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.

References

1. Meng B, Li Caihong, Wang W, Goszczynski D and Gonsalves D (2005) Complete genome sequence s of two variants of Grapevine rupestris stem pitting-associated virus and comparative analysis. J Gen Virol 86: 1555-1560.

2. Zhang Y-P, Uyemoto JK, Golino DA and Rowhani A (1998) Nucleotide sequence and RT-PCR detection of a virus associated with grapevine rupestris stem-pitting disease. Phytopathology 88: 1231–1237.

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	-

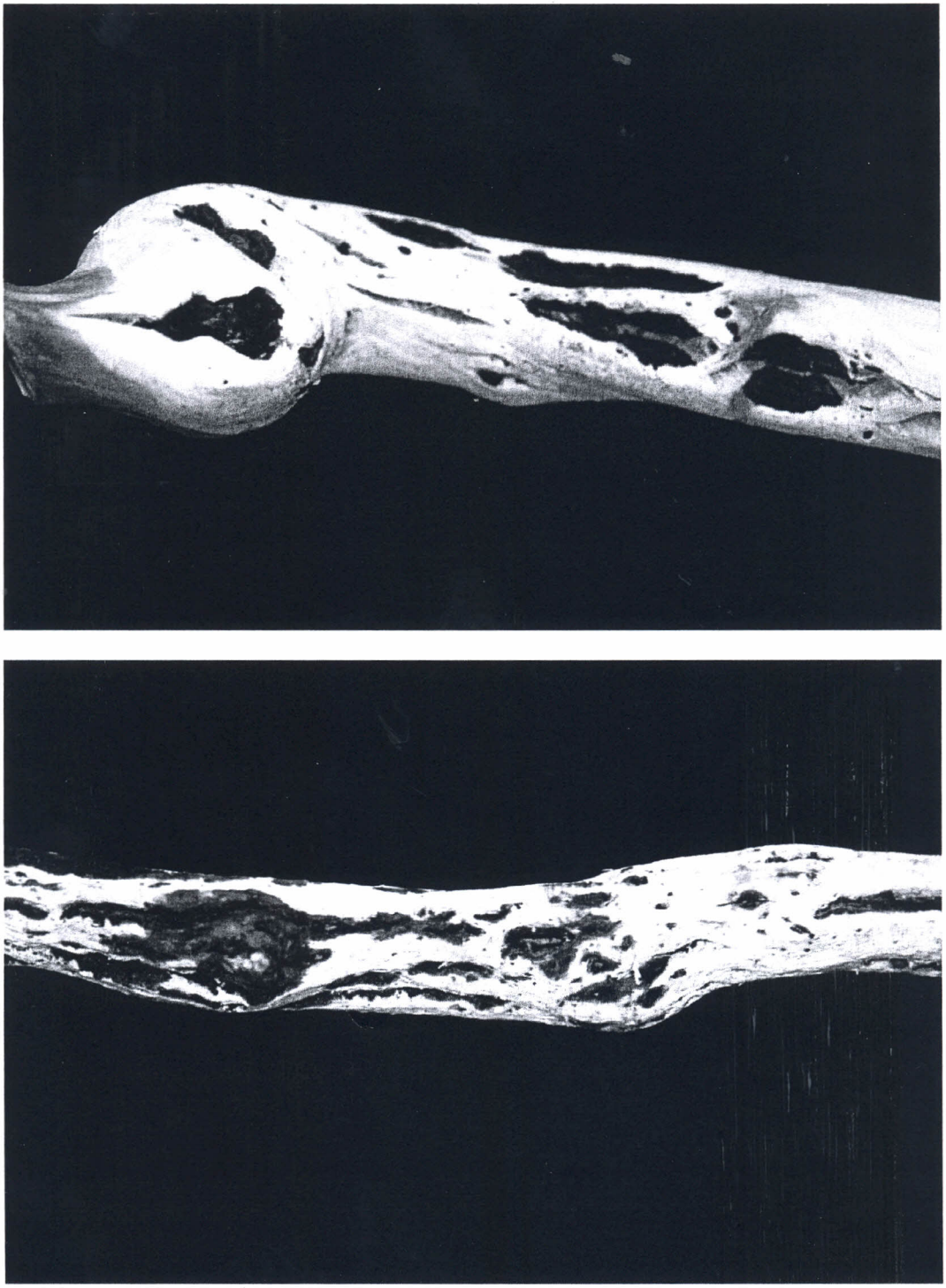

Figure 1. Woody cylinder of a Syrah plant affected with the disease. Pitting, grooving and dead tissue showing on the wood are similar to those that cause by some of the viruses in the rugose wood complex.

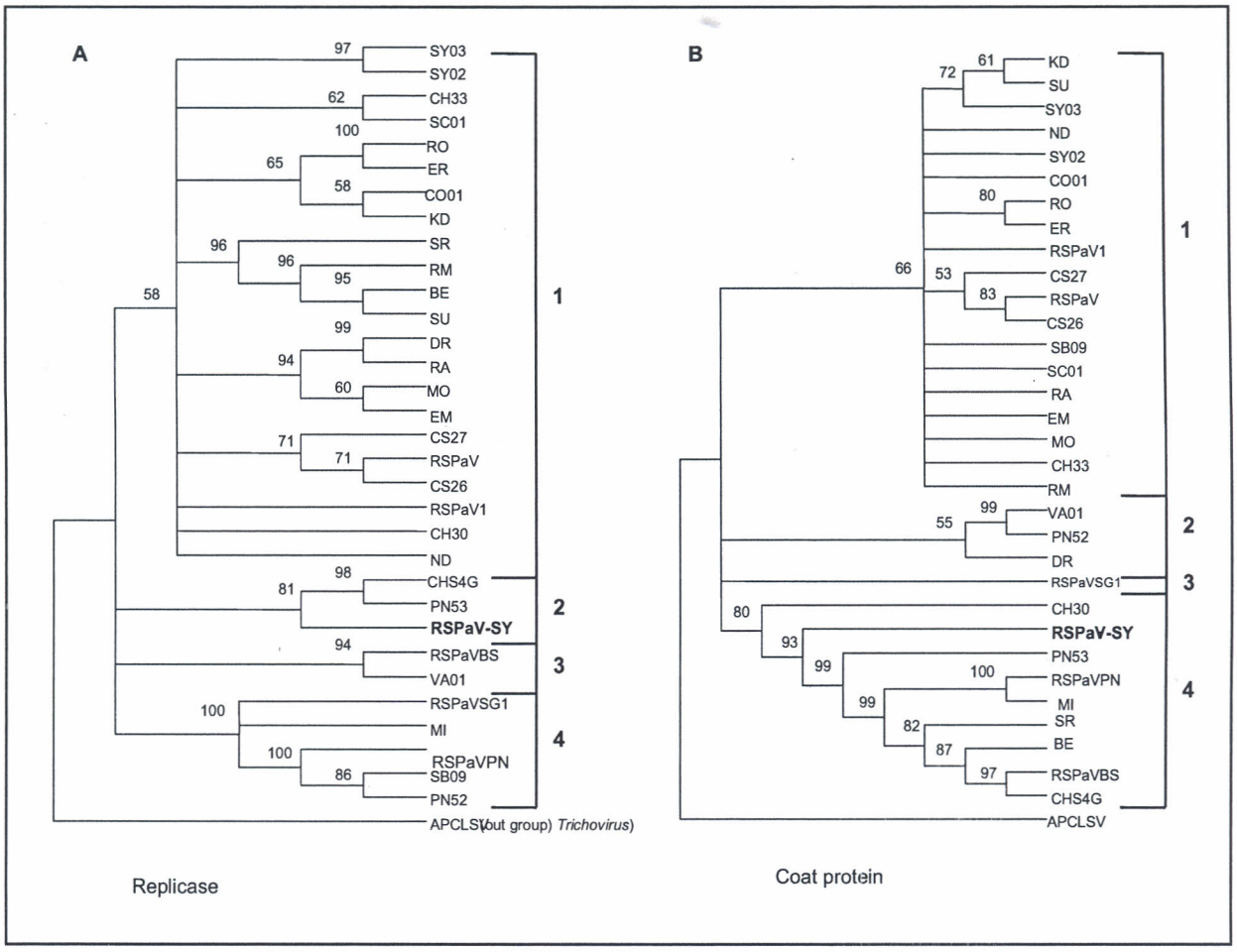

Figure 2. – Phylogenetic analysis showing the relationships among RSPaV-SY isolate and other 31 RSPaV isolates including the four isolates which their full length sequences are available in the database [RSPaV (AF026278); RSPaV-1 (AF057136); RSPaV-SG1 (AY881626) and RSPaV-BS (AY881627)]. For the comparison, 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.

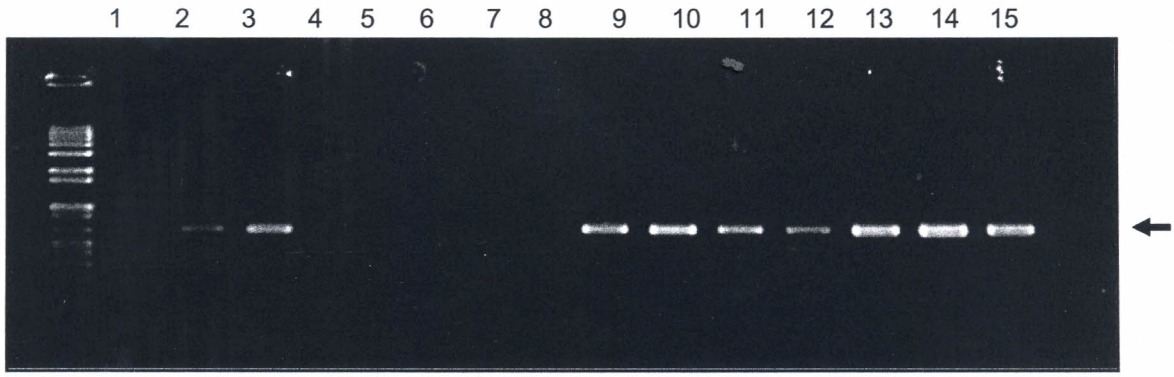

Figure 3. The RT-PCR analysis of number of Syrah samples showing the red leaf symptoms. Lane 1 is healthy control. Lanes 2-15 are samples from different Syrah plants. The primers SY-9F/8R were used for the test. The arrow shows the specific PCR products.