grown in the state. Multiple issues appear to be involved with the Syrah problems observed in different regions of the state. The proposed research will 1) aid in further defining the sources of these problems and 2) discern the role of management practices and pathogens in this problem in order to develop control practices.

Materials and Methods. Herbicide trial. In August 2005, green-grafts of Syrah clones 877 and 174, Shiraz 1, and Cabernet Sauvignon 8 (all on 110R) were planted at the UC Davis Viticulture and Enology Research Vineyard. The grapevines were planted on 6-feet x 10-feet vine by row spacing and trained to bilateral cordons on VSP trellising. The soil is Yolo silt loam (fine-silty, mixed, superactive, nonacid, thermic Mollic Xerofluvent). Vineyard weed control was by hand-hoeing in vine rows and mechanical mowing between rows.

Herbicides tested were Goal 2XL (oxyfluorfen), Surflan (oryzalin), and RoundUp Ultramax (glyphosate). Choice of compounds was based on information received from growers.

In January 2006, herbicides were applied (label rates) on selected replications of dormant grapevines with a CO_2-pressurized backpack sprayer. Treatments were 1) Surflan (1X = 6 lbs. ai/acre; 20 gallons per acre (GPA)); 2) Goal 2XL (1X = 2 lbs. ai/acre; 40 GPA); 3) RoundUp Ultramax (1X = 0.75 lbs. ai/acre; 20 GPA); 4) Surflan + RoundUp Ultramax (1X both materials; tank mix, 20 GPA); 5) Goal 2XL + RoundUp Ultramax (1X both materials; tank mix, 40 GPA), and 6) a water control. These applications were repeated on the same vines in February 2007.

Also in February 2007, previously untreated grapevines were sprayed with the following herbicide treatments: 1) 1X RoundUp Ultramax (20 GPA); 2) 1X RoundUp Ultramax applied twice at a ten day interval (20 GPA); 3) 2X RoundUp Ultramax (20 GPA); 4) 1X Goal 2XL (40 GPA); 5) 2X Goal 2XL (40 GPA); 7) Roundup Ultramax + Goal 2XL (1X both materials; tank mix, 40 GPA); 6) RoundUp Ultramax (20 GPA) followed ten days later by RoundUp Ultramax + Goal 2XL (1X both materials; tank mix, 40 GPA); 7) a water control . All treatments in 2007 were applied using an offset nozzle (model OCO2) attached to a pressurized sprayer on an ATV.

Assays for GTAs via graft-inoculations, grow-out test, and RT-PCR. In late Summer 2005, material collected from canes and roots of Syrah grapevines with and without symptoms of Syrah red leaf-necrotic stem was graft-inoculated onto six asymptomatic grapevine test plants, i.e., three test plants for root patch grafts and three for bud grafts. These were planted at Armstrong Tract, Department of Plant Pathology, UCD in May 2006. In addition, canes that were collected in Winter 2006 were own-rooted and planted in May 2006 for a “grow-out” assay to assess growth and behavior of symptomatic and asymptomatic collections. Concomitantly, all collections were RT-PCR assayed for grapevine viruses for which primers were available.

Results and Discussion. Herbicide trial. Plot surveys during September and October 2007 revealed a few grapevines exhibiting symptoms of Syrah red leaf-necrotic stems. Syrah 877 had 7 red-leaf vines and 2 with indeterminate, suspect symptoms; one suspect vine was a water control. Syrah 174 had one red leaf-vine and one suspect vine. Trunk specimens of a red-leaf grapevine of Syrah 174 and 877 were sacrificed, autoclaved, and bark removed. Both vines showed a necrotic band encircling the junction where the primary shoots emerged. In contrast, none of Shiraz 1 and Cabernet Sauvignon 8 grapevines developed red-leaf. It is anticipated that incidence of red-leaf grapevines may increase following repeat herbicide applications. **Biological assays and grow-out tests**. Graft-indexed and own-rooted grapevines have exhibited normal green leaves, cane growth and development. All the above experiments will be continued. Attempts to culture pathogenic bacteria or fungi have produced negative results. Results of molecular virus assays will be presented by Dr. Adib Rowhani.

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Appendix Contents

Miscellaneous References

Appendix I

Citation:

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SYRAH DECLINE

Report on the meeting of the National Working Group – 11 April 2005

Anne-Sophie RENAULT-SPILMONT, Serge GRENAN and Jean-Michel BOURSIQUOT

ENTAV - Domaine de l'Espiguette - **30240 LE GRAU DU RO**

Abstract

As every year, the main results obtained on Syrah decline were presented during the meeting of the National Working Group at ENTAV.

The research of a potential pathogenic agent is in progress: in 2004 the studies were related to the possible implication of a phytoplasma in this disease. These first analyses led to the detection of phytoplasma belonging to groups I (Yellow Aster) and XII (Bois Noir) in several samples with and without symptoms. Further experiments will be conducted to conclude on their potential role in Syrah decline. In the same way, detailed observations were carried out on the transmission tests. Cracks at the graft union were observed on various scion/ rootstock combinations including Syrah^{+} grafted both onto 110R and *vinifera*. It is too early to speak of transmission, but these results raise the issue of a possible accelerated reproduction of the symptoms in the offspring of declining plants.

Experiments using various grafting techniques gave interesting results. As cracks have been observed with all grafting methods, one can exclude the grafting techniques as being the primary cause; nevertheless their long-term impact on leaf reddening and death has to be evaluated.

Analyses of vineyards surveyed since 1997 brought spatio-temporal information on the relationship between symptoms and the importance of the vineyard environment on mortality.

Understanding the impact of rootstocks and clones on symptom expression is essential for future vineyard establishments. Since information had already been obtained on the importance of the rootstock, the observations carried out in 2003 and 2004 were essentially related to the clone aspect. Numerous and reproducible results were obtained which shed light on the behavior of the Syrah clones with respect to Syrah decline. This is obtained in case of a rootstock origin particular to this design. No clone seemed to be symptom-free but clones could be ranked in three groups according to their disease susceptibilities. Nevertheless, more data are needed concerning the respective impact of the clone of Syrah and the rootstock on symptom expression.

Water stress is one of the potential worsening factors studied. Observations carried out in 2004 in an experimental vineyard seem to indicate a relationship between water stress and foliar reddening preceding death. At least, one year of additional observations seems necessary to conclude on this aspect.

The last topic presented concerned preliminary studies necessary before conducting a survey. The goal is to identify factors responsible for the evolution of the cracking plants towards leaf reddening and death, rather than the original factor inducing crack formation.

The inquiry framework (plot sampling, questionnaire form, …) is currently operational.

This set of different experiments did not result in a conclusion on the etiology of Syrah decline. Nevertheless, we have a more precise understanding of the causes of the disease: some hypotheses are being gradually ruled out, as others appear more promising. These hypotheses are currently under investigation.

Key words

Syrah decline, phytoplasma, grafting, clone, survey

1 - RESEARCH ON POSSIBLE CAUSES

1-1 A pathogenic agent

1-1.1 Tests of transmission

Understanding the etiology of Syrah decline remains a priority. Among potential causes, we are investigating the possibility of a pathogenic agent: our observations on transmission trials are currently in progress. The purpose of these trials were to evaluate transmission of the typical symptoms of Syrah decline (leaf reddening and cracking at the graft union) to other grapevine clones, either indicators or genetically similar to Syrah, i.e. cuttings taken from stocks of declining Syrah (RENAULT-SPILMONT *et al*. 2003). Two tests were established for this objective: the first at the site Piolenc (Vaucluse) in 2001 and the second at Claret (Herault) in 2003. Other complementary tests were prepared in 2004 and should be set up this year.

The first results were collected from the "oldest" site, which is still only 4 years old. The first observations carried out in 2002 and 2003, which were also confirmed in 2004, showed expression of the first symptoms of cracks at the graft union on a number of the scion-rootstock combinations (Table 1).

In this trial, we tested various clone-rootstock combinations with either dormant (GL) or green-wood (GBH) grafting methods. Regarding Syrah grafted to the rootstock 110R, we observed cracks for all three Syrah/110R combinations less than four years after planting. This very early occurrence was particularly pronounced in the 16 Syrah/110R dormant grafted vines, which all showed cracks at the graft union ("Associations Syrah/110R", third combination in Table 1). Among these, one vine showed leaf reddening and three vines died during the winter. In these cases, we cannot confirm transmission of a causal agent because decline occurs with "traditional" Syrah-rootstock combinations; but this unexpected result lead us to wonder about the e**ventual magnification and/or acceleration of the symptoms when cuttings are taken from declining vines.** Symptoms seem less prominent for the two other Syrah/110R combinations using the green-wood grafting method ("Associations Syrah/110R + GBH" in Table 1). For all three Syrah/110R combinations, a very low number of vines were used and therefore this observation cannot be extrapolated to the rest of the vineyard. Nevertheless, if these observations were confirmed, Syrah decline would tremendously impact Viticulture production. Mr. MINODIER (Department of Agriculture, Ardèche) stresses that, as a preventive measure, in Ardèche, for the past four years, the trade union for grapevine selection had enforced the elimination of mother-vines showing symptoms of cracks and leaf reddening. It is still too early to see the eventual positive effects of this action.

The Syrah/*V.vinifera* combination ("Association vinifera/ Syrah" and "Association Syrah/vinifera" in Table 1) also showed some interesting results, even though a significant number of vines grafted onto *V. vinifera* died following attacks of phylloxera reducing the number of observable vines significantly. The Syrah/*V. vinifera* combination was chosen based on the assumption that the cracks resulted from an incompatibility between the rootstock and Syrah; incompatibility which should not be seen by grafting on a genetically more similar rootstock, i.e. *V. vinifera*. On the five associations present (Syrah as the scion or rootstock), to date, only three vines of Syrah grafted onto Pinot Noir showed only slight cracks. We will need a few additional years to conclude. A similar observation was seen on the double-grafting ("double-greffes" in Table 1), where crack symptoms were identified on Syrah grafted either on Cabernet Sauvignon or on Syrah clone 300. These last two observations show that the grafting of Syrah with *V. vinifera*, and with *a fortiori* the same species, does not prevent the appearance of the symptoms which seem to exclude the hypothesis of genetic incompatibility. To evaluate this hypothesis of genetic incompatibility, tests of double-grafting (with intermediates of Marsanne and Mourvedre) were established in 2004 at two sites in Vaucluse.

New observations will be carried out this year at Claret and a follow-up of more complete tests, using the green-wood grafting method, should bring additional information in a few years. To complete our observations, the same trials were set up with dormant material taken from vines with cracks and leaf reddening symptoms (B1 in Table 1). Cuttings were taken in January 2004 from a site located near Matelles (Herault) where symptoms were mapped every year. We kept track for each vine of their mother vine of origin. Concerning the hypothesis of transmission of the symptoms by a pathogenic agent, experiments of transmission via chip-bud have been set up. With this technique, one tries to express the symptoms (cracks and leaf reddening) on indicator clones by separating the transmission from the grafting, i.e. the pathogenic agent cause from the physiological causes.

1-1.2 Research on phytoplasm

In many woody species, the occurrence of decline can be attributed to phytoplasms. In grapevines, two phytoplasms have been identified to be responsible for diseases: Yellow Aster and Bois Noir. Yet, we cannot exclude the existence of another phytoplasm responsible for Syrah decline: we therefore found it essential to study this hypothesis.

Phytoplasms are heterogeneously distributed within

a plant, which can lead to an inability to detect them at a given time. Therefore, multiple dates and kind of samples were carried out. The samples were taken in 2003 (spring, summer, fall) at two sites located in Pic Saint Loup (Herault). Vine stocks with symptoms (B0 = green vines with cracks and B1 = red vines with cracks) and without symptom (AO) were sampled for analysis. In addition to the traditional analysis on petioles/veins, extracts of flowers (or bunches) were also analyzed. After universal markers were calibrated, the identification of the phytoplasm was done by comparing profiles obtained after enzymatic digestion or by sequencing and searching matches in a database.

Results obtained are in Table 2. The results were split into two categories, either as number of positive samples, or as number of positive vines. A vine is considered positive when at least one sample taken from the vine is positive. **The analyses revealed the presence of phytoplasms in a considerable high number of samples: 33% of the samples of vines with cracks and leaf reddening symptoms.** These phytoplasms were also found in samples without any symptoms, but in a slightly smaller proportion (19%). If we consider the total number of positive grapevines: A0 (healthy vines) had 5 out of 10 tested, B0 had 7 out of 10 tested, and B1 had 13 out of 20 tested. The presence of phytoplasms in vine stocks without symptom shows that it is not possible to establish a direct link between the presence of these potentially pathogenic agents and Syrah decline.

We then identified the phytoplasm using sequencing. Among the 10 identified sequences, 6 correspond to group XII (Bois noir) and 4 to group I (Aster Yellow). This group is related to the clover phyllody found in many plants, and has been occasionally found in Chilean, Italian grapevines and Eastern Europe, but has never been found in France.

The presence of this phytoplasm has not yet been connected with disease or precise symptoms on grapevine. With the present knowledge, a definite link between the presence of this phytoplasm and Syrah decline can therefore not be established. New samples will be taken in the fall 2005 to check for the presence of the phytoplasm in all areas where Syrah decline is present.

In addition to this work on the phytoplasm, research on the implication of one or more pathogenic agents continues. Even though bacteria and fungi were eliminated as the primary cause for Syrah decline, viruses or viroïds are still potential causal agents.

TABLE 1

Symptom expression in the transmission trial (Piolenc); observations on the different grafted plants

A0 : healthy vines ; B0 : cracking vines with green leaves ; B1 : cracking vines with reddening leaves ;
M-B : dead cracking vines ; crev : global cracking vines (B0+B1+MB)

Syrah+ and 110R + : wood taken from declining vines ; GBH : green grafting

greffon	*Porte-greffe*	*Total*	*A0*	*B0+B1*	*MB*	*crev*
Association Syrah / 110R						
Syrah 300 / 110R$^+$ (GBH)		19	13	6	0	6/19
Syrah$^+$ / 110R + (GBH)		20	12	8	0	8/20
Syrah$^+$ / 110R		16	0	13	3	16/16
Association Syrah / vinifera						
Syrah$^+$ / Pinot Noir		7	4	3		3/7
Syrah$^+$ / Mondeuse		7	7	0		0/7
Association vinifera / Syrah						
Durif / Syrah$^+$		5	5			0/5
Syrah 300 / Syrah$^+$		4	4			0/4
Syrah 300 / Syrah$^+$ (GBH)		17	17			0/17
Double-greffes						
Syrah$^+$ / CS / 110R +		12	10	2		2/12
Syrah$^+$ / Syrah 300 / 110R +		6	4	2		2/6
Syrah$^+$ / Syrah 300 / 110R		15	8	7		7/15

1-2 Problems due to grafting

Because the first symptoms appear at the level of the graft union, our second research objective was to explore problems due to grafting: graft incompatibility, physiological or mechanical problem.

Field trials testing different grafting techniques were set up at two sites - Moussac (Gard) and St-Mathieu de Treviers (Herault). Two types of grafts and four methods of hormone applications were performed. The grafts were completed by either dormant (LPG) or by green (GBH) grafting, planted at one year interval. The details of these methods were presented in a previous report (*Prog. Agric.Vitic 2002*). In 2004, out of the 4 blocks, the rate of cracked plants increased at both field sites and particularly at St Mathieu de Treviers, where currently only a little more than 50% of seedlings are showing no symptoms (Table 3). We observed at the same time an increase in the number of stocks with leaf reddening (B1) and an increase in mortality especially at Moussac, where 5 vines died in 2004 (MB = dead cracking vines). As often is the case with Syrah decline, the vines did not grow after pruning. One might wonder about the acceleration in the rate of occurrences after one year of apparent stabilization: is there a connection between the symptoms and the fact that 2003 was a very dry and hot summer?

We also analyzed symptom expression as a function of different bench-grafting procedures (English or Omega grafting, and hormone concentrations). Results are presented in Figure 1. As can be seen, **cracks are observed with all bench- grafting methods and with all hormone concentrations.** The rates of vines cracking vary from 30% to 55% depending on the bench-grafting method.

The average values obtained for each bench-grafting method are relatively similar at both sites, except for ARB (English grafting + wax with hormones) and AH2 (English grafting + wax with hormones + 4% wax at the cutting base) (Figure 1). These averages do not take into account the great variability within each site between the various blocks.

According to the analysis of variance, there are no significant differences between the two grafting treatments, nor between the four hormone treatments and the control at the two sites for all the variables recorded (A0 (healthy vines), B0 (cracking vines with green leaves), B1 (cracking vines with reddening leaves), MB (dead cracking vines)).

Cracks were observed for all grafting methods used. From these data, it is therefore not possible to conclude that omega grafting or an excessive concentration of hormones is the primary cause for crack development. Nevertheless, the long-term impact of these two types of grafting and the different hormone treatments on leaf reddening and grapevine mortality must be evaluated.

Concerning the two other grafting methods, cracks on the grapevines were observed at Saint Mathieu de Treviers: 4 in green grafting (GBH) and 1 in dormant grafting (LPG). Because with both of these latter grafting methods it seems that symptoms are slower to appear, additional years of observations will be necessary to draw a clearer conclusion.

2 – RELATIONSHIPS BETWEEN THE DIFFERENT SYMPTOMS

2-1 Spatial distribution and evolution of disease symptoms

Detailed ratings about each stock were carried out in 1997 to 2001 at 38 sites located in 7 departments. We asked whether there is a spatial organization of diseased grapevine, i.e. are they organized randomly or is there a pattern, and how this spatial organization evolves. We also asked whether there exists a temporal sequential evolution of symptoms at different stages of the disease (cracks, leaf reddening, and mortality). These analyses were carried out in collaboration with stations at INRA of Avignon (V CHRISTIAN & J CHADOEUF, Biometrie) and of Montpellier (JP PEROS), and the CIRAD (F BONNOT).

The first analysis concerned the distribution of diseased grapevines (RENAULT-SPILMONT *et al.* 2003). **We analyzed 87 pairs of site-year and show an effect of space aggregation along the vine row for all three symptoms ranked (cracks, leaf reddening, and mortality).** It was concluded that the rare aggregation formation between rows excludes a priori the implication that the pathogenic agent is from the soil. Disease aggregation along the row might result from the propagation of a pathogenic agent during

TABLE 2

Phytoplasma detection in Syrah samples: positive samples for total tested samples and quantity of positive plants.

A0 : healthy vines ; B0 : cracking vines with green leaves; B1 : cracking vines with reddening leaves

Souches	*Echantillons positifs*	*Souches positives*
A0	8/42 (19%)	5/10
B0	9/42 (21%)	7/10
B1	15/46 (33%)	13/20

cultural practices (pruning…) originating from a few diseased grapevines or localized soil heterogeneity. This phenomenon of disease aggregation did seem to affect each site equally. Yet, we cannot tell whether the fact that we did not detect aggregation in certain sites was due to the lack of statistical power (sampling number too small) or to the real absence of aggregation. For all the tests, the distribution of the values obtained at the different sites is far from a uniform distribution, which leads us to conclude that aggregation within sites is present at different levels. All ranges of the possible critical values are represented. Part of this distribution could be explained by the site ages or by the pruning techniques of each sample followed (the more the number of followed vines at a site, then the more one is likely to detect an aggregation), but that does not seem sufficient to explain the variability observed.

We then focused on the time course of the disease, where it is critical that data was collected correctly and consistently. With that in mind, we selected sites that had kept good records to decrease any "background noise" created by erroneous data. The reliability of the records was checked over the various years (consistency in the dead and cracked grapevines) and corrections were made when that was possible. When there were too many variations, the sites were eliminated and 15 sites were left out of the original 24 followed. Two approaches for analysis were then used: a general descriptive study, then a study by site and by year:

- the probability of leaf reddening is more likely in B0 (cracking vines with green leaves) than in A0 (healthy vines) grapevines

- the probability of death is more likely for B0 (cracking vines with green leaves) than in A0 (healthy vines) grapevines

- the probability of death is more likely for B1 than in B0 (cracking vines with green leaves) grapevines

In general, this information is in accordance with the proposed model of Syrah decline evolution: **the appearance of cracks (B0) occurs from grapevines that were previously healthy (A0), then signs of leaf reddening (B1) begin to develop, followed by death within a relatively short period of time** (RENAULT-SPILMONT *et al.* 2003).

In addition to the general descriptive study, analysis was performed for each site. We analyzed the probability of death of B1 grapevines according to the site and the year (potential year effect). The analysis showed that for 2 consecutive years the probability was highly variable according to site with values oscillating between 6% and 80%; nevertheless for the majority of the sites this probability is comprised between 10% and 30%. On the other hand, we realized that within our sites, and for the time they were followed (4 years maximum) that the probability of dying was relatively constant within each site.

In addition there did not appear to be a year effect,

TABLE 3

Global syrah decline symptom evolution in both experimental plots
STM = Saint Mathieu, MO = Moussac
A0 : healthy vines ; B0 : cracking vines with green leaves ; B1 : cracking vines with reddening leaves ;
M-B : dead cracking vines

		A0	*B0*	*B1*	*M-B*
Moussac	2001	82 %	16 %	2 %	0
	2002	74 %	22 %	4 %	0
	2003	69 %	30 %	0	0,3 %
	2004	61 %	31 %	7 %	1,6 %
St Mathieu	2001	77 %	22 %	<0,1 %	0,3 %
	2002	72 %	28 %	--------	0,3 %
	2003	71 %	26 %	2 %	0,6 %
	2004	54 %	42 %	3,2 %	0,8 %

with the average probability varying only 20% to 26% between 1997 and 2000. Globally, these analyses highlight a **very important environmental site effect.** We also calculated an average probability of mortality per type of grapevine for 2 consecutive years. The values obtained on the rated sites are less than 1% for the A0 (healthy vines) grapevines, 2% to 4% for the B0 (cracking vines with green leaves) grapevines and 19% to 26% for the B1 grapevines. However, it is currently not possible to go any further with this data.

2-2 Rate of apparition of cracks

At the same time, to better understand the relationship between the various symptoms, precise observations were taken at the graft union of 14 successive grapevines taken within the same vineyard in September 2003: the grapevines were Syrah clone 100 grafted on 140Ru planted in 1987. After thoroughly describing the graft unions (size, presence and location of cracks…), we cut them into successive discs, polished and bleached to observe their growth rings. It is understood that the cracks correspond to cambial dysfunction (RENAULT-SPILMONT *et al.* 2003). Looking at the growth rings makes it possible to date the year when the cambium completely stopped functioning resulting, by contrast with the neighboring area, in apparition of cracks (RENAULT-SPILMONT *et al.* 2004 - Figure 11). These are macroscopic observations witnessing an end in cambium production, i.e. an absence of new vessels. We could then date when the cambium ceased functioning and decipher whether it occurred on the various grapevines at the same time, or if it appears in successive years witnessing a transmission of the disease according to the distance between the successive grapevines. The distribution of the grapevines and their respective states are indicated in Table 4.

The first problems were detected 3 to 9 years after planting (Table 4). Interestingly, even though the grapevine S3 was the earliest to die (in 2003), it was not the first having cambium dysfunction. Also, grapevine S5, for which the dysfunction goes back to 1990/91 (one of the oldest), is still in the B0 stage (green grapevine with cracks). Concerning grapevines 5, 6, 7 and 8 - S5 showed signs of dysfunction first, followed sequentially by the others, each one year apart, from 1990 to 1995 (Table 4). However, this did not occur in the other direction (grapevines 4, 3, and 2) (Table 4). The assumption that transmission occurs via neighboring grapevines in the vineyard cannot be confirmed by these observations.

3 – EFFECTS OF SYRAH DECLINE ON DIFFERENT SYRAH CLONES

A significant research effort on the effect of syrah decline on different syrah clones was initiated in 2003

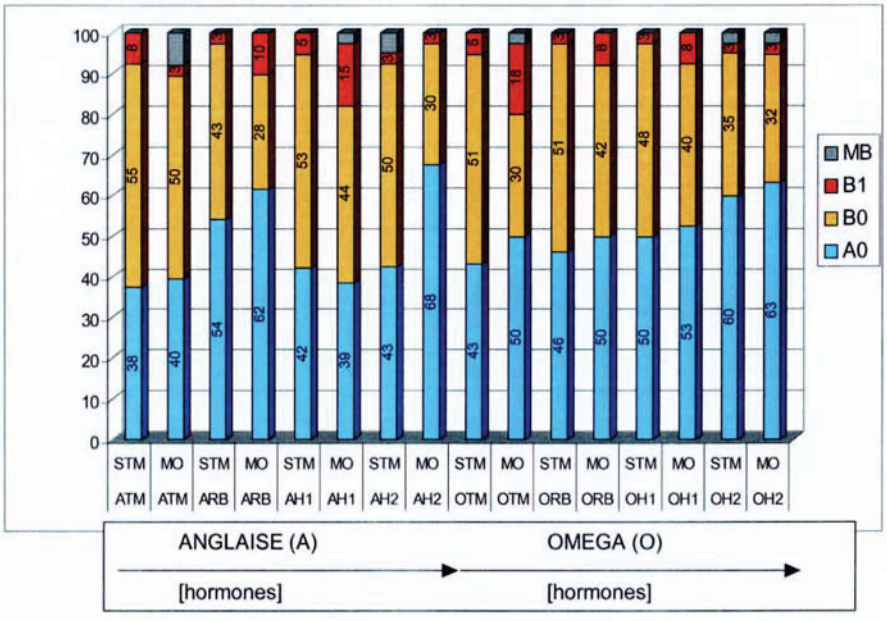


FIGURE 1

Symptom expression depending on the grafting modalities: TM: control (no hormone); RB: wax with hormones; H1 wax with hormones + 2% hormone at the base; H2 wax with hormones + 4% hormone at the base

and continued into 2004. Forty sites between the ages of 4 to 25 years were followed in 7 départements: Ardeche, Aude, Drome, Gard, Herault, the Pyrenees-Orientales, and Vaucluse. These sites consisted of 27 mother-vines (VMG) and 13 experimental sites. The experimental sites were initially established for agronomy studies concerning clones: they were therefore planted with Syrah clones of the same origin. The mother vines were chosen using the following criteria : there must exist at least 2 clones grafted on the same rootstock, and the vines should have been established the same year to compare their behavior. Precise observations were recorded on these sites to rate for each grapevine the presence or absence of one or more Syrah decline symptoms. Grapevine mortality was calculated as the number of dead, missing, or young vines (replacement). Therefore this acts, under these criteria, as the total mortality or the absence of stocks, including all the different causes. One-hundred grapevines per clone were precisely rated in the mother-vines sites, which was then the maximum number of grapevines to observe in experimental sites.

We rated 5 rootstocks - SO4, 140Ru, 110R, 161-49C, and 3309C. This number was limited to avoid multiplying factors of variance. We also made sure that each clone rated was grafted on most of these different rootstocks. Various types of analyses were then reported in order to synthesize all these data, some examples are shown below.

Results obtained from all sites were used to calculate the minimum, maximum, median, and interquartile range for cracking vines (B0+B1) and dead vines (Figure 2). From this representation we drew several conclusions: clones 747, 470, 524, and 471 are characterized by lower average rates of cracks and reduced mortality (3% to 6%). While clones 73, 381, 383, 99, 301 and, to a lesser extent, clone 382, show higher rates of cracks (on average close to 50%) and a higher mortality (on average 20% or higher) (Figure 2). The mortality rate is variable according to each site as represented by broad boxes. For example, the average mortality rate for clone 383 was 25%, but varied from 0 to 54% according to site! The rest of the clones are characterized by an intermediate behavior, as for clone 300, which showed an average rate of cracks (35%) but a reduced mortality (9%), or an irregular behavior as for clone 174.

We then ran multiple correspondence analysis (MCA), which takes into account all the factors of variation of the different sites, such as the age of the site and the rootstock (Figure 3). Clones 470, 524, 747 and to a lesser extent 471 are clustered with the variables A5 (highest rate of healthy plants), B1 (lower rate of cracks) and M1 (lowest mortality). On the other hand, clones 73, 99, 381 and 383 are grouped with the variables A1 (lowest rate of healthy plants), B3 (medium rate of cracks) and M5 (highest mortality). Clones 301 and 382 are associated with A2 (low rate of healthy plants), B5 (highest rate of cracks) and M4 (high mortality).

The majority of clones shows reproducible results with all the tests and often can be categorized. From the high number of observations and the reproducibility of the results, there are trends in the behavior of Syrah clones with respect to Syrah decline. **No clone is completely immune to the disease, but there is a gradation in the expression of symptoms.** We confirmed and completed our first conclusions from the previous year for a certain number of clones we had tested. From these, there are **significant and reproducible differences between clones,** which can be **classified in three different categories** of behaviors (represented by arrows in Figure 4). The results were expressed as percentages (average and standard deviation) of grapevines without symptoms (blue bars) and of dead or missing grapevines (striped bars). The observations were carried out on 6 to 23 sites per clone, as indicated between brackets under each number of its respective clone (Figure 4). Clones 470, 471, 524 and 747 usually have few symptoms of syrah decline, with 75% to 95% of grapevines without cracks and leaf reddening. In addition, these clones had a relatively low rate of mortality (3 to 6%) (Figure 4). On the other hand, clones 73, 99, 301, 381, 382, 383 generally had high rates of symptoms (cracks and leaf reddening) and a higher mortality. The other clones had intermediate or irregular behaviors, variability represented by the error-bars are

TABLE 4

Detailed observations realized on graft unions from 14 consecutive plants taken from Saint Mathieu de Tréviers (34): symptom description and time evaluation of the first cambial dysfunction that can be observed macroscopically

n° de la souche	symptômes	année 1er problème
S0	B0	1994
S1	B1	1990
S2	B1	1993/94
S3	B1 (mort)	1992/93
S4	B1	1993/94
S5	B0	1990/91
S6	B0	1991/92
S7	B0	1993/94
S8	dB0	1995/96
S9	A0	/
S10	B0	1996
S11	dB0	2001
S12	B0	1997/98
S13	dB0	1993/94

seen, with respect to Syrah decline in the sites studied – this is especially true for clone 174 (Figure 4).

The importance of the Syrah clone in Syrah decline is unquestionable. ***But is it solely the effect of the clone or an interaction effect of the clone and the rootstock? Or could it possibly be a particular combination of different pathogenic agents brought together by the scion and the rootstock?***

4 – A STUDY OF POTENTIAL FACTORS THAT WORSEN SYMPTOMS

4-1 Vine Training

We studied the effects of the vine training system as a potential aggravating factor in a trial set up in an experimental site at Crozes-Hermitage (Drome). At this site, multiple factors of variation are present: planting density (i.e. row and vine spacing), total leaf surface in the case of "espalier" training where the height, i.e. its total leaf surface, of the vine will vary, and the clones used (there were 4 different clones on the rootstock 3309C). The "goblet" (head training) training was also part of this trial.

From the first trials we conducted in 2003, we observed no significant effect of the total leaf surface on the different symptoms of Syrah decline under the conditions selected, which included goblet (head training) (RENAULT-SPILMONT *et al.* 2004). In 2004, we studied under "espalier" training, the impact of the total leaf surface on the different symptoms of Syrah decline. Even though no significant effect on symptoms has been observed concerning the variation of leaf surface area, one could question the role of planting density on the establishment of the root system and therefore on water and mineral intake of the vines. Even though these factors might not act directly on the cracks formation, their effect on the leaf reddening and subsequent death should be

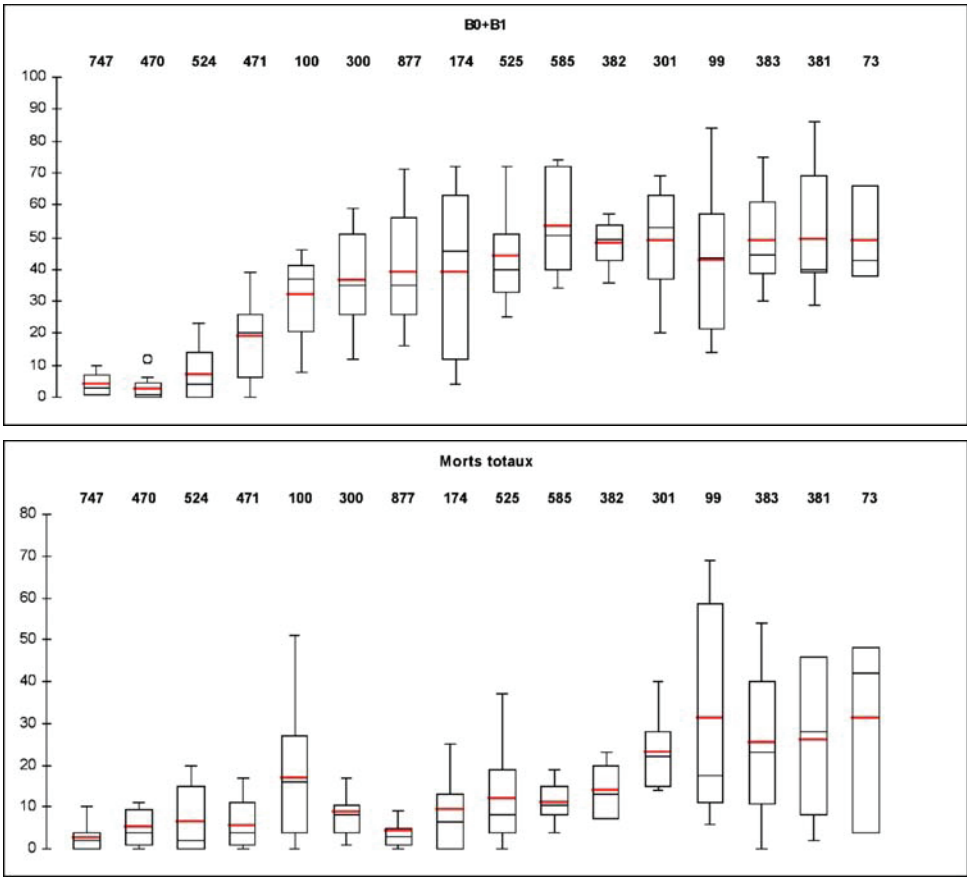


FIGURE 2

Boxplot representation of Syrah clones behavior

The minimum, the maximum, the median, the mean (red line) and the interquartile range are represented for cracking vines (B0+B1) and dead vines (global death = dead + missing + young plants)

studied. The observations carried out on clone 383 were unfortunately very variable and therefore inconclusive. We will collect more observations in 2005.

4-2 Water supply

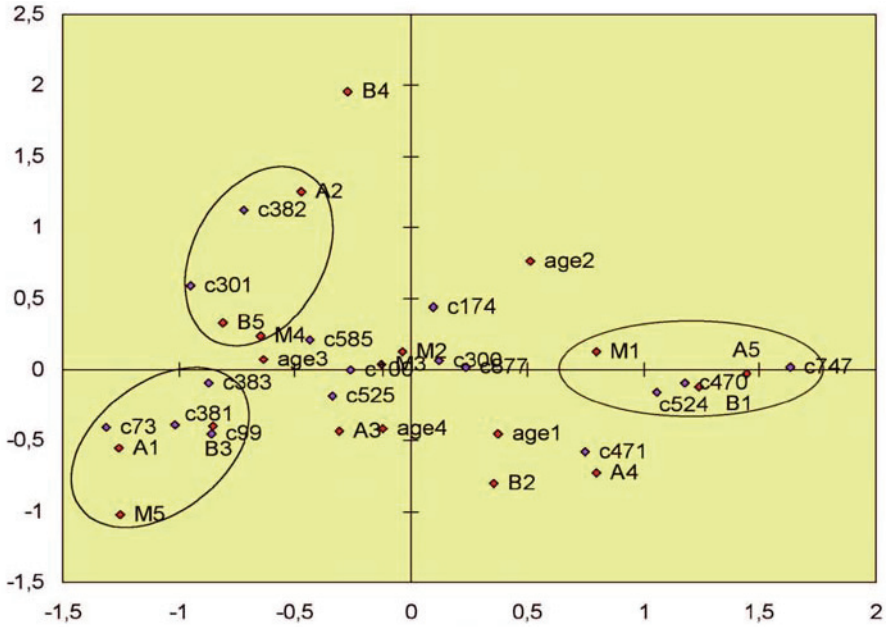
In addition to the vine training system used, the water intake might influence the expression and aggravation of symptoms, specially leaf reddening and death. The "Chambre of Agriculture du Gard" is evaluating the influence of water supply, i.e. different irrigation treatments, on the site of Asperes. In this site, where the hydric potentials have been recorded and the water profile is well understood for the different irrigation treatments, ratings started in 2003. The hydric constraints, i.e. at this site the depth of the ground, did not affect the rate of cracks and death (RENAULT-SPILMONT *et al.* 2004). Because very few leaf reddening symptoms were recorded in 2003, observations were continued in 2004; this year symptoms were more important on the control treatment (31 % of vines) than on the three irrigation treatments (14 to 20%). Additionally, in the control treatment, the majority the leaf reddening appeared on zones with more shallow ground.

A few interesting observations can be made from this site. Cracks and death observed to date do not seem correlated with water supply. On the other hand, in 2004, leaf reddening might have been linked with water stress: differences between non-irrigated control treatment and irrigated treatments as well as a gradient in symptoms from the wettest to the driest zone were observed. We will carry out new ratings in 2005 to track the evolution of vines with leaf reddening symptoms and evaluate the new distribution of leaf reddening according to water constraints.

4-3 Set up of a new investigation

The first investigation was carried out in 1997 to evaluate the importance of various factors in the expansion of Syrah decline. Problem of sample size and return of uncompleted questionnaire forms resulted in inconclusive results.

Current knowledge has led us to re-initiate this



A1: less than 25% of vines "healthy"
A2: 25% to 50% of vines "healthy"
A3: 45% to 65% of vines "healthy"
A4: 65% to 85% of vines "healthy"
A5: more than 85% of vines "healthy"

B1: less than 15% of vines with crevasses
B2: 15% to 35% of vines with crevasses
B3: 35% to 50% of vines with crevasses
B4: 50% to 65% of vines with crevasses
B5: more than 65% of vines with crevasses

M1: less than 5% of vines died
M2: 5% to 13% of vines died
M3: 13% to 20% of vines died
M4: 20% to 30% of vines died
M5: more than 30% of vines died

Figure 3

Behavior of Syrah clones towards decline (ACM representation)

investigation focusing on more significant, according to us, areas. The goal of the current investigation is not to assess, as before, the different factors responsible for Syrah decline but rather to identify those that influence evolution of the vine from the cracking stage to the leaf reddening stage and subsequent death stage. To avoid the same mistakes, we found it necessary to carry out a study preliminary to this second investigation.

This study was a Master's project carried out by 5 students from Agro Montpellier from February 2004 to January 2005. The goals were to work on the problem of sampling, to set up a database with information from the selected sites, write a more tailored questionnaire form and think about appropriate statistical analyses of the data. To better understand the factors responsible for leaf reddening and death, one should choose a sample of sites with more homogeneous cracks and clones/ rootstocks combinations used. For time, availability, confidentiality and reliability purposes, it was necessary to go towards the mother-vines. This strongly reduced the number of sites available, which led us to widen our criteria for selection. Six clones of Syrah (99, 100, 174, 300, 383, and 525) and 4 rootstocks (110R, SO4, 3309C, and 140Ru) were selected. We chose these types of vines because they can be found with large number of cracks in many sites and are largely representative of the three regions of interest (Languedoc-Roussillon, Provence Alpes Cote Azur, Rhone-Alpes). In addition to these criteria, we selected sites at least 7 years old: all these criteria taken together, we were constrained to only 199 sites. The investigation is currently set up but starting the actual investigation will necessitate a significant investment in terms of man power (rating, form questionnaire, statistical analysis) and budget.

5 – FUTURE PLANNED TESTS AND STUDIES IN PROGRESS

Trials on preventive treatments of Syrah decline are currently established. The first question is about the replacement of declining vines and re-grafting adult vines, after the decline of the scion. A first site in Gard, re-grafted 3 to 12 years ago by the grower, was followed in 2004. It was a site highly infected with Syrah decline where 34 grapevines were re-grafted (scions came from 3 different sites), among which some, for more than 10 years without showing any cracks. This observation was interesting enough, that we found it appropriate to established specific tests. Grapevines with B1 symptoms (cracking vines with reddening leaves) will be cut under the graft union and subsequently grafted with 2 clones of Syrah: the goal is to test whether the type of vine material used for the scion can have an influence on symptoms expression (rate, intensity).

We will also test the impact of mother vine on its descendants. This follow-up experiment will be compared to the one at Piolenc (see 1-1-1). Canes were taken this winter from grapevines with A0 (healthy vines), B0 (cracking vines with green leaves), or B1 (cracking vines with reddening leaves) symptoms in 2 sites in Vaucluse mapped precisely. These canes were grafted onto SO4 rootstock and will be planted in 2006 to rate their evolution on the long-term.

On a site established in 2004 with Syrah clone 300 grafted on 140Ru, we will quantify the protection of the graft union and pruning wounds by application of the fungicide "Escudo" (flusilazole + carbendazime). These tests will evaluate the potential aggravating effect of fungi on symptoms.

We will also evaluate symptoms on sites planted with vines that have been hot water-treated to quantify the effect of this treatment on Syrah decline. Currently this treatment is used only against phytoplasms in France, however other countries also use it against bacteria and fungi. Treated and non-treated Syrah clone 383 will also be compared.

To further understand the impact of how specific plant material influences the expression of the symptoms, different combinations of various clones of Syrah on various rootstocks (plant material of the same origin) will be set up. A total of 9 combinations (3 clones x 3 rootstocks) will be planted in each of the 7 sites located in 6 departments. The objective, on one hand, is to study the behavior of "intermediate" clones grafted with "less sensitive" rootstocks and, on the other hand, to evaluate the importance of environmental factors at each site, two controls scion/ rootstock combinations being established at all the sites.

Research on the cause of Syrah decline is still significant with, on one hand the study of the grafting recovery of Syrah and, on the other hand, the research on causal agents. Concerning grafting recovery of Syrah, we remind that previous observations showed low recovery after grafting Syrah on 140Ru by comparison with Cabernet Sauvignon and Grenache Noir even though it is difficult to establish a direct relation with the latter appearance of cracks (RENAULT-SPILMONT *et al.* 2003). To investigate further this question, the same type of study was set up with 2 other rootstocks, 5BB and SO4, which, while being genetically close, presented different behaviors in the vineyard. Observations showed a few differences in behavior that must be studied in more detail. Regarding grafting recovery, we plan to examine additional Syrah clones which showed more extreme behaviors.

These experiments have not made it possible to yet define the causes of Syrah decline. However, the

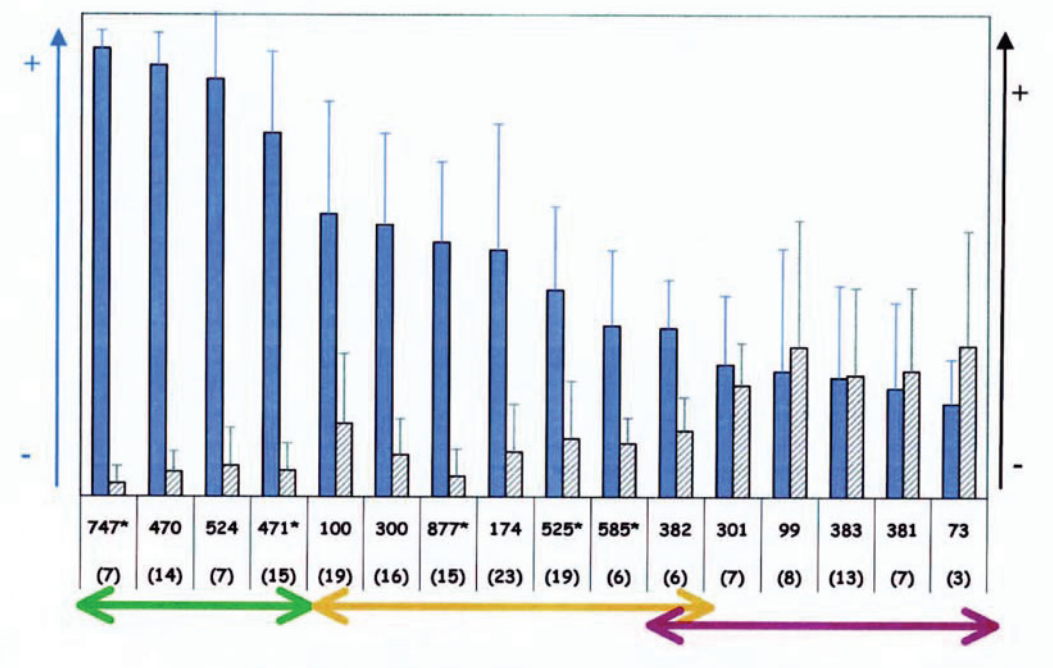


FIGURE 4

Syrah clones behavior towards decline, synthesis of the 2003/2004 observations
Mean values and standard deviations observed for healthy and dead plants

Plants without any symptoms

Dead plants (all causes possible)

* For these clones, more than half of the observations were realized on young plots (less than 7 years old)

( ) Number of the observations realized

understanding of the disease progresses and the range of possible pathogenic agents has been narrowed.

The transmission tests are starting to give interesting information: we are confident they will be conclusive. Neither the omega grafting nor the hormone treatment is causing the disease. Yet, Syrah presents, compared to other type of vines, difficulties recovering after grafting which could be correlated to the appearance of later symptoms.

Important information was obtained on the effect of plant material on Syrah decline which will eventually allow us to provide adequate advice to the winegrowers.

Syrah decline appears to be extremely complex and might be of multifactorial origin; research is ongoing to better determine these different factors.

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Appendix II

Citation:

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THE ASSOCIATION OF *GRAPEVINE VIRUS A* WITH AUSTRALIAN SHIRAZ DISEASE AND ITS APPARENT SPREAD IN A COMMERCIAL VINEYARD

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Introduction

Vitis Vinifera cv. Shiraz (syn. Syrah) is the most popular red wine variety in Australia. In Australia, a disease with the following symptoms has been observed in both own rooted and grafted Shiraz vines: delayed bud burst, stunted and zigzag growth of canes, poorly lignified wood. Also leaves, which hang on the vines during the winter, turn red and become brittle. *Grapevine virus A* (GVA) was detected in these vines using the reverse-transcription polymerase chain reaction (RT-PCR) assay (Symons & Habili, 2000; Habili & Randles, 2004). These symptoms resemble those described for Shiraz disease (SD) in South Africa (Corbett & Wiid, 1985), where GVA has also been detected in the infected vines (Goszczynski,& Jooste, 2003). Natural spread of the SD associated GVA by mealybugs has been reported from South Africa (Goszczynski & Jooste, 2003).

We have named the disease Australian Shiraz Disease (ASD) to differentiate it from its counterpart in South Africa. A similar disease has been found in Merlot and Ruby Cabernet in Sunraysia (Victoria). We have already reported the spread of GVA in our experimental vineyard (Habili *et al*., 2003). Here we report its apparent spread in a commercial vineyard in the Adelaide Hills (South Australia).

Materials and Methods

Dormant cane samples of the grapevine were sent to Waite Diagnostics from all the viticultural regions of Australia and were tested for GVA using RT-PCR as described (MacKenzie *et al*., 1997; Shi *et al*., 2003).

For the RT-PCR assay, a pair of GVA specific primers, GVA-H7038, 5'-AGG TCC ACG TTT GCT AAG-3' and GVA-C7273, 5'-CAT CGT CTG AGG TTT CTA CTA T-3' derived from the sequence of the ORF5 (putative RNA binding protein) of the Italian isolate of the virus (GenBank Acc. # X745433, Minafra *et al.*, 1994) was used.

The Shiraz vineyard was monitored for symptoms in the late autumn when the GVA associated symptoms were most conspicuous.

Results and Discussion

Table 1 shows the percentage of samples that tested positive for GVA has been on the rise since 2001. This is a cause for concern, and it maybe linked to our previous report that the virus was spreading

Table 1. The *Detection of Grapevine virus A* by RT-PCR in Australian grapevine samples sent to Waite Diagnostics for virus testing.

Year	*Total tested*	*+ve samples*	*%*
2001	728	25	3.4
2002	557	65	11.6
2003	1309	116	9.0
2004	1279	213	16.6

naturally, but at a slow rate, in our experimental vineyard (Habili & Randles, 2003).

Recently, another hot spot for the natural spread of ASD was detected in a Shiraz vineyard in the Adelaide Hills (South Australia). The Shiraz vines in that vineyard showed the typical symptoms of Australian Shiraz Disease. The RT-PCR assay detected GVA only in the symptomatic vines, while the apparently healthy neighboring vines of the same row tested negative for the virus. I also tested the samples for 11 other viruses and the results were negative (see also Habili & Randles, 2004). Up to date, no mealybugs were observed in the vineyard. The collection of data for the temporal and spatial analysis of GVA in Shiraz is in progress.

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Appendix III

Citation:

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DETECTION OF A PHYTOPLASMA BELONGING TO GROUP I IN DECLINING SYRAH

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Introduction

Syrah is one of the most important grape varieties cultivated in southern French vineyards. Since the 1990s, specific symptoms were described on this variety. This disorder, so-called "Syrah decline", is characterized by grooving and cracking at the graft union and leaf-reddening during autumn. Several studies have been initiated on physiological and pathological aspects (5,6). This set of experiments did not allow to identify the Syrah decline origin so far. However, some of the previously described symptoms (leaf-reddening and problems of wood maturation) appear similar to some of the physiological disorders classically observed in grapevine yellows. Consequently, we have decided to research a potential phytoplasma in Syrah declining plants.

Materials and Methods

Grapevine samples were collected in 2003 from a vineyard of Syrah located in Languedoc-Roussillon. Numerous samples, made of petioles/veins and flowers/clusters, were taken away on each plant in order to increase the possibility of detecting this pathogen agent. They were collected in May, July and October: 1g of each sample was frozen until analysis. They were taken away from plants showing different degrees of Syrah decline: vines without any symptom; vines showing only swelling and cracking at the graft union without leaf reddening; and vines expressing both cracking at the graft union and foliar reddening

DNA of the various samples was extracted using the phytoplasma enrichment procedure described by Daire *et al.* (1) with some minor modifications. A nested PCR procedure was used with combination of two phytoplasma-universal primer pairs P1/P7 followed by U5/U3 (2, 3, 4). In order to identify and determine the phytoplasma group, some PCR products were submitted to sequencing. The sequences obtained were analysed with the Blast software available on NCBI (National Center of Biotechnology Information, http://www.ncbi.nlm.nih.gov/blast/). They were aligned to other 16SrRNA sequences available in the databank and pairwise comparisons were realised to calculate percentage sequence similarities. Three of them, chosen to be representative of these sequences, were used to establish a phylogenetic tree.

Results and Discussion

32 of the 130 samples collected from Syrah plants from May to October 2003 showed one band at the expected size of 880 bp after the nested PCR. Positive samples were found in both symptomatic and symptomless plants in the following proportions:

- 19% (8/42) of the samples taken from vines without any symptom
- 21% (9/42) of the samples extracted from "cracking vines"
- 33% (15/46) of the samples taken away from "cracking and reddening vines"

Positive samples were detected at the three sampling dates but in an increasing proportion since May to October.

Nine of the PCR products obtained, chosen to be representative of all of the different samples (tissue, sampling period, degree of symptoms) were sequenced. They were then analysed by comparison with other sequences available on NCBI. Very strong similarities (more than 98%) were obtained with numerous 16SrRNA phytoplasma sequences, belonging to group I and group XII, identified in many different plants. These nine sequences show from 96 to 100% similarities to each other. Two sets can nevertheless be distinguished, the first one constituted of 4 sequences belongs to group I whereas the second one belongs to group XII. Thus, these analyses confirmed that the positives samples were infected with two distinct phytoplasmas: one belonging to 16SrI group (Aster yellow) and the other belonging to16SrXII-A group (Stolbur). No correlation could be established between the type of sample (tissue, sampling date and degree of symptoms) and the group belonging. It is worth noting that it is the first time the 16SrI group phytoplasma is reported on grapevine in France.

These experiments allowed us to identify phytoplasmas of two different groups in declining Syrah. However, there is still no obvious correlation between phytoplasma infection and Syrah decline; further investigations are in progress to be able to conclude.

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Appendix IV

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EMERGING GRAPEVINE DISEASES

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From the grape growing regions of the world, reports continue to be published of grapevine diseases previously unknown or only recently discovered in a new place. In some cases, these emerging diseases can be associated with well known grapevine viruses. In other cases, a previously unknown virus is discovered. Often, in the early stages of reporting, diseases of unknown aetiology are reported that may be caused by grapevine viruses and/or graft transmissible agents (GTAs).

Often, these disease reports refer to symptoms which involve the graft union or mimic symptoms of graft incompatibility. Since no substantiated examples of graft incompatibility in *Vitis* species have been reported due to strictly genetic causes (A. Walker, personal communication), a virus aetiology is strongly suggested when graft union disorders are associated with a vineyard disease. It is logical that since most commercial grapevine rootstocks have been selected for compatibility with *Vitis vinifera* and have been in commerce for many years, it is unlikely that they would demonstrate previously unreported genetic incompatibilities with *V. vinifera* scions.

Decline of Grafted Plants

Chilean table grapes have historically been grown on their own roots, due to the lack of phylloxera. As the industry has matured, however, nematode populations have increased and other replant problems have developed. As a result, growers are increasing turning to grafted plants. From 2000-2003, 'Thompson Seedless' grafted on nematode resistant "Freedom' and 'Harmony' rootstocks showed severe decline symptoms. Virus testing of symptomatic vines in these vineyards using a combination of ELISA and PCR revealed high rates of infection with GLRaV-2 and GFkV GVA was also found in 'Thompson Seedless' on Harmony rootstock but not on Freedom (8). This pattern of mild virus symptoms in infected *V. vinifera* grown on its own roots showing severe symptoms when grafted on Freedom and Harmony is consistent with previous reports (See Virus Induced Rootstock Decline below).

Grapevine Rootstock Stem Lesion-associated Virus

A disease of 'Redglobe' grapevines was described in California. Redglobe is a very popular table grape developed by University of California grape breeder Harold Olmo which is worldwide in popularity. In the early 1990s, the California table grape industry moving from vines without rootstock to grafted vines. A University of California rootstock trial was established with a large number of rootstock cultivars, many of them normally used in California for wine grapes. It was observed that Redglobe grafted to the rootstocks 5BB, 5C, 3309C, and 1103P declined and died within two years of planting. However, no symptoms were produced on Cabernet Franc in the classic 2-year woody index when grafted with this selection of Redglobe. Further experiments demonstrated that a GTA was involved in the disease which was readily transmitted to Cabernet Sauvignon causing a stem lesion on the susceptible rootstocks (14, 15).

This GTA was cloned using double-stranded RNA extracted from "Redglobe" stock which had exhibited the disorder. A new virus was identified which had 73% sequence homology with the HSP 70 gene of GLRaV-2. Weak cross reactions occurred in both Western blots and ELISA using antisera for GLRaV-2 obtained from Sanofi. (11).

Surveys have found this virus in wine grape cultivars in California (13) and in French wine grape clones imported into the USA (Golino, unpublished). A survey of 2045 vines in the Foundation Plant Services collection at UC Davis has found only two selections which tested positive for the virus; both were selections produced by the same breeder suggesting that infected rootstock might have been used in his program. (Rowhani, unpublished).

A survey in Italy of table grape varieties from the USA showed a high rate of infection with GLRaV-2 and GRSLaV (M. Borgo, personal communication). Analyses on about 110 samples of table grape varieties (cv. Redglobe and others) tested 99% tested positive for GLRaV-2 by ELISA (furnished by Agritest, BARI, Italy). Further analyses were conducted on another 18 Red Globe accessions with ELISA and RT-PCR with primer pairs specific for GLRaV-2 and GRSLaV. Serological assays for GLRaV-2 gave positive results for all the samples except two. PCR tests results were positive for only GRSLaV in 50% of samples, positive for GLRaV-2 for 22% of samples, 22% of samples were positive for both viruses, and 1 sample (6%) was negative also in RT-PCR assay

Ilarvirus from Greece

In 1994, virus symptoms were observed on a hybrid grapevine in a collection in Athens (3). Symptoms included mosaic, stunting, decline, and death; fruit and seeds were abnormal. No known grapevine viruses could be associated with the diseased vine. Herbaceous indicators were inoculated successfully in serial passage from *Gomphrena globosa* to *Chenopodium quinoa* and tobacco. From *C. quinoa*, a new ilarvirus was isolated which demonstrated similarities to tobacco streak virus.

Syrah Decline

A unique problem has been observed in southern French vineyards on the variety Syrah, a very important winegrape variety in this region (1, 9, 10). The decline is distinguished by swelling and cracking of the graft union and strong leaf reddening symptoms early in the summer. The graft union is enlarged and deep grooves can be observed when the bark

is removed. Scions of affected vines frequently die shortly after symptoms develop. Efforts are underway to determine whether this disease is graft transmissible. All rootstocks and clones seem to be involved although a higher rate of disease is observed with some clones (J.-M. Boursiquot, personal communication). An extended survey of 77 vineyards was unable to identify any cases of the disease in California vineyards (6).

Vine Decline Syndrome in Argentina

In Argentina, the transition from an industry growing grapevines on their own roots to vineyards propagated on rootstock is also resulting in increased virus problems (7). In the last decade, rootstocks have come into common use. In some vineyards, leafroll symptoms have been observed. PCR surveys of symptomatic vines indicate a high level of infection with GLRaV-2 and possible associations with GRSLaV. Studies are continuing to determine the full range of virus types.

Virus Induced Rootstock Decline

Newly replanted grape (*V. vinifera*) vineyards in California in the 1990s were observed failing with disease symptoms characteristic of virus infection (4). This epidemic occurred during a planting cycle which involved a dramatic change in rootstock genotypes. Disease was associated with vineyards using certified rootstock, field grafted with scion buds from apparently healthy commercial vineyards. It has been demonstrated that affected vineyards were impacted by mixed infections of GLRaV and vitivirus infection, most often GVB. This virus combination causes what has been called Virus Induced Rootstock Decline (VIRD). The severity of the disease is highly dependent on rootstock genotype. It has been shown that *V. vinifera* on its own roots and the rootstocks AXR-1 and St. George are fairly resistant to the virus combination. Freedom and Harmony are very susceptible, which is important to note since they are frequently used when nematode populations are high in areas where previous vineyards have been propagated without rootstock (5).

Other Emerging Diseases

A work in progress from New Zealand describes an incompatibility syndrome on Merlot. Molecular investigations of infected plants suggest the presence of a previously uncharacterized closterovirus (2). Nucleotide sequence comparisons suggest a close relationship to GLRaV-2.

A young vineyard near Conegliano, Italy, has been discovered in which Mourvedre grafted on *V. riparia* 'Gloire de Montpellier' is exhibiting symptoms of graft incompatibility, degeneration, and thickening of the graft union. ELISA tests gave negative results for ArMV, GFLV, Fleck, GVA, GVB, and GLRaV-1-2-3-6-7. RT-PCR assays tested negative with primer pairs specific for GLRaV-2, GRSLaV and GVA. Work is continuing to identify a causal agent. (M. Borgo, personal communication).

Woody indexing and PCR surveys in California (13) have identified 5 additional putative GTAs from diverse samples demonstrating disease symptoms.

Common Elements

The development of improved diagnostics and molecular cloning techniques has revolutionized the diagnosis of previously unrecognized grape virus diseases. It is now possible to rapidly assess the possible involvement of known grapevine viruses in emerging vineyard disease cases using PCR and other laboratory diagnostics. If no known virus can be implicated, purification, cloning and sequencing of viral RNAs from symptomatic plants often result in the efficient identification of causal agents.

Many of the current cases of grapevine virus disease problems involve transitions in growing practices in specific grape growing reagions. Where rootstock has been recently introduced (Argentina, Chile) or rootstock cultivars have changed (California), endemic viruses which had been latent or mild under past growing conditions may become severe. This is broadly attributed to the differing genetic susceptibilities of grapevine rootstock cultivars to the wide diversity of grapevine viruses.

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Appendix V

Citation:

Lima, M.F., R. Alkowni, A. Rowhani, J.K. Uyemoto, D.A. Golino and A-S. Renault-Spilmont. 2003. Genomic study of two *Grapevine rupestris stem-pitting-associated virus*-like isolates. Ext. Abstr. 14th ICVG Meeting. Locoronto, Italy, p. 125. Retrieved October 22, 2007 from http://www.icvg.ch/archive.htm

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August 16, 2007
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GENOMIC STUDY OF TWO *GRAPEVINE RUPESTRIS STEM PITTING-ASSOCIATED VIRUS*-LIKE ISOLATES

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Grapevine rupestris stem pitting-associated virus (GRSPaV) is a component of the rugose wood complex. It is a graft-transmissible virus and detectable on *Vitis rupestris* cv. St George, in which induces basipetal pitting bellow grafted bud chip (3). GRSPaV is a filamentous particle of about 800 nm long and its genome consisted of a single-stranded, positive sense RNA and classified in *Foveavirus* genus with *Apple stem pitting virus* as type species (1). RT-PCR methodology was developed for detection and identification of the virus based on the available genome sequences (2, 5). This methodology was used to detect isolates of GRSPaV which were showing great nucleotide sequence variability in their different genes (3, 2). In our study reported here, two different GRSPaV-like isolates were detected from different symptomatic grapevines and their genomes were sequenced and studied. Specific RT-PCR detection method was developed for each isolate.

During field surveys in Californian vineyards, symptomatic grapevines were collected and tested by RT-PCR for a panel of grapevine viruses. In this investigation, two different GRSPaV isolates were detected using the primers RSPC-48 and RSPV-49 (5). One of these viruses was isolated from a syrah selection showing graft union disorders and will refer to as "SY-RSP-isolate". The second virus was isolated from a Pinot Noir vine showing stem lesions on the rootstocks and solid reddish colour in the fall on leaves and will refer to as "PN-RSP-isolate". cDNA libraries were made for each isolate using random priming cDNA synthesis kit (Invitrogen) and dsRNA as a template (4). Double-stranded DNAs were cloned using TA cloning Kit, and clones were selected and sequenced on both directions. The overlapping clone sequences were analyzed using the Wisconsin GCG software package (Genetic Computer Group, Madison, WI) and the National Center for Biotechnology Information (NCBI) for database.

Nucleotide and amino acid analyses of both isolates showed sequence identities ranging from 70% to 89% and from 81% to 95% in amino acid sequence to GRSPaV and among themselves, respectively. The open reading frame corresponding to the position encodes the virus capsid protein of SY-RSP- isolate showed identities of 82% and 91% in nucleotide and amino acid, respectively, to that of GRSPaV. These significant variations among the isolates made it possible to design specific primers for detection of each virus isolate, hence, will assist in tests used for better understanding of the aetiology of these isolates, and investigate the possible association of each virus isolate with its corresponding field symptoms. Further surveys are underway.

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Appendix VI

Citation:

Renault-Spilmont, A-S, S. Grenan and J-M. Boursiquot. 2003. Syrah Decline in French vineyards. Ext. Abstr. 14th ICVG Meeting. Locoronto, Italy, p. 144. Retrieved October 22, 2007 from http://www.icvg.ch/archive.htm

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August 16, 2007
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SYRAH DECLINE IN FRENCH VINEYARDS

A.S. Renault-Spilmont , S.Grenan and J.M.Boursiquot

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Syrah is one of the most important grape varieties cultivated in southern French vineyards. Since the 1990s, a unique problem has been observed by grape growers and researchers on Syrah plants: leaf reddening and swollen graft unions. The scions of affected vines declined and died more or less rapidly. By contrast, the rootstock often stays alive and canes can be observed suckering below the union.

Syrah decline is characterized by two symptoms on mature plants:

-swelling and cracking at the graft level (5-6 cm), very specific of the syndrome

-early leaf reddening (from July)

The graft union becomes enlarged and the wood hard. After peeling the bark, deep and parallel grooves can be observed in this specific localized area. The vines can also show a premature discoloration of the leaves during the spring, becoming red in autumn.

All rootstocks and clones are known to demonstrate this problem although there are some indications that their sensitivity might vary.

Development of the symptoms is very different depending on the site. Mostly, strong symptoms appear on 8-10 years old vines. But in the few last years, symptoms seem to be observed on more young plants than previously, perhaps due to more careful observation. Four year-old vines are now recorded to show typical symptoms.

Syrah vineyards have been surveyed and some sites have been followed since 1999. Each plant is identified and observed from one year to another in the aim of describing the spatial and temporal evolution of the problem. Statistical analyses of these records will aid in better understanding of symptom development. In a first approach the symptoms seem to propagate along the row.

To find a putative pathogen, disease associated viruses were sought with ELISA and biological indexing tests. The virus tests were performed on GFLV, GLRaVs, GFkV, GCBV, GVA, RSP and KSG. No correlation could be established between one or more viruses' presence and the previously described symptoms. PCR tests with different primers are in course. Furthermore, experiments were conducted to determine if the problem was associated with a graft transmissible agent. Some interesting results were obtained several months after green grafting as leaf reddening was sometimes observed with Syrah or rootstock taken from diseased vines. No symptoms at the graft union have been observed so far but experiments are on-going.

As the primary symptoms of Syrah decline involve the graft union, studies were conducted to compare different grafting techniques. Experiments were made comparing bench grafted Syrah ("long-whip" and omega cut), field grafted Syrah (with and without hormone applications) and green grafted plants. Five years after establishment, many plants show cracked and swollen unions but none have died yet. No significant difference could be found between these grafting techniques up to now.

To understand cracking morphology, several graft unions were dissected for observation under a microscope. Precise observations in the cracking areas suggest a dysfunction of the cambial zone with a disruption of the local area. We are trying to determine the origin of this disruption.

The previously described symptoms might be similar to those observed in incompatible grafted fruit trees. To confirm this hypothesis, an important experiment is currently conducted to describe the first events after vine grafting. The process of graft union development was studied in Syrah compared to two other grape varieties (Cabernet-Sauvignon and Grenache) used as controls. Histological studies are being performed on the first events following grafting; callus proliferation, cambium formation and vascular connections are compared among the varieties. The first results seem to indicate that the level of vascular connections is lower during the healing of Syrah than for the other two varieties.

As explained previously, two types of symptoms are associated with Syrah Decline. The relation between those two symptom types needs to be well established. Careful observations in a number of different situations showed that many plants show only cracking without leaf reddening. This has led us to suggest the hypothesis that two different factors could be implied in this problem: the first one would be involved in the cracking of the wood and a second one is responsible for inducing the leaf reddening and the death of the plant.

The problem of Syrah Decline appears to have no simple explanation. We believe that the problem is very complex, and may involve multiple factors. Results of our experiments with possible graft transmission of a potential pathogen agent are waited with hope. In the meantime, our research will go on.

Appendix VII

Citation:

Golino, D. 2001. “A graft union disorder of Syrah.” FPMS Grape Program Newsletter, October. 2001, p. 13.

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August 8, 2007
Dr. Deborah Golino
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A Graft Union Disorder of Syrah

by Dr. Deborah Golino, Director of FPMS, UC Davis

Viticulturists have observed a phenomenon in Syrah vineyards in the last couple of years which suggests that this very popular variety may be subject to special propagation problems. Leaf reddening, swollen graft unions, and stem necrosis symptoms have been seen in some California Syrah vineyards. These kinds of symptoms are most often associated with genetic incompatibility and/or virus infection.

Dr. Andy Walker, UC Davis Department of Viticulture and Enology, reported on this phenomenon at the Syrah Symposium held as part of the June 2001 American Society for Enology and Viticulture annual meeting in San Diego. He noted that the cause of the Syrah phenomenon is unknown but several conditions have been associated with the symptoms observed including: poor graft unions, crown gall infection, genetic incompatibility, traditional viruses, new viruses, viroids, and environmental interactions.

Walker reported that problems with Syrah have also been seen in France. French Syrah vines with red leaves and swollen graft unions are dying within 1-2 years. These symptoms are associated with Syrah grafted to a number of rootstocks including: 110R on lime soils, 140Ru, *Vitis berlandieri* x *V. riparia*, and *V. riparia* x *V. rupestris* in France.

This winter, Dr. Walker, Dr. Adib Rowhani, and I plan to apply for research funding to study this problem. The project to be proposed will include a statewide survey of Syrah sites with the problem, as well as analysis of clone source, rootstock variety, site, and management techniques associated with the Syrah phenomenon. Tests will also be conducted to look for any associated graft transmissible agent(s).

Above: Syrah on 101-14 Mgt with a swollen and necrotic graft union associated with graft union disorder.

Appendix VIII

Citation:

Renault-Spilmont, A-S. and J-M. Boursiquot. 2002. “Syrah decline in French vineyards.” FPMS Grape Program Newsletter, October. 2002, p. 22-23.

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Syrah Decline in French Vineyards

by Anne-Sophie Renault-Spilmont and Jean-Michel Boursiquot
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BECAUSE OF ITS GREAT POTENTIAL to produce quality wine, Syrah is one of the most important grape varieties cultivated in southern French vineyards. Since the 1990s, a unique problem has been observed by grape growers and researchers on Syrah plants: leaf reddening and swollen graft unions. The scions of affected vines declined and died more or less rapidly. By contrast, the rootstock often stays alive and canes can be observed suckering below the union.

Symptom description

Syrah decline is characterized by two symptoms on mature plants:

-swelling and cracking at the graft union (Fig. 1)
-early leaf reddening (from July)

The graft union becomes enlarged and the wood hard. After peeling the bark, deep and parallel grooves can be observed in this specific localized area. The vines can also show a premature discoloration of the leaves during the spring, becoming red in autumn.

All rootstocks and clones are known to demonstrate this problem although there are some indications that their sensitivity might vary.

Figure 1: Swelling and cracking at graft union. *(Photo courtesy of ENTAV)*

Development of the problem

Development of the symptoms is very different depending on the site. In the last few years, symptoms seem to be observed on more young plants than previously, perhaps due to more careful observation. Four year-old vines are now recorded to show typical symptoms.

Syrah vineyards have been surveyed and some sites have been followed since 1999. Each plant is identified and observed from one year to another with the aim of describing the spatial and temporal evolution of the problem. Statistical analyses of these records will aid in better understanding of symptom development.

As explained previously, two types of symptoms are associated with Syrah Decline. The relationship between those two symptom types needs to be well established. Careful observations in a number of different situations showed that many plants show only cracking without leaf reddening. By contrast, very few plants showing only leaf reddening (without cracking) could be found. This has led us to suggest that two different factors could be implied in this problem: the first one would be involved in the cracking of the wood and a second one (different from the first) is responsible for inducing the leaf reddening and the death of the plant.

To understand cracking morphology, several graft unions were dissected for observation under a microscope. Precise observations in the cracking areas suggest a dysfunction of the cambial zone with a disruption of the local area. We are trying to determine the origin of this disruption.

Current studies and preliminary results

A pathogen?

A study was set up to identify this disorder and tests were carried out to look for any associated transmissible agent(s).

Disease associated viruses were sought with ELISA and biological indexing tests. The virus tests were performed on traditional grapevine viruses responsible for Leafroll, Fanleaf, Fleck, Corky Bark, Rupestris Stem Pitting and Kober Stem Grooving. No correlation could be established between one or more viruses' presence and the previously described symptoms. No phytopathogenic bacteria (Crown Gall, Bacteria Blight, Pierce's disease) could be found.

As far as the fungi, some of them associated with wood diseases were found in symptomatic plants but also in control vines. Thus, it does not seem that their presence could be correlated with the specific Syrah decline. Nevertheless, these fungi involved in wood diseases might play a second role in increasing or quickening the decline of already weakened plants. The cracking may be a point of entry for penetration of these fungi. They could also induce necrosis, leading to plant death. The possible involvement of these fungi with Syrah decline will be further studied by a field experiment.

Furthermore, experiments were conducted to determine if the problem was associated with a graft transmissible agent. Some interesting results were obtained several months after green grafting as leaf reddening was sometimes observed with Syrah or rootstock taken from diseased vines. No symptoms at the graft union have been observed so far but experiments are on-going.

An incompatibility?

The previously described symptoms might be similar to those observed in incompatible grafted fruit trees. To confirm this hypothesis, an important experiment is currently being conducted to describe the first events after vine grafting. The process of graft union development was studied in Syrah compared to two other grape varieties (Cabernet-Sauvignon and Grenache) used as controls. Histological studies are being performed on the first events following grafting; callus proliferation, cambium formation and vascular connections are compared among the varieties. The first results seem to indicate that the level of vascular connections is lower during the healing of Syrah than for the other two varieties.

Possible grafting factors?

As the primary symptoms of Syrah decline involve the graft union, studies were conducted to compare different grafting techniques. Experiments were made comparing bench grafted Syrah ("long-whip" and omega cut), field grafted Syrah (with and without hormone applications) and green grafted plants. Five years after establishment, many plants show cracked and swollen unions but none have died yet. No significant difference could be found between these grafting techniques up to now.

The problem of Syrah Decline appears to have no simple explanation. We believe that the problem is very complex, and may involve multiple factors. Results of our experiments with possible graft transmission of a potential pathogen agent are awaited with hope. In the meantime, our research will go on.

Appendix IX

Citation:

Battany, M., A. Rowhani, and D. Golino. 2004. “Syrah in California: Decline or Disorder?” Practical Winery & Vineyard, May/June, p.1-7.

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GRAPEGROWING

Affected Syrah in the foreground: third leaf Syrah "Shenandoah" clone on 5C rootstock, with strong symptoms. Adjacent block (background) not affected.

AMERICAN VINEYARD FOUNDATION

SYRAH IN CALIFORNIA
Decline or Disorder?

BY **Mark Battany, Adib Rowhani, & Deborah Golino**

Growers of Syrah in California vineyards have observed a variety of symptoms in recent years that suggest this popular variety may be subject to unique problems. Reports include cases of leaf reddening, scorching, swollen graft unions, and stem necrosis symptoms. These symptoms are often associated with genetic incompatibility and/or virus infection.[5]

Some viticulturalists have expressed concern that affected vineyards might be showing symptoms of the disease known in France as "Syrah Decline." At this time, the cause of that disease is unknown.

The authors of this report conducted an extensive survey of California Syrah vineyards in 2002 and 2003. The survey did not confirm the presence of Syrah Decline in California. Rather, most affected vineyards were found to have problems that resulted from environmental stress or poor cultural practices.

In addition, on the Central Coast, significant acreage is affected by a problem we are calling "Syrah Disorder." We believe Syrah Disorder is a separate problem from Syrah Decline and propose some possible causes later in this report.

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Affected vine on left, unaffected vine on right. When vines are affected, generally the entire canopy shows symptoms rather than individual leaves and shoots.

The French situation

Syrah Decline is a well-documented problem in France.[1,6,7] It is characterized by swelling and cracking of the graft union and early leaf reddening (as early as July; see Figures I and II). French scientists at ENTAV have been studying this problem since 1999 with efforts to determine whether there is any correlation between Syrah Decline and other conditions, such as poor graft unions, crown gall infection, genetic incompatibility, traditional viruses, new viruses, fungi, viroids, and environmental interactions.

At this time, our French colleagues have concluded that the problem has no simple explanation. Consequently, they are investigating solutions involving a number of possible causes, including new, unknown viruses.

California statewide survey

In 2002, we began our statewide survey of Syrah sites to determine whether the disease Syrah Decline, as reported from France, occurs in California. Our goals were to visit Syrah sites in which problems have been observed, including graft union abnormalities and reddening of leaves; to test selected samples for pathogens by using molecular and biological assays; and to establish a small field trial with Syrah grafted onto select rootstocks to attempt to induce observed symptoms.

Swelling and cracking at the graft union characterize the Syrah Decline disease in France.

Growers, UC faculty, and Cooperative Extension staff were asked to help us identify possible sites based on symptoms. Seventy-seven Syrah vineyards in 10 California counties were surveyed and inspected in 2002 and 2003. Counties included Calaveras, El Dorado, Madera, Merced, Napa, Sacramento, San Joaquin, San Luis Obispo, Sonoma, and Stanislaus.

Twelve clones, seven field selections, and 12 different rootstock varieties were represented (Table I), and more than 50 different combinations of clone and rootstock were observed (Table II). Geography ranged from central San Joaquin Valley to Sierra foothills to coastal valleys, with temperatures as high as 110ºF and as low as 19ºF.

All vineyards were drip irrigated, and many vineyard managers reported practicing deficit irrigation to control vigor. Many were planted as green-growing benchgrafts, some as dormant benchgrafts, and others as field-budded vines.

Observed symptoms included mild and severe vertical cracking on the

Table I: Survey of Virus Test Results in California Syrah Vineyards

	Clone/ Selection	# Vineyards Surveyed	Rootstocks	Virus test results
1	Syrah FPS 01	4	110R, 99R, Freedom, Teleki 5C,	No virus detected.
2	Syrah FPS 07	4	101-14, 420A, Schwarzmann, SO4	No virus detected except RSP in 2 vineyards.
3	Syrah ENTAV-INRA 99	2	3309C, 99R	No virus except RSP in 2 vineyards.
4	Syrah ENTAV-INRA 100	3	3309C, Freedom, Teleki 5C	No virus except RSP in 2 vineyards.
5	Syrah ENTAV-INRA 174	6	110R, Freedom, SO4, Teleki 5C	GLRV2 detected in 1 vineyard. RSP detected in 5 vineyards.
6	Syrah ENTAV-INRA 383	1	110R	No virus detected.
7	Syrah ENTAV-INRA 877	4	101-14, 3309C, Freedom, Teleki 5C	No virus detected except RSP in 4 vineyards.
8	Syrah Estrella	15	101-14, 110R,1103P, 140Ru, 44-53M, Freedom, Kober 5BB, Teleki 5C, own roots	No virus detected except RSP in 1 vineyard.
9	Syrah Noir	8	101-14, 110R, Freedom, Kober 5BB, Teleki 5C	GLRV2 detected in 2 vineyards; GLRV9 detected in 2 vineyards; GVA detected in 1 vineyard; GV detected in 1 vineyard; RSP detected in 8 vineyards.
10	Syrah Beaucastle	2	110R, 44-53M	No virus detected except RSP in 2 vineyards.
11	field selections/ unknown	18	039-16, 99R, 110R,101-14, 3309C, Freedom, Schwarzmann, SO4, Teleki 5C,	GLRV2 detected in 2 vineyards; GVA detected in 1 vineyard; GV detected in 1 vineyard; RPS detected in 5 vineyards.
12	Shiraz FPS 01	3	99R,3309C, SO4	No virus detected except RSP in 1 vineyard.
13	Shiraz	8	101-14,1103P, 140Ru, 420A, Schwarzmann	No virus detected except RSP in 2 vineyards.
Total	More than 12 clones/selections	78	13 different rootstocks	GLRV 2, GLRV 9, GV, GVA detected in less than 4% vineyards; RSP detected in approximately 50% vineyards.

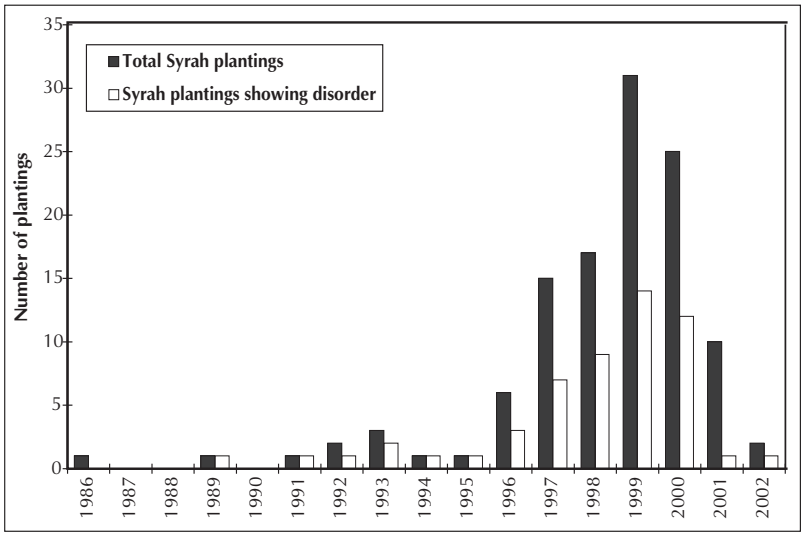

Figure I. Incidence of Syrah Disorder on the Central Coast in 2003, by year of planting.

trunk, interveinal burgundy red leaves (typical of leafroll-virus symptoms), leaf-margin reddening and necrosis, and swelling at the graft union.

No strong pattern emerged between specific management practices, site, clone, rootstock, or type of planting material, and observations of symptoms or reports of problems. Individual healthy vineyards were observed planted with all the common Syrah clones, making it unlikely that a disease carried with propagative materials was involved.

In many cases, symptoms in problem vineyards could be attributed to specific factors, including frost damage of young vines, water stress (including lack of water to young vines and poor drainage), poor planting and training techniques (Figure III, IV), and other factors causing root stress, including known presence of Armillaria root rot.

Only in the Central Coast were problem vineyards observed for which no obvious environmental or management problem existed. These vineyards will be discussed in more detail below.

Infection with known viruses (based on test results) did not seem to be a common factor in problem grapevines identified by vineyard managers.

More than 1,500 PCR tests were completed on 132 samples. All samples were tested for viruses that are associated with graft union disorders: leafroll type 2, grapevine virus A (GVA), grapevine virus B (GVB), grapevine virus D (GVD), Rootstock Stem Lesion-associated Virus (GRSLaV), and Rupestris Stem Pitting-associated Virus (GRSPaV). Selected samples were tested for additional viruses. A summary of results is presented in Table I.

Statewide, results were very similar. Approximately 95% of the samples tested negative for viruses typically associated with graft-union disorders. Several samples tested positive for leafroll type 2, leafroll type 9, and for vitivirus.

The general vitivirus primer used for these tests is experimental, detecting the vitiviruses GVA, GVB, and GVD. One would normally expect that a sample testing positive for the vitivirus primer would also test positive for GVA, GVB, or GVD.

However, this was not the case in this study. We are continuing to work on this inconsistency, to explore if there is a different, as yet unidentified, vitivirus in these samples. Although these viruses are sometimes associated with graft-union disorders, we did not get consistent positive results among samples from the same vineyard, neither were they correlated with symptoms.

Approximately 50% tested positive for Rupestris Stem Pitting-associated Virus (RSP). RSP, believed to be a mild virus, is known to be widespread in California. It is not known to cause the symptoms associated with Syrah Decline.

Many sites tested negative for all viruses, yet showed reddening. However, these vines also suffered from large cracks and splits in the bark, which appeared to be due to frost damage. Such damage can also cause leaf reddening. In other cases, plants with no obvious red leaf symptoms tested positive for GLRaV-2, apparently harboring a latent infection.

In general, far less virus was found in Syrah than is usually found in some major grape varieties grown in California. In previous survey work, Chardonnay, Zinfandel, and Cabernet Sauvignon have been tested from diverse vineyard locations and are much more likely to be infected with both leafroll viruses and vitivirus.

We believe that Syrah in California is relatively free of virus problems because it has become popular relatively recently, so nearly all the clonal material in the trade has come from clean stock programs and been tested by formal importation programs.

Syrah Disorder on the Central Coast

Although most problem Syrah vineyards we visited could be explained by

Leaf reddening can occur as early as July when a vine is affected by the French Syrah Decline.

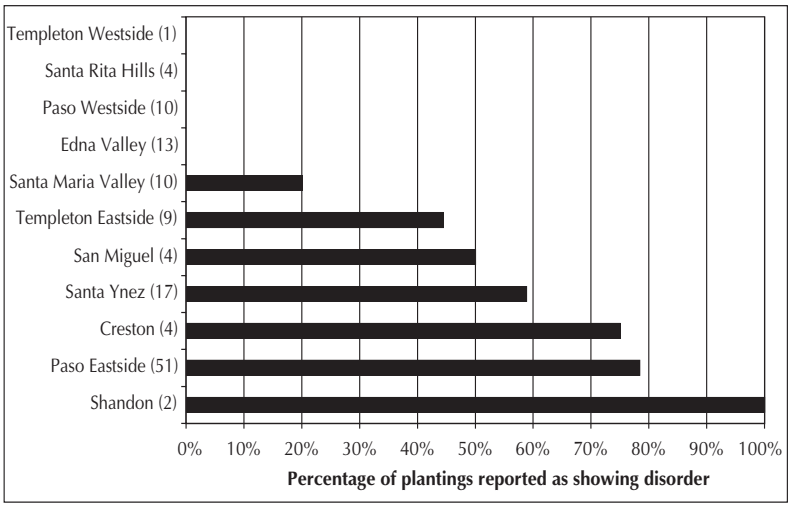

Figure II. Incidence of Syrah Disorder on the Central Coast in 2003, by location. The numbers in parenthesis refer to the sample size for each category.

cultural or environmental factors, Syrah grown in the warm climate areas of the Central Coast has suffered an increasing incidence of a generally uniform disorder during the past three seasons. This disorder, which we call Syrah Disorder, appears to be relatively unknown in other parts of California, but has affected significant acreage in this area.

Typical symptoms of Syrah Disorder include:

- Leaf reddening and senescence beginning around veraison or soon thereafter, with visual leaf symptoms that somewhat resemble a blend of leafroll virus, severe potassium or magnesium deficiency, salt burn, and/or severe water stress;
- Limited ripening of the fruit beyond veraison; the resulting juice has very low soluble solids, high pH, high potassium, and very poor color;
- Undesirable, difficult-to-market fruit, and reduced wine quality.
- Adjacent blocks of other varieties under the same management and environmental conditions do not display any similar characteristics to those above.

There are approximately 4,400 acres of Syrah grown in San Luis Obispo and Santa Barbara counties. We surveyed the area's Syrah growers after the 2003 harvest regarding Syrah Disorder. Forty-five growers responded, representing 125 individual plantings of Syrah, Shiraz, and Syrah Noir.

This brief, non-scientific survey requested the planting year, rootstock, clone, location, certification status, and whether or not symptoms were observed in 2003 for each individual planting. Based on the survey results, there were no correlations between occurrence of the disorder and specific clones, rootstocks, or year of planting (Fig. I).

There was, however, an obvious correlation with location: The hotter, drier areas in the Central Coast showed more occurrences of the disorder, while the cooler, wetter areas showed few or no occurrences. For example, in the warmest inland areas east of Paso Robles, 78% of the plantings showed the disorder, and likewise in the warm inland area of Santa Ynez Valley, the percentage was 58% (Fig. II).

Non-certified material was more likely to be affected (Fig. III). Several growers who have had successive poor years with Syrah have resorted to removing the plantings.

Some of the possible causes of the disorder that have been receiving attention among growers include:

- An as-yet unknown virus or other pathogen;
- Graft-union disorders;
- Nutritional imbalances, particularly potassium, magnesium and phosphorus;
- Physical root impairment, such as J-rooting;
- Increased vine water stress due to recent drought, salinity, and changing irrigation practices;

Based on our 2002 statewide virus survey, we believe that some of the factors above can be eliminated as possible causes of the disorder. Our testing did not indicate that a known virus was responsible for the symptoms that growers are reporting. The majority of growers in the area reported that there were no graft union problems in their affected vineyards.

There does not appear to be any obvious correlation between the disorder and soil or vine nutrient status. Distribution of the disorder across a wide variety of soils tends to decrease the likelihood that a specific nutrient imbalance is the primary cause, particularly when other varieties perform perfectly well under the same conditions, and the same Syrah plantings may have performed well in previous years. Physical root impairments due to improper planting methods could contribute to the symptoms, but again, it is unlikely that only Syrah was planted improperly while other varieties were not.

Perhaps more significantly, over the past five years this region has experienced a significant rainfall deficit compared to historical averages. This time period also coincides with increased adoption of deficit irrigation practices by the local winegrape industry.

Additionally, the groundwater quality in many areas of the Central Coast is fairly marginal, with high salt levels. Without adequate leaching either from winter rainfall or by application of an extra leaching fraction with irrigation, these salts accumulate in the root zone and lead to increased vine water stress over time. The cumulative effect of dry winters, reduced irrigation, saline irrigation water, and increasing soil salinity should notably

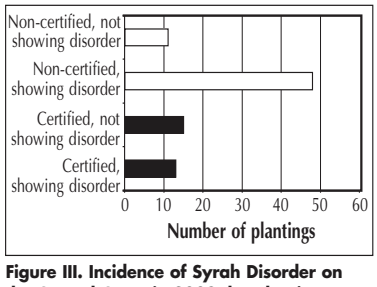

Figure III. Incidence of Syrah Disorder on the Central Coast in 2003, by planting material certification status.

Red leaf symptoms—may be an early stage of the disorder.

increase vine water stress, with all other factors being equal.

Possible primary factor

The affected areas are also characterized by extreme daily fluctuations in both temperature and evapotranspiration (ET). Daily temperature swings of 60ºF are typical, and peak afternoon ET rates can be very high, driven by high temperatures, low humidity, and the strong solar radiation characteristic of the region. These factors, along with the results of some recent research regarding the unusual physiological response of Syrah to drought (discussed below), seem to suggest that excessive water stress may be a primary factor causing Syrah Disorder.

H.R. Schultz compared the drought responses of Grenache and Syrah in France and Germany.[12] He showed that Syrah, which originated in the relatively humid climate of the Rhône Valley, has a very different drought response mechanism than Grenache, which originated in northern Spain. Grenache exercised rapid and significant stomatal control under increasing water stress, which placed a lower limit on the leaf water potential attained; this is an example of "near-isohydric behavior." This type of response is considered more typical of vines that have developed an adaptation to severe drought.

Schultz demonstrated that Syrah, in contrast, exercised very little stomatal control, instead responding to drought by continually lowering its leaf water potential while maintaining full transpiration; this is an example of "anisohydric behavior." Such a response may be more typical of vines that evolved where drought is rarely severe; that corresponds well to Syrah's area of origin.

In earlier work, Schultz compared the drought response of Syrah and Grenache in France and determined that Syrah showed very little adaptive response to water stress other than lowering the leaf water potential at which leaf turgor was lost.[13]

Schultz also noted that, for Syrah under severe drought conditions, there was a very fine line between adequate production and death of a vine. A practical message from Schultz's work is that Syrah's drought response is efficient when water stress remains below some maximum threshold, but as water stress exceeds this, the limited stomatal response and progressively lower leaf-water potential may make the variety more prone to vascular failure (cavitation), ultimately causing a cessation in the xylem sap flow.

T. Winkel and S. Rambal conducted similar research on the varieties Carignane, Merlot, and Syrah in France.[15] They also found that the Syrah exhibited relatively little stomatal response to drought. They further noted that Syrah protected its vascular integrity by reducing the total transpiring surface via a reduction in leaf area.

In earlier work, Winkel and Rambal determined that Carignane had a much greater stomatal sensitivity to changes in air humidity as compared to Syrah, which they attributed to the different evolutionary origins of the two varieties.[16] They concluded that this difference in behavior reflected the recognized poorer drought adaptability of Syrah.

The degree of water stress experienced by Syrah in different parts of the world can be evaluated by comparing pressure chamber readings of midday leaf-water potential from some published research trials in other regions with values reported by Central Coast growers. Both Schulz in France and Ginestar in Australia reported maximum midday leaf pressure chamber readings of approximately 18 bars for their "dry" irrigation research treatments.[4]

By comparison, several Central Coast growers have reported midday readings of 21 or 22 bars in their vineyards. Research by C.G. Dundon and R.E. Smart in Australia measured values of up to 23 bars prior to harvest in a dry-farmed Shiraz vineyard. According to the authors, "extraordinary levels of stress were experienced by the vines" at this site, which led to a substantial amount of leaf necrosis.[2]

Based on the above research, the drought-response mechanism of Syrah appears to make it prone to xylem cavitation and/or leaf-area reduction when under excessive drought conditions. Such a response could then manifest itself as the symptoms that we associate with Syrah Disorder on the Central Coast.

Growers have commented that the onset of symptoms often occurs very rapidly, with entire vines becoming severely affected within just one or two days. This type of response is consistent with a significant and rapid change in vascular function, particularly at some location lower on the trunk.

Interestingly, research by ENTAV in France has shown that Syrah tends to form fewer vascular connections between the rootstock and scion after grafting as compared to Cabernet Sauvignon and Grenache.[6] If this trait remains as vines mature, it could predispose the variety to vascular failure when under severe stress.

Growers have also noted that individual vines that are affected in one season may not show symptoms the following

Red-brown leaf symptoms—may be a more advantaged stage of the disorder.

Poor training of young vines can result in constriction of the trunk, which will ultimately cause red leaf symptoms and stunting.

season, and vice-versa; thus vines can recover from the disorder, which would also be expected with a transient vascular failure.

From a practical irrigation management perspective, the anisohydric drought response of Syrah effectively masks the degree of water stress experienced by the vines. By lowering its leaf-water potential while keeping its stomata open, Syrah maintains relatively strong leaf turgor pressure and continues to transpire large amounts of water, which keeps the leaves cool and the photosynthesis rate high.

The resulting firm, cool leaves and continual growth suggest to growers conducting visual and tactile inspections that the vines are not under significant stress, when in fact just the opposite may be true. Additionally, with its open stomata, Syrah will be transpiring relatively more water than other varieties under drought conditions, effectively removing more water from the soil and ultimately worsening its own condition.

The demonstrated varietal susceptibility to severe drought, recent dry winters, reduced irrigation amounts, marginal irrigation water quality, and the intrinsic climate of the inland areas of the Central Coast, in part, may explain why we have seen an increasing incidence of Syrah Disorder in this area in recent years.

However, the fact that many of the recently affected vineyards have, in the past, produced high quality grapes without showing any disorder-symptoms suggests that this is a transient and correctable phenomenon. Growers with susceptible plantings should consider paying extra attention to irrigation and salinity management practices to avoid severe vine-water stress, and ideally monitor vine-water status throughout the season with a pressure chamber.

While other as-yet unknown causal factors may be involved in this disorder, the pattern of occurrence on the Central Coast suggests that excessive vine water stress is a primary factor in its ultimate expression.

Future plans

Our 2002–03 Syrah virus survey did not find Syrah vineyards with symptoms that matched the French Syrah Decline symptoms. In general, the majority of Syrah plantings are relatively virus-free compared to varieties, such as Cabernet Sauvignon, Chardonnay, and Zinfandel, all of which have been established in California longer.

Over 1,500 PCR tests were completed on 131 samples from 77 surveyed vineyards. Approximately 95% of the samples tested negative for viruses typically associated with graft union disorders. Virus testing results were very similar statewide, which would be expected since the same clones and selections are available to all of California's growers, and viruses usually move with propagating materials.

The majority of problem sites could be explained by vineyard site or management issues. At this writing, Syrah growers should be relieved to know that if Syrah Decline exists in California, it is very rare.

Authors Golino and Rowhani had the opportunity to visit French vineyards with Syrah Decline. The French cases did not resemble cases in California. The swelling at the graft union of vines with the French Syrah Decline syndrome is solid and woody — something which has not been seen in samples of Syrah submitted to us from California.

We continue to take an interest in the cause of the French Decline problems. We are collaborating with Dr. Jean-Michele Boursiquot, ENTAV, France, and his colleagues to investigate the possibility that an unknown virus is causing French Syrah Decline.

Because we are not convinced that authentic Syrah Decline has been found in California, we plan additional work with samples from France that are frequently identified to demonstrate Syrah Decline. Both French samples and California field samples will be used for dsRNA extraction and cloning in hopes of identifying a causal agent of Syrah Decline.

Extracted dsRNAs can be virus-specific and are often used to find new viruses in plants, including grapes.[11] If a dsRNA can be associated with Syrah Decline, it can be used to create a cDNA library, which can be used both in detecting the agent of disease (by developing reliable PCR primers) and in characterizing its genome.

Grafting experiments are ongoing to try to recreate the symptoms of Syrah Decline. We have established a small field trial with symptomatic Syrah grafted onto select rootstocks to attempt to induce observed symptoms.

Most critical in our opinion, new work needs to be initiated on the Central Coast to determine the cause of the widespread and economically significant Syrah Disorder. Water-relations

Budding tape should be removed from all young vines as soon as the graft is healed, to prevent overgrowths like the one shown here.

research noted earlier provides a solid direction to pursue in the 2004 season.

A proposal has been submitted to conduct a thorough investigation into the water relations of Central Coast Syrah across a range of irrigation levels, to determine if the disorder symptoms are, in fact, correlated with increasing water stress.

Plantings of Syrah along with Cabernet Sauvignon as a comparison variety will be monitored periodically for leaf- and stem-water potential, stomatal conductance, and leaf chlorophyll content. Vines will also be monitored continually for trunk sap flow, leaf temperature, and soil moisture.

A primary goal of new research will be to find a measurable vine parameter that can be used practically to predict if and when the vines are approaching the onset of the disorder.

Acknowledgements

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Many growers on the Central Coast have shared their individual experiences, theories, and valuable time in helping us evaluate Syrah Disorder, and a group of proactive Central Coast growers has pledged additional funding to the American Vineyard Foundation specifically to address this issue. Our thanks to all for giving us the help and resources needed to effectively address this emerging issue. ■

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Appendix X

Citation:

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Characterisation of grapevines visually infected with Shiraz disease associated viruses

by Piet Goussard and Henk Bakker,
Department of Viticulture and Oenology, Stellenbosch University
Based on an Honours project by Henk Bakker (2005)
Key words: *Shiraz disease, anatomy, morphology, physiology*

Introduction

The destructive effect of Shiraz disease noticeable on affected grapevines of predominantly cultivars such as Shiraz, Merlot noir, Gamay noir and Malbec, is a well-known phenomenon in the SA wine industry for many years. Earlier assumptions that its appearance was due to the presence of virus and/or virus like entities in plant material, have been scientifically confirmed by research undertaken mainly since 1990 (Engelbrecht a.o, 1990; Burger & Spreeth, 1993 Carstens, 1997; Goszczynski, 2001). The latest findings (Goszcznski, 2006) indicate that molecular variants of grapevine virus A (GVA) play a significant role at the commencement of and further development of Shiraz disease in certain vineyard blocks. In contrast with leafroll, where re-infected vines of certified plant material can survive and continue to be quite productive for a number of years (provided there is proper management) (Carstens, 2001 a.o), vines infected with Shiraz disease display a sudden and drastic expression of typical visual symptoms, accompanied by a meaningful deterioration of their in-vineyard performance (Engelbrecht a.o, 1990; Carstens, 1999; Goszczynski, 2001 a.o). Infected vines never recover and normally succumb to the disease within a period of three- to a maximum of five years. Although well-known vectors like mealy bug (as with leafroll) are also active in the transfer and spread of the disease (Goszczynski & Jooste, 2003) there is a radically different pattern, as Shiraz disease reflects a typical and unique distribution pattern and tempo (Engelbrecht a.o, 1990).

With the above-mentioned as background the purpose of the project (which stretched over a year) was to characterise vines in a given vineyard block according to the occurrence of Shiraz disease with regard to two specific categories, viz (i) visually infected and (ii) visually not infected. Apart from general performance, the anatomical, morphological and physiological changes/reactions caused by Shiraz disease and expressed symptomatically, were also studied. It was envisioned overall to formulate explanations for the influence of above-mentioned changes/reactions on general in-vineyard performance.

Materials and methods

Experimental vineyard block

The specific vineyard block of 1 ha was established with certified material of Shiraz/Richter 99 on a Glenrosa soil type at Nietvoorbij (sloping to the west with north-south rows). The vineyard was trellised (five-wire extended Perold with moveable foliage wires) and has since its establishment been subjected to supplementary irrigation. A total of 20 vines in this block formed the subject of the study. Out of this group 10 vines were classified as visually infected and 10 vines as visually not infected.

Anatomical, morphological and physiological changes/reactions

Morphological reactions/changes with regard to vine size, leaf characteristics, size and mass of bunches and growth reactions (representative of primary shoots and laterals) in terms of leaf surface, mass and length of shoots and internodal lengths were investigated in both categories. Anatomical investigations were based on changes/reactions induced in primary shoots (representative of apical, central and basal sections) and in the peduncles. Physiological changes/reactions associated with Shiraz disease included measurements of the pH, sugar- and acid concentrations of grapes, as well as the starch content of canes. Standard procedures were used for all the analyses/measurements. Due to the limited duration of the project and use of the particular vineyard for longer- term ARC research project(s), no statistical calculations of applicable data or root investigations could be made.

Results

Morphological reactions/changes

Table 1: The effect of Shiraz disease on growth responses of visually infected and visually not infected vines.

Growth response	Visually infected	Visually not infected
Bunch mass*	38.74 g	198.15 g
Leaf area (primary shoots)*	79.21 cm^2	197.68 cm^2
Leaf area (lateral shoots)*	27.32 cm^2	74.12 cm^2
Shoot lengths (primary shoots)***	75.56 cm	128.97 cm
Shoot lengths (lateral short)***	32.08 cm	48.31 cm
Internode lengths (primary shoots)***	4.88 cm	9.76 cm
Internode lengths (lateral shoots)***	3.78 cm	6.31 cm
Shoot mass (primary shoots)***	26.61 g	83.18 g
Shoot mass (lateral shoots)***	5.00 g	11.26 g
* Mean of bunches derived from 10 vines (1 bunch/vine/infections status)		
** Mean of leaves derived from 6 shoots sampled at apical, middle and bacal parts per infected status		
*** Mean of total shoot numbers from 10 vines per infection status		

Table 2: The effect of Shiraz disease on grape composition at harvest and starch content of canes at winter pruning.

Physiological parameter	Visually infected	Visually not infected
pH*	3.38 (23°C)	3.93 (22°C)
Acid concentration*	7.83 g/L/	5.66 g/L
Sugar concentration*	22.5°B	23.9°B
Starch content *	16.85 mg/g dry material	26.69 mg/g dry material
* Mean of bunches derived from 10 vines (1 bunch/vine/infections status)		
** Mean of total shoot numbers from 10 vines per infection status		

Grapevine sizes and shoot characteristics

Typical of vines associated with Shiraz disease (visually infected), they appeared droopy, with a considerable visual decline in vigour, which together accentuated their smaller size (Fig 1). However, the most obvious characteristic of these vines was the presence of green, rubbery shoots that suffered a lack of lignification. These shoots had numerous lenticells (from the base), which in most cases appeared so closely together that the converging pattern seemed like a longitudinal crack (Fig 2). As the season progressed, there was a total lack of lignification in some shoots, with discolouration from green to nearly black, followed by die-off during the winter. Re-growth of the apical parts was observed during winter, after most of the leaves had already been shed. In most cases the shoots were still green and where lignification did occur, it was abnormal and limited to the basal parts.

Leaf characteristics

Typical discolouring patterns occurred in leaves of visually infected vines (from the outside edge to a complete red colouring) (Fig 4). Together with this, it was observed throughout that in the above-mentioned cases leaf-fall occurred very late, beginning in fact only at the end of June, as opposed to the visually uninfected vines where the process had already run its course by this time (Fig 5).

Interflorence and berry characteristics

Bunches on visually infected vines were the exception rather than the rule. Smaller bunches with berry sizes and characteristics varying from small, green and underdeveloped to practically normal were characteristic and resulting from, inter alia, weak berry set and small, underdeveloped interflorences (Fig 6). A severely reduced crop was reflective of lower bunch masses (Table 1), as well as the presence of suckers, without bunches, which in many cases appeared after allocated bearers had died (Fig 7).

Fig 1. The canopy of vines visually infected with Shiraz disease is characterised by green, rubbery, droopy shoots (February 2005).

Fig 2. Green unlignified shoots with abundant lenticells (February 2005).

Fig 3. Re-growth of visually infected vines as observed during July 2005.

Leaf areas, shoot masses, shoot- and internodal lengths

The two categories differed considerably with regard to leaf areas (determined during harvesting), shoot mass, as well as shoot- and internodal lengths (determined during winter pruning) (Table 1) and these differences were strongly indicative of the presence of visually smaller vines affected by Shiraz disease.

Anatomical reactions/changes

The cambium and cambium derivatives of shoots as well as peduncles were affected, which led to disrupted differentiation patterns in especially the secondary phloem, but also in the secondary xylem (Figs 8, 9, 10). Anatomical changes were characterised by: (i) an indentation of the cambium between the xylem rays, in the direction of the pith, which lent a wavy appearance to the former; (ii) differentiation of abnormally high levels of phloem at the expense of xylem; (iii) absence of secondary phloem fibres; (iv) no or insufficiently developed cork cambium and cork layers; (v) insufficiently developed or absence of primary phloem and (vi) atypical xylem with small compressed trachea. These anatomical changes were representative of entire shoots (basal to apical).

Physiological reactions

The physiological changes in terms of berry composition and starch content of shoots are indicated in Table 2. Using the average concentrations of sugar and acid, pH and starch content as parameters, clear differences could be detected in the categories of visually infected versus visually not infected vines.

Discussion and conclusions

Morphological and physiological changes/reactions as induced in grapevine by Shiraz disease, correspond to the literature (Engelbrecht et al., 1990; Burger & Spreeth, 1993; Carstens, 1997, 1999; Goszczynski, 2001, 2006). Information flowing from this study explained the clear interaction between above-mentioned responses and an unacceptable in-vineyard performance by infected vines. Globally speaking, the cause of these problems may probably

Fig 4. Typical colouring patterns of leaves of visually infected vines. The evenly coloured red lamina was characteristic (June 2005).

Fig 5. While leaf-drop would have been completed under normal circumstances, the process was severely retarded in visually infected vines (Early July 2005).

Fig 6a. Poorly developed interflorences on visually infected vines (left) in comparison with the desired situation on visually not infected vines (Fig. 6b) (October 2005).

be ascribed to anatomical reactions/changes with an adverse influence on essential physiological processes and expressed as abnormal morphological characteristics in such vines. These reactions can be summarised in the following explanation:

Grapevine- and shoot characteristics

The curtailed life span of vines with droopy, rubbery and unlignified shoots following infection by Shiraz disease may, apart from other factors, be caused by an absence of secondary phloem fibre, but especially by insufficient cork cambium and subsequent insufficient formation of cork layers. Such unlignified (green) shoots have difficulty surviving the winter and generally suffer die-off. Abnormal phloem development limits/hampers translocation of photosynthetic products such as carbohydrates to important storage areas like roots, stems and cordons. These reserves play a cardinal role during budding and initial growth during the subsequent growing season. In addition to this, the photosynthetic capacity of infected vines (protection of chlorophyll in leaves and drastic decline in leaf surfaces) gets hampered, leading to a similar situation. This study confirms a decline in photosynthetic capacity by, amongst others, drastically lowered starch content in the shoots of infected vines.

Grape composition

Lower sugar concentrations in the grapes of infected vines may be ascribed to a decline in leaf area (lowering the photosynthetic capacity) as well as hampered translocation of sucrose to the bunches as a result of abnormal phloem development in shoots and peduncles. Consequently, abnormal phloem can also have a hampering effect on the translocation of potassium to the bunches, as manifested in the higher titrateable acid- and lower pH levels of infected vines.

Bunch characteristics and crop sizes

Although the roots were not examined, it may be expected that the lowered photosynthetic capacity and reduced accumulation of reserves (current study) may have a negative impact on the development of roots (fewer root tips) of infected vines. It is known that cytokinins, gibberellins and auxins in particular, are closely

Fig 6b. Desired developed interflorences on visually not infected vines (October 2005).

Fig 7. Die-off of an allocated main bearer (two buds) during winter followed by the development of unfertile suckers in the following growing season contribute to a drastically reduced crop.

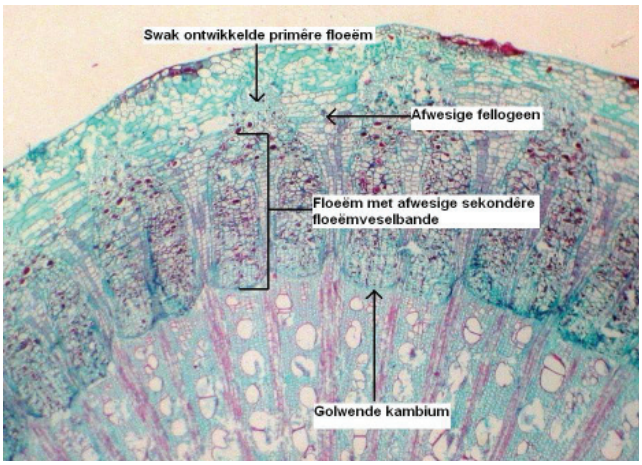

Fig 8. Anatomical composition of a basal shoot section of a visually infected vine. Characteristic is the wavy cambium, weakly developed primary phloem and absence of secondary phloem fibres, as well as phellogen (April 2005).

involved in berry-set and subsequent growth and development. These hormones do not act independently, but maintain a very fine balance and interchange to ensure optimal grape quality and -quantity. It may also be argued that the production of less cytokinins, due to fewer root tips of infected vines, may cause an imbalance. That, together with insufficient reserves, may lead to smaller berries and bunches. Cytokinins also play an important role in regulating the initiation of interflorence primordia (Srinivasan & Mullins, 1979). Smaller interflorences, as observed, may therefore be linked to lower Cytokinins levels that cause reduced fertility and a smaller crop, largely due to an hormonal imbalance in infected vines.

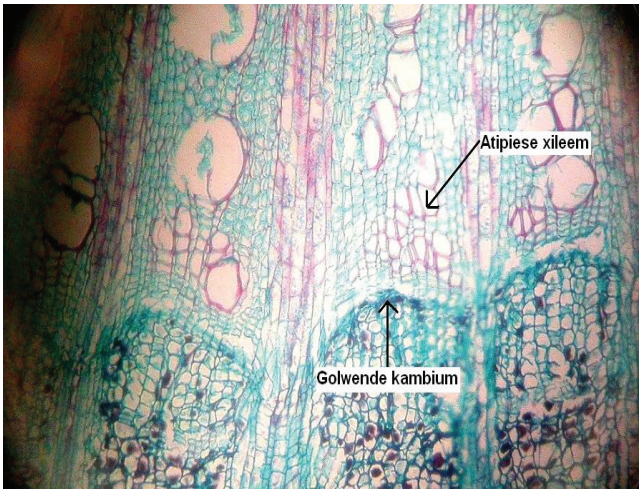

Fig 9. Changes/reactions of the secondary xylem caused by Shiraz disease. It is characterised by atypical capillaries (April 2005).

Leaf area and shoot masses

It is accepted that Shiraz disease may lead to smaller vines and shorter shoots with fewer and smaller leaves. Vines lack vigour, which in turn causes lower reserves, less cytokinins and reduced photosynthesis capacities.

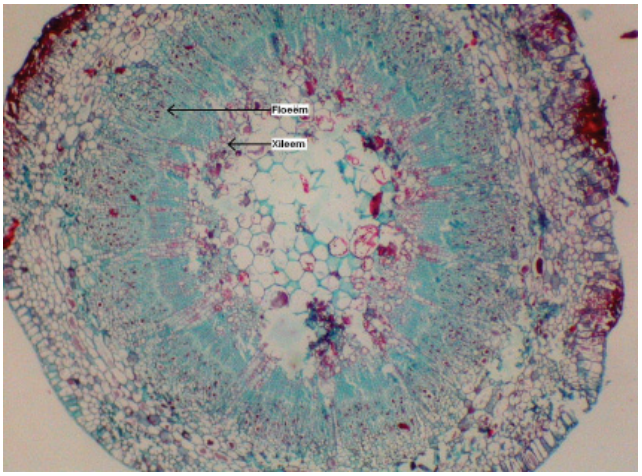

Fig 10. Differentiation of abnormally large phloem tissues, at the expense of xylem in the penucles of infected vines (March 2005).

Synopsis

Against the above background, anatomical changes/reactions of the cambium and the resultant disturbed differentiation and functioning of cambium derivatives can be singled out as important, if not major, causes of inadequate and impaired physiological activities, that in their turn lead to abnormal morphological characteristics. It is known that, for instance in the case of leafroll infected vineyards, as long as recommended management practices are adhered to, vines may not merely be able to “co-exist” with the disease, but may even be able to produce fruit of acceptable quality and quantity. In the case of Shiraz disease, this is definitely not possible. The only recommended practice is to rid the vineyard of infected vines without delay and preferably to burn them. All things considered one of the most important lessons gleaned from this study is the unpredictable way in which the disease targets its prey and its bizarre pattern of spreading before Shiraz disease takes its toll - irrespective whether the specific vineyard was established with certified material or not. Intensive research is under way to formulate scientifically based explanations for (i) the peculiar spreading pattern; (ii) reasons why the “disease” mainly attacks Shiraz and Merlot noir; (iii) the occurrence of other vectors apart from mealy bug and (iv) the virus and/or virus-like entities associated with Shiraz disease. Good progress has been made with regard to (iv) above and it is hoped that these findings may contribute to the clearing up of uncertainties surrounding the other aspects.

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Summary

Grapevines (Shiraz/Richter 99) were characterised according to their responses to Shiraz disease infection. Specific data relating to anatomical, morphological and physiological responses to Shiraz disease, which was manifested by varying degrees of visual symptoms, was gathered. These data included determinations of bunch- and shoot mass, shoot and internode lengths, ripening of grapes as well as grape composition (sugar- and acid concentrations and pH levels) and total leaf area at harvest. Morphological investigations included all the aerial parts of the vines, while anatomical investigations focused primarily on shoots/canes and peduncles. The latter investigation revealed that important physiological processes are hampered as a result of severe anatomical changes/responses, leading eventually to abnormal morphological characteristics of visually infected vines.

Appendix XI

Citation:

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Molecular analysis of a California strain of *Rupestris stem pitting-associated virus* isolated from declining Syrah grapevines

Brief Report

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Summary. The sequence of the genome of a Rupestris stem pitting-associated virus (RSPaV) isolated from a declining Syrah grapevine in California, designated the Syrah strain (RSPaV-SY) was determined. The genome of this strain had an overall nucleotide identity of 77% in comparison with RSPaV sequences in GenBank; the coat protein was the most conserved gene among RSPaV sequences and the replicase was the least conserved gene. Phylogenetic analysis of partial coat protein and replicase gene sequences showed RSPaV-SY clustered independently from the majority of RSPaV isolates.

*

Rupestris stem pitting (RSP) is one disease in the rugose wood disease complex of grapevines [2]. The other diseases are corky bark, Kober stem grooving, and LN33 stem grooving [4]. RSP is characterized by pitting symptoms below inoculum chips in St. George grapevines (*Vitis rupestris* Steele). Chronic infections produce stunting and slow decline [3]. The virus associated with the disease is Rupestris stem pitting-associated virus (RSPaV), which is a member of the genus *Foveavirus* [5] in the family *Flexiviridae* [1]. RSPaV is restricted to grapevines, is not mechanically transmissible [5], and is not known to spread naturally. The virus is found in pollen grains [9] and seeds [10], but these sources do not give rise to infected seedlings [6]. The objectives of this study were to sequence the genome of a strain of RSPaV isolated from a diseased Syrah grapevine (RSPaV-SY) and to use it for sequence comparisons with other RSPaV strains and for design of specific primers to assay field collections for the presence of this virus strain.

The source of RSPaV-SY was a field selection of *V. vinifera* L. variety Syrah exhibiting weak growth and red canopy with an enlarged scion trunk immediately above the graft union. Prior to sacrificing the grape plant, canes were collected and two-node cuttings were rooted and maintained in a greenhouse as source plants. Extracts of leaf petioles were tested for RSPaV, grapevine leaf roll-associated viruses -1, -2, -2 redglobe, -3, -4, -5, -7, and -9; grapevine viruses -A, -B, and -D; grapevine fanleaf virus, Arabis mosaic virus, and tomato ringspot virus in a one-step RT-PCR assay [9]; only RSPaV was identified.

Double-stranded RNA was purified from bark tissues and leaf petioles [11] and a dsRNA of ca. 8.7 kbp in size was detected, which was used to construct a cDNA library [12]. Complementary DNA clones were d(A) tailed [13] and cloned into the Topo TA cloning vector (Invitrogen-Life Technologies). Selected clones were sequenced on both sense and anti-sense strands using vector primers T7 and T3. Existing gaps were completed using specific PCR primers designed based on known nucleotide sequences. The 5′ and 3′ terminal sequences were obtained using a 5′ rapid amplification of cDNA ends kit (Invitrogen-Life Technologies) and RT-PCR with oligo d(T)-priming, respectively. Sequence data were analyzed using the BLAST program of the National Center for Biotechnology Information (NCBI). For primer design and ORF search, the DNAsis Max Program package version 2.0 (Hitachi software Engineering Co., Middlesex, UK) was used. The RSPaV-SY sequence was assigned the accession number AY368590 in GenBank.

The complete genome of RSPaV-SY was 8,725 nt long and had a genome organization similar to RSPaV with six ORFs ([12]; Fig. 1). The RSPaV-SY nucleotide sequence showed a genomic sequence identity of 77% to each of the previously published sequences of four other RSPaV isolates (RSPaV, RSPaV-1, RSPaV-BS, and RSPaV-SG1) [7, 8, 12]; these four isolates were more closely related to each other than to RSPaV-SY, sharing a genomic sequence identity varying from 83% between RSPaV-BS and RSPaV-SG1 [8] and 98% between RSPaV and RSPaV-1 [7, 8]. The coat protein gene (ORF 5; see Fig. 1) of RSPaV-SY

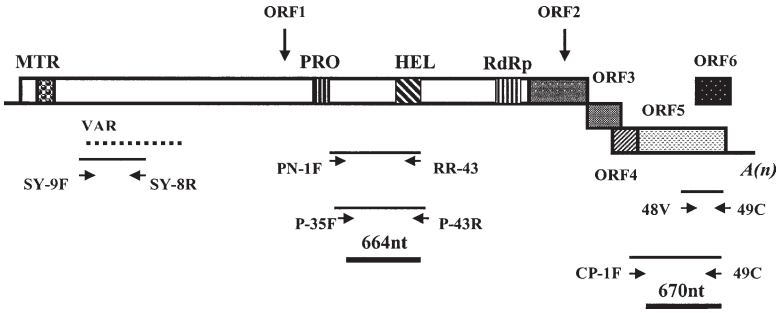

Fig. 1. Genome organization of the Rupestris stem pitting-associated virus, Syrah strain. Six open reading frames (ORF) were identified in the genome. The solid lines are the location of primers used in this report. Their respective sequences are given in Table 1. The thick lines show the size and location of the regions in the replicase and coat protein genes (ORFs 1 and 5) used for phylogenetic analysis. The broken line shows the location of the variable region (VAR) identified in the ORF1, which encodes for the replicase gene

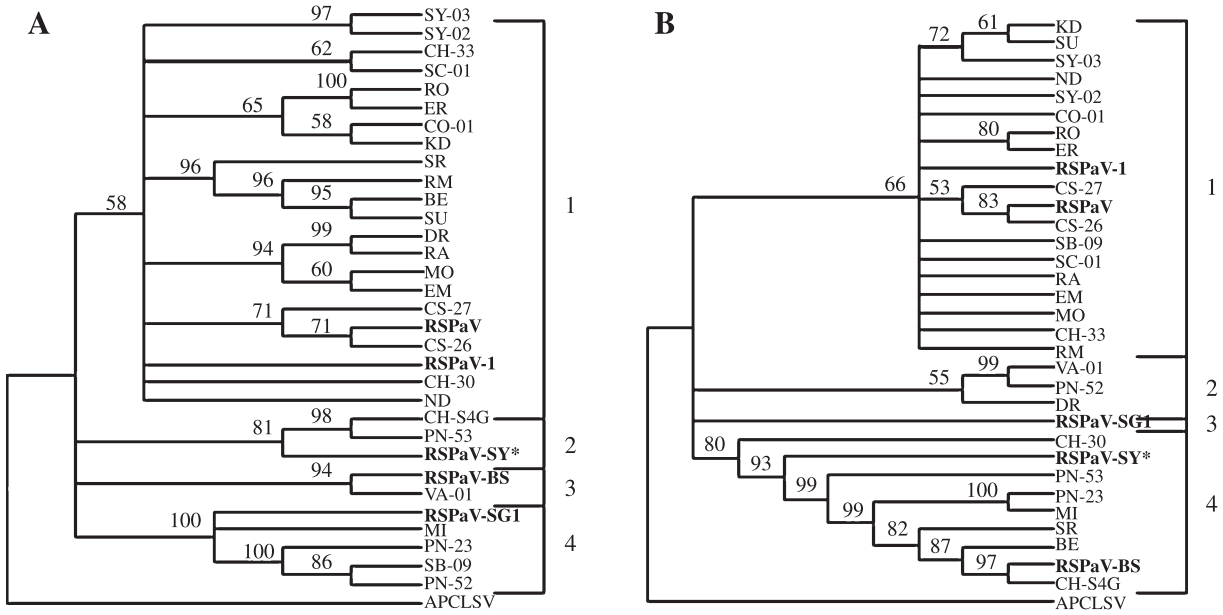

Fig. 2. Phylogenetic analysis showing the relationships among RSPaV-SY isolate (emboldened and marked with an asterisk) and other 31 RSPaV isolates including the four isolates (emboldened) for which their full-length sequences are available in the database [RSPaV (AF026278); RSPaV-1 (AF057136); RSPaV-SG1 (AY881626) and RSPaV-BS (AY881627)]. For the comparison, fragments of the replicase gene were amplified by RT-PCR using primers P-35F/P-43R or PN-1F/RR-43, and the overlapping 664-nt fragments were used in the phylogenetic analysis of ORF 1 (**A**). For the coat protein analysis, fragments were obtained with primers RSP-49C and CP-1F (Table 1). However, due to difficulties in sequencing this product in some isolates, an internal forward primer, CP-3F, was designed based on sequences of several isolates and used on the remaining isolates. Complete sequences of the resulting products (670 nt in length) were utilized in the phylogenetic analysis of the coat protein (**B**). Apricot pseudo-chlorotic leaf spot virus [APCLSV (AY713379)] in the genus *Trichovirus* was used as an out group. The phylogenetic trees were generated by the Mega 2.1 program, with assistance of the Clustal X and Genedoc programs. Horizontal distances are proportional to sequence distances. The dendrogram was bootstrapped 1000 times. Bootstrap scores are on relevant horizontal branches. Branches with less than 50% bootstrap support are presented as polytomies. RSPaV isolates used in this investigation were from the following grape selections and the corresponding database accession numbers (replicase, coat protein) are presented in parenthesis: *DR* = Damas Rose (AM180520, AM180426); *ER* = Emile Royal (AM180539, AM180438); *EM* = Emperor (AM180532, AM180419); *RM* = Red Malaga (AM180533, AM180424); *RA* = Rangspray (AM180534, AM180422); *RO* = Royal (AM180535, AM180427); *MI* = Murma Isium (AM180536, AM180423); *KD* = Kara Dzhidzhigi (AM180537, AM180437); *ND* = Noir D'automne (AM180531, AM180421); *BE* = Bellino (AM180538, AM180420); *MO* = Monukka (AM180540, AM180433); *SU* = Sultana (AM180541, AM180443); *SR* = Sultanina Rose (AM180542, AM180432); *CS-26* and *CS-27* = Cabernet Sauvignon-26 (AM180543, AM180440) and -27 (AM180521, AM180435); *CH-30* and *CH-33* = Chardonnay-30 (AM180522, AM180425) and -33 (AM180523, AM180439); *PN-23*, *PN-52* and *PN-53* = Pinot Noir-23 (AM180519, AM180418), -52 (AM180529, AM180436) and -53 (AM180524, AM180430); *SY-02* and *SY-03* = Syrah-02 (AM180525, AM180442) and -03 (AM180518, AM180431); *CO-01* = Coucoceira-01 (AM180526, AM180441); *VA-01* = Valdiguie-01 (AM180527, AM180428); *SB-09* = Sauvignon Blanc-09 (AM180528, AM180417); *SC-01* = Schiopp.-01 (AM180530, AM180434); and *CH-S4G* = Chardonnay-S4G (AM181038, AM180429)

shared the highest nucleotide and amino acid identities (83–84% and 91–92%, respectively) with other RSPaV sequences in the database [7, 8, 12], while the replicase gene (ORF1) shared the least sequence identity (75–76% and 85% at the nucleotide and amino acid levels, respectively). The most significant variation occurred in a region of 331 amino acid residues located between methyltransferase (MTR) and protease (PRO) conserved domains (See Fig. 1 – ORF1 dotted line). Amino acid sequence identity in this region was only 52–54% between RSPaV-SY and RSPaV/RSPaV-1, RSPaV-BS and RSPaV-SG1. The RSPaV-BS and RSPaV-SG1 variants shared 74 and 73% amino acid identity with RSPaV, respectively, in this region of ORF1. In contrast to the replicase gene, the 5′ and 3′ untranslated regions of the RSPaV-SY and these other RSPaV isolates shared 91–96% and 91–92% nucleotide identities, respectively. Further comparisons of the replicase gene between RSPaV-SY and the type virus of the genus *Foveavirus*, apple stem pitting virus (ASPV) [1] revealed 59% nucleotide and 42% amino acid identities.

Phylogenetic analyses of ~650-nt regions of both the replicase and coat protein genes (Fig. 1) were performed to determine the relationships between RSPaV-SY and 27 other RSPaV isolates from different grape varieties in a UC Davis collection (see legend Fig. 2). Analysis using the replicase gene sequence revealed that the RSPaV isolates segregated into four branches (Fig. 2A). The first branch was comprised of 22 isolates, including the previously sequenced RSPaV and RSPaV-1 isolates (which shared very close nucleotide identity of 98% of their complete genome). RSPaV-SY isolate, which has shown significant variability on the ORF1, clustered with only two other isolates, PN-53 and CH-S4G (branch 2). Branch 3 is formed by RSPaV-BS and VA-01 isolates, and RSPaV-SG1 with four other isolates was clustered on the fourth branch. The phylogenetic tree generated from the more conserved coat protein sequence differed to some extent in the groupings evident in the replicase sequence tree (Fig. 2B). Two main groups of isolates were identified in this analysis. One was formed by 17 isolates (branch 1) and the other by 9 isolates (branch 4). Most of the isolates in branch 1 were grouped together in both phylogenetic analyses. Branch 4 of this tree contained isolates from branches 2, 3 and 4 of the replicase gene tree. Overall, the RSPaV-SY sequence clustered with few of the other isolates in the collection and, in this regard, the previously reported genomic sequences also represent different phylogenetic groupings. Within either of the phylogenetic trees, the clustering reflected neither the geographic regions of isolation nor the grape varieties from which the isolates were made.

To investigate incidence of RSPaV-SY isolate in commercial vineyards, by RT-PCR, two pairs of primers targeting two different genes were used (Fig. 1; Table 1). One set of primers, RSP 48V/49C, was designed from a more conserved region (coat protein) and used as RSPaV-universal primers, which amplified a 331-bp fragment. The second pair, SY9F/SY8R, was designed from a more variable region (replicase gene) of the RSPaV-SY, which specifically detects this strain, and amplified a fragment of 628 bp. Among 383 plants tested, including 70 Syrah plants, 245 (64%) tested positive using RSPaV-universal primers (48V/49C); 59 of

Table 1. List of oligonucleotide PCR primers used in this study. Primers obtained from other sources are listed in the reference column

	Sequence	Location in the genome	Product size (nt)	Reference
48V	5′AGCTGGGATTATAAGGGAGGT3′	8,180–8,200	331	unpublished
49C	5′CCAGCCGTTCCACCACTAAT3′	8,509–8,528		
P-35F	5′ATGGTTGCATGATCACAGCCA3′	3,545–3,566	776	this work
P-43R	5′AGTGGCCAGCCTTCAATCC3′	4,300–4,319		
PN-1F	5′GATGGATACAAGTTACGGGC3′	3,442–3,462	868	this work
RR-43	5′ACATCCCACCCTTCCTTCTT3′	4,289–4,308		
CP-1F	5′GGTTTGAAGGCTTTAGGGGT3′	7,709–7,728	817	this work
49C	5′CCAGCCGTTCCACCACTAAT3′	8,506–8,526		unpublished
SY-9F	5′AGGATTCCAAACTGTAGAGCAA3′	2,083–2104	628	this work
SY-8R	5′TTGGTCGTCATCTTCCAGTT3′	2,689–2,710		
CP-3F	5′TGAAGAAATTGATTATC3′	7,741–7,757	–	this work

the positives were Syrah plants. Using the RSPaV-SY-specific primers (SY-9F/SY-8R), 36 (14.7%) of the RSPaV-positive samples were also positive for RSPaV-SY, including 29 of the positive specimens from Syrah plants. Thus, roughly half of the diseased Syrah plants, but very few of the other infected grape varieties (7/186), were infected with the SY strain. To confirm the specificity of the RSPaV-SY primers, twenty of the amplicons generated by these primers were sequenced and found to share about 90% nucleotide identity to RSPaV-SY and about 73% to RSPaV sequences in the database. Among the Syrah specimens in the collection tested, four asymptomatic plants (without any canopy symptoms) were included in these assays and all of the four plants tested positive for the 48V/49C universal and one for RSPaV-SY (SY-9F/SY-8R) specific primers. Research is continuing to investigate the correlation between the virus and the disease.

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